© 2019. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

RESEARCH ARTICLE

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

Tatiana S. Filatova[1,2,*], Denis V. Abramochkin[1,2,3] and Holly A. Shiels[4]

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 acclimatory response within a single eurythermal fish species, the European shorthorn sculpin (*Myoxocephalus scorpio*). 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 kept at 12°C ('summer-acclimated'); (2) summer-caught fish kept at 3°C ('cold acclimated'); and (3) fish caught in March ('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_{Kr} and I_{K1}) following winter acclimatization, with cold 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 further analysis shows that ionic current remodelling is primarily due to changes in ion channel density rather than current 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 scorpio*

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 Gesser, 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 acclimatory response of both chambers of the heart within a single eurythermal fish species, the European shorthorn sculpin (*Myoxocephalus scorpio*) 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).

[1]Department of Human and Animal Physiology, Lomonosov Moscow State University, Leninskiye gory, 1, 12, Moscow, Russia 119234. [2]Department of Physiology, Russian National Research Medical University, Ostrovityanova str., 1, Moscow, Russia 117997. [3]Ural Federal University, Mira 19, Ekaterinburg, Russia 620002. [4]Faculty of Life Sciences, Core Technology Facility, 46 Grafton Street, University of Manchester, Manchester M13 9NT, UK.

*Author for correspondence (filatova@mail.bio.msu.ru)

T.S.F., 0000-0003-0131-1911; D.V.A., 0000-0001-5751-8853

Received 25 February 2019; Accepted 15 July 2019

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

List of symbols and abbreviations

AP	action potential
APD25, APD50 and APD90	action potential duration at 25%, 50% and 90% level of repolarization, respectively
G	conductance
I	registered current at a given membrane potential
I_{Ca}	calcium L-type current
I_{Na}	fast sodium current
I_{K1}	inward rectifier potassium current
I_{Kr}	rapid delayed rectifier potassium current
RMP	resting membrane potential
TTX	tetrodotoxin, selective fast sodium current blocker
V_{rev}	reversal potential of the current
$V_{0.5}$	half activation potential
τ	time constant of inactivation

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 [*Myoxocephalus scorpio* (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 m^3) 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 Ca^{2+}-free low-Na^+ solution (in mmol l^{-1}): 100 NaCl, 10 KCl, 1.2 $KH_2PO_4{\cdot}2H_2O$, 4 $MgSO_4{\cdot}7H_2O$, 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, 0.66 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

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_{Kr} and I_{K1}) 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_{Ca}) and fast sodium current (I_{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, GmbH, 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_2GTP 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_{Kr} was blocked with E-4031 (1 µmol l^{-1}), I_{K1} was blocked with Ba^{2+} (1 mmol l^{-1}), I_{Ca} was blocked with nifedipine (10 µmol l^{-1}) and I_{Na} was blocked with TTX (0.5 µmol l^{-1}). I_{Ca} and fast sodium current I_{Na} amplitudes (pA) were analysed as the difference between the peak current and current at the end of the pulse. I_{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_{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 (τ) for I_{Ca}, I_{Na} and I_{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_{Kr} time constant of inactivation was measured from the tail current:

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

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

$$y = \frac{1}{1 + \frac{\exp(V - V_{50})}{S}}, \quad (2)$$

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:

$$G = \frac{I}{V - V_{rev}}, \quad (3)$$

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_{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 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

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

Table 1. Relative heart mass and the capacitance of cardiac myocytes isolated from shorthorn sculpin

	Summer-acclimated	Cold-acclimated	Winter-acclimatized
Relative heart mass (%)	0.245±0.0182	0.219±0.0275	0.239±0.0248
Atrial myocyte capacitance (pF)	26.82±1.415	25.05±0.764$^{\#}$	28.92±0.814$^{\#}$
Ventricular myocyte capacitance (pF)	28.2±1.184*	27.21±0.922$^{\#}$	34.36±1.194*,$^{\#}$

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.

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 ventricle 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 acclimatization to

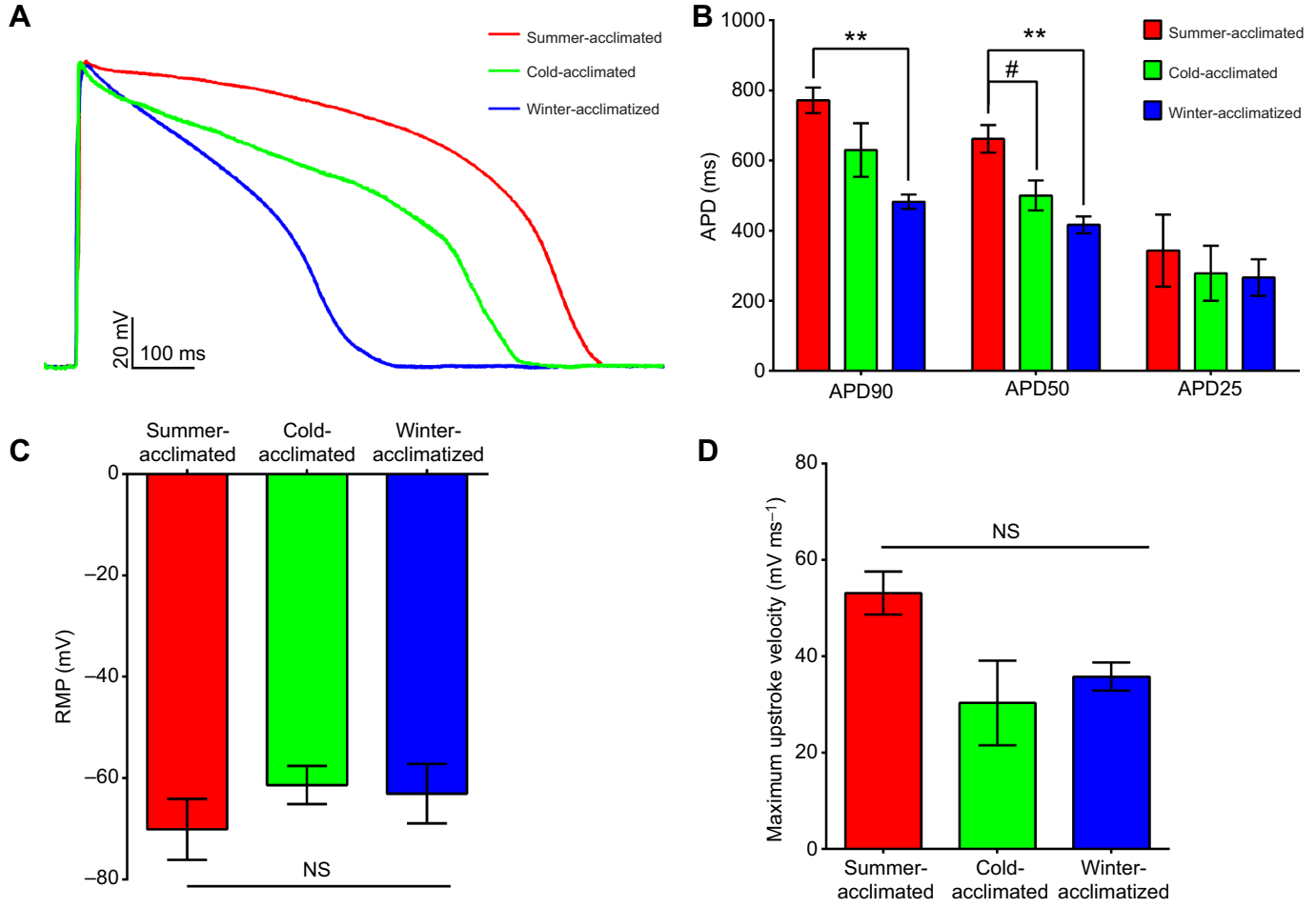

Fig. 1. Ventricular action potential configuration. (A) Representative recordings of ventricular action potentials (APs), (B) means±s.e.m. for the APD at 90%, 50% and 25% repolarization, (C) resting membrane potential and (D) maximum upstroke velocity in isolated ventricular myocytes from summer-acclimated 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 (3°C, blue, n=8 myocytes from 6 animals). Statistically significant difference between summer-acclimated and winter-acclimatized groups (**P<0.01); between summer-acclimated and cold-acclimated groups ($^{\#}P$<0.05). 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.

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

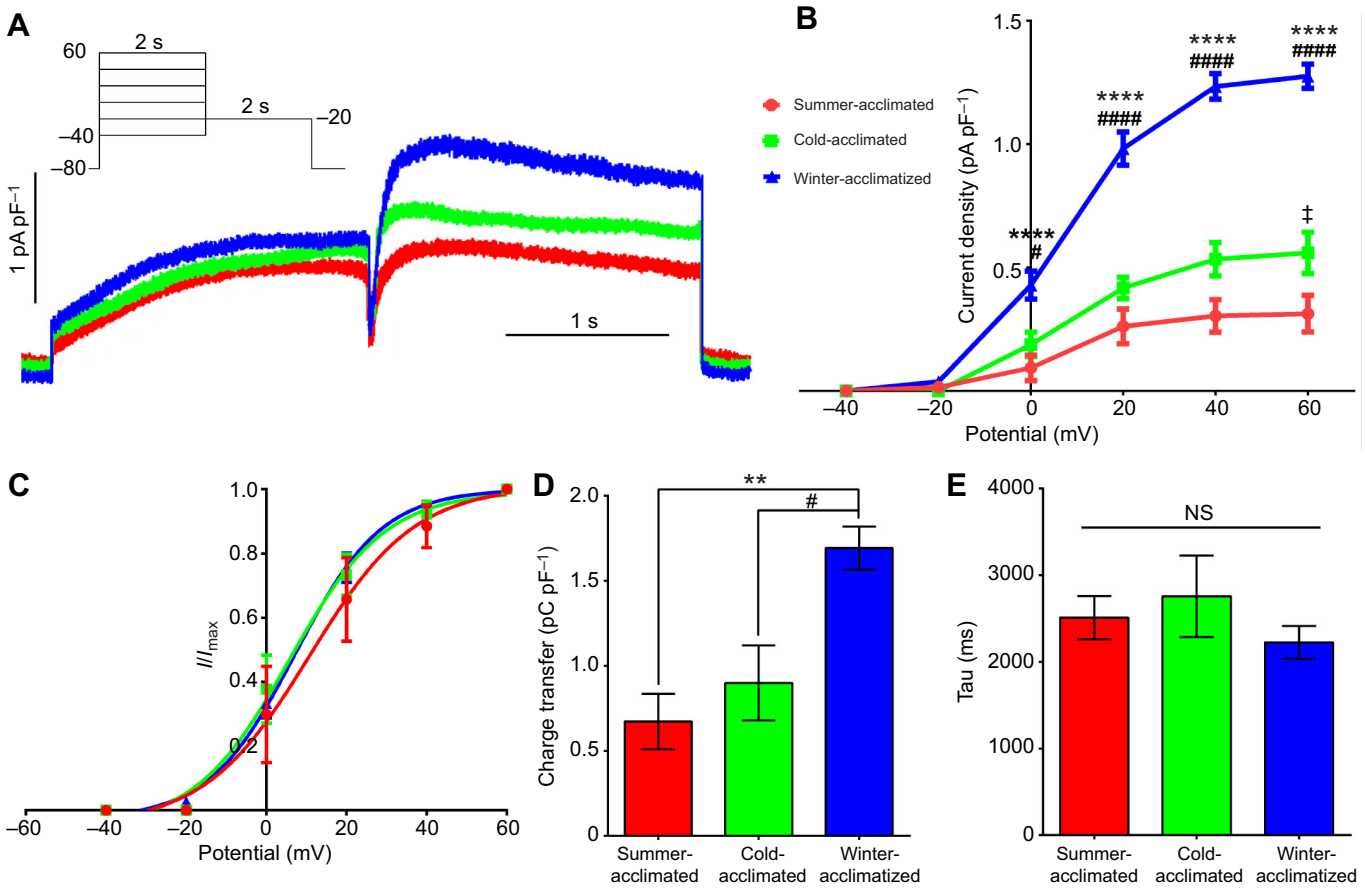

Fig. 2. Characteristics of I_{Kr} in sculpin ventricle. (A) Representative delayed rectifier potassium current recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of the I_{Kr} tail current in isolated ventricular myocytes from summer-acclimated (12°C, red, n=11 myocytes from 6 animals), cold-acclimated (3°C, green, n=9 myocytes from 5 animals) and winter-acclimatized sculpins (3°C, blue, n=12 myocytes from 7 animals). Statistically significant differences between: summer-acclimated and winter-acclimatized groups (**P<0.01, ****P<0.0001); winter-acclimatized groups and cold-acclimated groups ($^{\#}P$<0.05, $^{\#\#\#\#}P$<0.0001); summer-acclimated and cold-acclimated groups ($^{‡}P$<0.05); NS, not significant.

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 compared with summer-acclimated fish. Similarly, the charge carried by the I_{Kr} current was 47.7±11.6% higher in atrial cells and 151.6±18.8% higher in ventricular cells from winter-acclimatized compared with summer-acclimated fishes. Unlike seasonal acclimatization to the cold, cold acclimation for 6 weeks in the laboratory did not significantly affect this key repolarizing current, which was not different from that in the atrium or ventricle of the summer-acclimated cohort (except at peak activation, 60 mV, Fig. 2B).

Fig. 2C,E, Fig. 3C,E and Table 2 show that neither thermal acclimation nor seasonal acclimatization affected steady-state activation or time-dependent inactivation of the I_{Kr} tail current in ventricular and atrial myocytes, respectively. This suggests that the changes in I_{Kr} current density and charge transfer are due to altered current density levels, not a change in the way the channel conducts current.

Inward rectifier current (I_{K1})

The inward rectifying potassium current (I_{K1}), 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_{K1} 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-acclimated 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_{Kr}, cold acclimation did not significantly modify the current density of I_{K1} when compared with the summer-acclimated cohort.

L-type calcium current (I_{Ca})

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

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

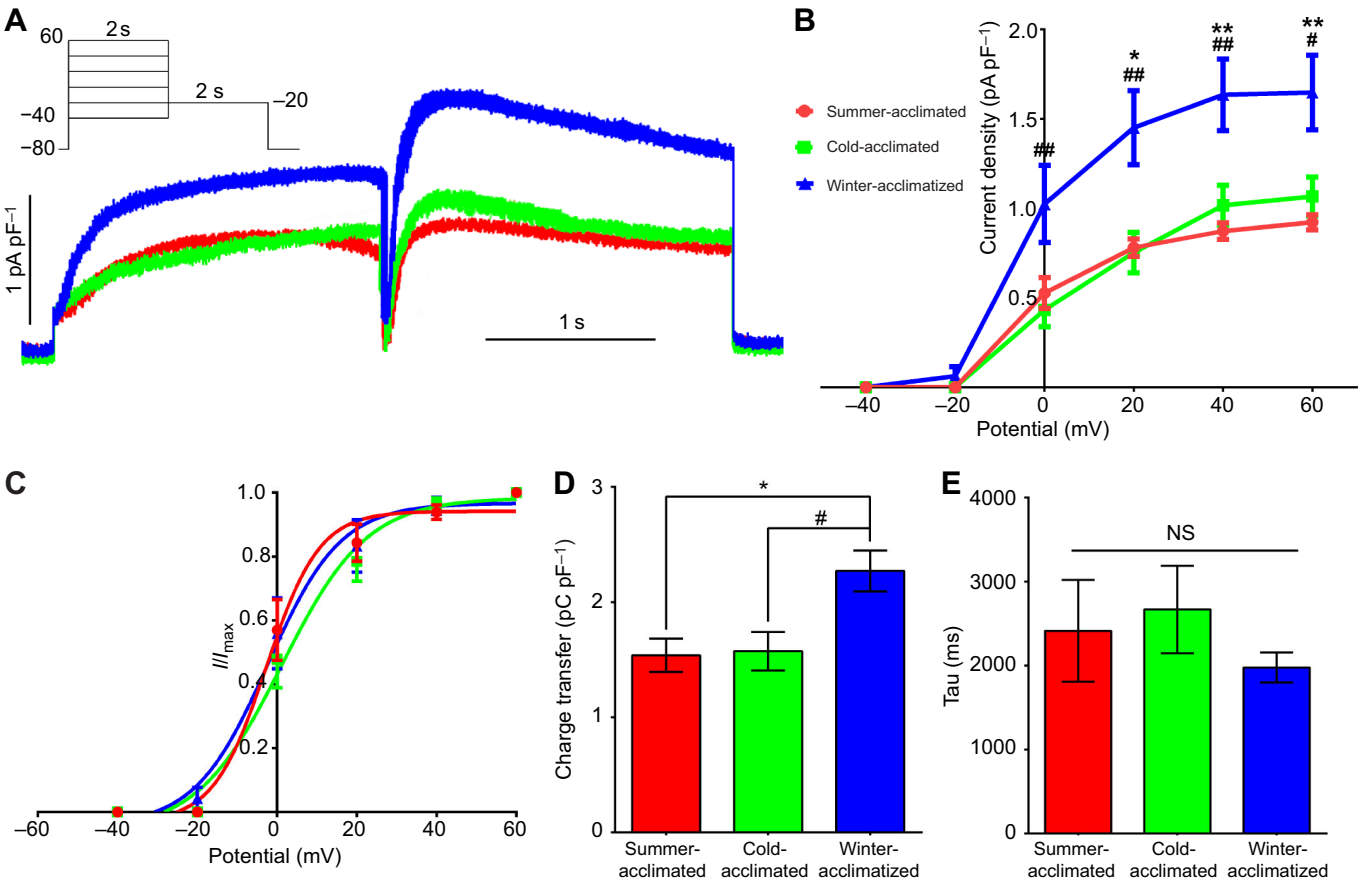

Fig. 3. Characteristics of I_{Kr} in sculpin atrium. (A) Representative delayed rectifier potassium current recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of I_{Kr} tail current in isolated atrial myocytes from summer-acclimated (12°C, red, n=11 myocytes from 6 animals), cold-acclimated (3°C, green, n=9 myocytes from 6 animals) and winter-acclimatized sculpins (3°C, blue, n=12 myocytes from 7 animals). Statistically significant differences between: summer-acclimated and winter-acclimatized groups (*P<0.05, **P<0.01); winter-acclimatized groups and cold-acclimated groups ($^{\#}P$<0.05, $^{\#\#}P$<0.01); NS, not significant.

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_{Ca} 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

		Ventricle		Atrium	
		$V_{0.5}$	Hill slope	$V_{0.5}$	Hill slope
I_{Kr}	Summer-acclimated	10.808±4.853	13.159±4.776	−2.694±1.89	6.658±2.257
	Cold-acclimated	5.733±2.999	11.938±2.891	1.196±2.475	10.692±2.452
	Winter-acclimatized	7.134±1.407	10.856±1.284	−2.354±2.751	9.136±2.772
I_{Ca}	Summer-acclimated	−17.754±1.844*	8.811±1.904	−19.96±2.405*	8.246±2.861
	Cold-acclimated	−14.317±1.297$^{\#}$	8.211±1.124	−13.494±3.571	9.537±3.114
	Winter-acclimatized	−8.855±1.214*,$^{\#}$	6.412±0.718	−10.249±1.045*	6.836±0.646
I_{Na}	Summer-acclimated	−37.24±0.524	2.838±0.411	−34.162±0.536*,‡	5.097±0.456
	Cold-acclimated	−38.448±1.045	2.465±1.334	−38.074±1.032$^{\#}$,‡	5.373±1.065
	Winter-acclimatized	−36.284±0.436	2.874±0.267	−40.467±0.243*,$^{\#}$	4.27±0.288

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.

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

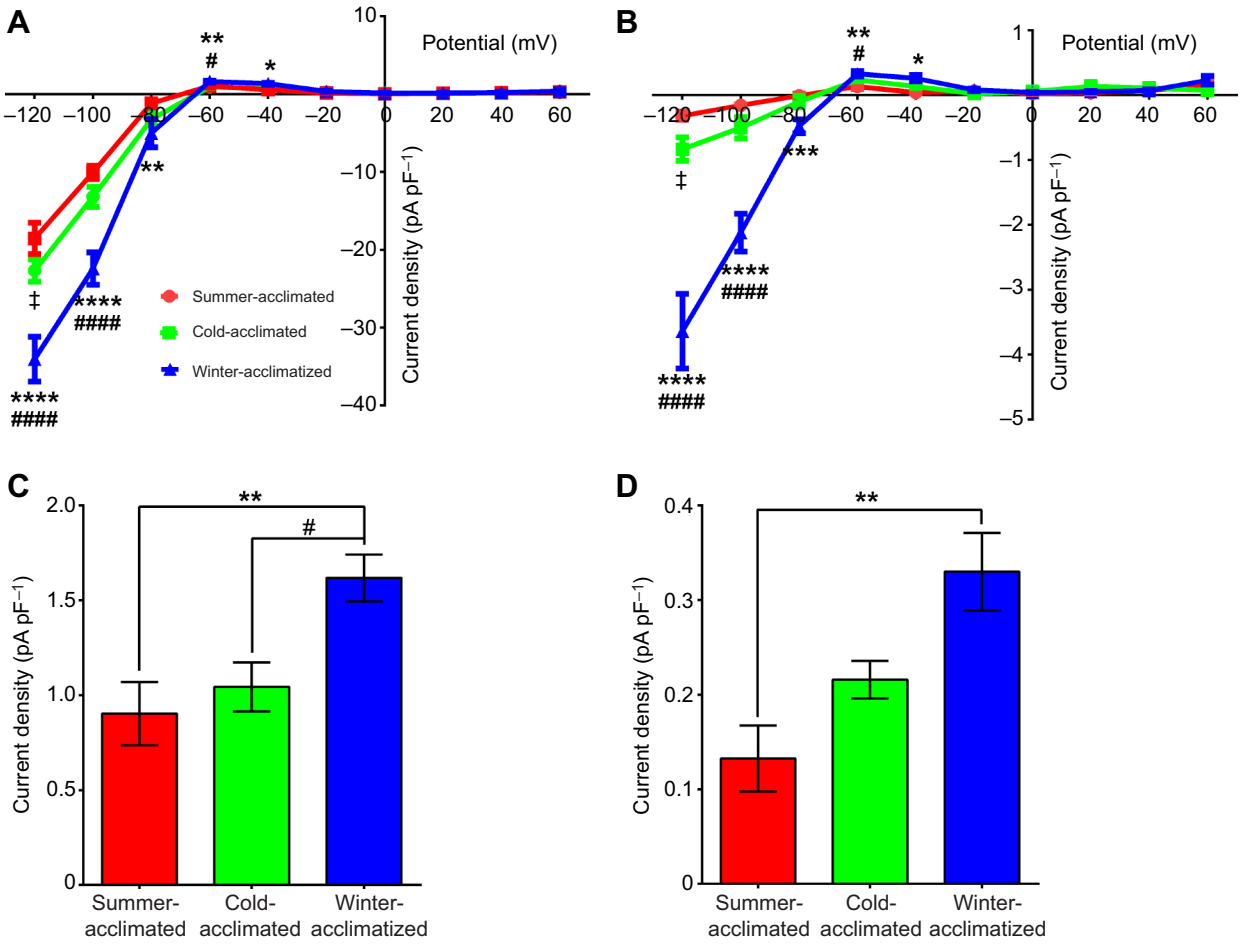

Fig. 4. Characteristics of I_{K1} in sculpin ventricle and atrium. (A,B) Current–voltage relationships of I_{K1} current and (C,D) amplitude of outward current at holding potential −60 mV in isolated ventricular and atrial myocytes (respectively) from summer-acclimated (12°C, red, *n*=6 and 11 myocytes from 6 animals), cold-acclimated (3°C, green, *n*=7 and 10 cells from 6 animals) and winter-acclimatized sculpins (3°C, blue, *n*=8 and 12 myocytes from 5 animals). Statistically significant differences between: summer-acclimated and winter-acclimatized groups (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001); winter-acclimatized groups and cold-acclimated groups ($^{\#}P$<0.05, $^{\#\#\#\#}P$<0.0001); summer-acclimated and cold-acclimated groups ($^{\ddagger}P$<0.05).

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 (τ_{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 τ_{fast} nor τ_{slow} differed between the groups; no changes were observed in the steady-state activation characteristics of I_{Na} (Fig. 7D,E,F).

In atrial myocytes of the summer-acclimated group 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 τ_{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

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

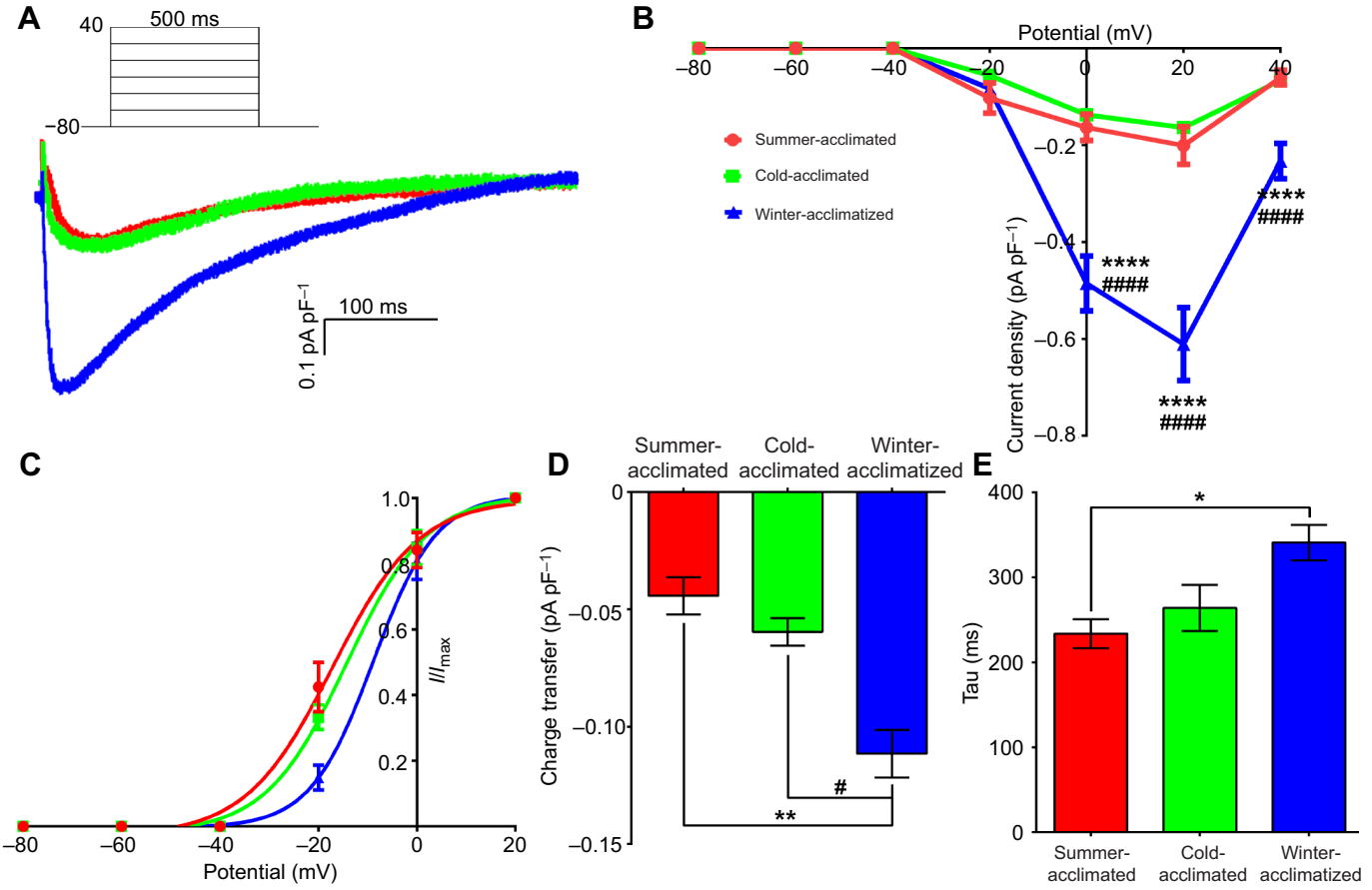

Fig. 5. Characteristics of I_{Ca} in sculpin ventricle. (A) Representative recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of I_{Ca} current in isolated ventricular myocytes from summer-acclimated (12°C, red, n=9 myocytes from 5 animals), cold-acclimated (3°C, green, n=11 myocytes from 5 animals) and winter-acclimatized sculpins (3°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-acclimated groups ($^{\#}P$<0.05, $^{\#\#\#\#}P$<0.0001).

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 (*Myoxocephalus scorpio*) 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 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_{Kr}. This appears to be a ubiquitous mechanism for AP shortening

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

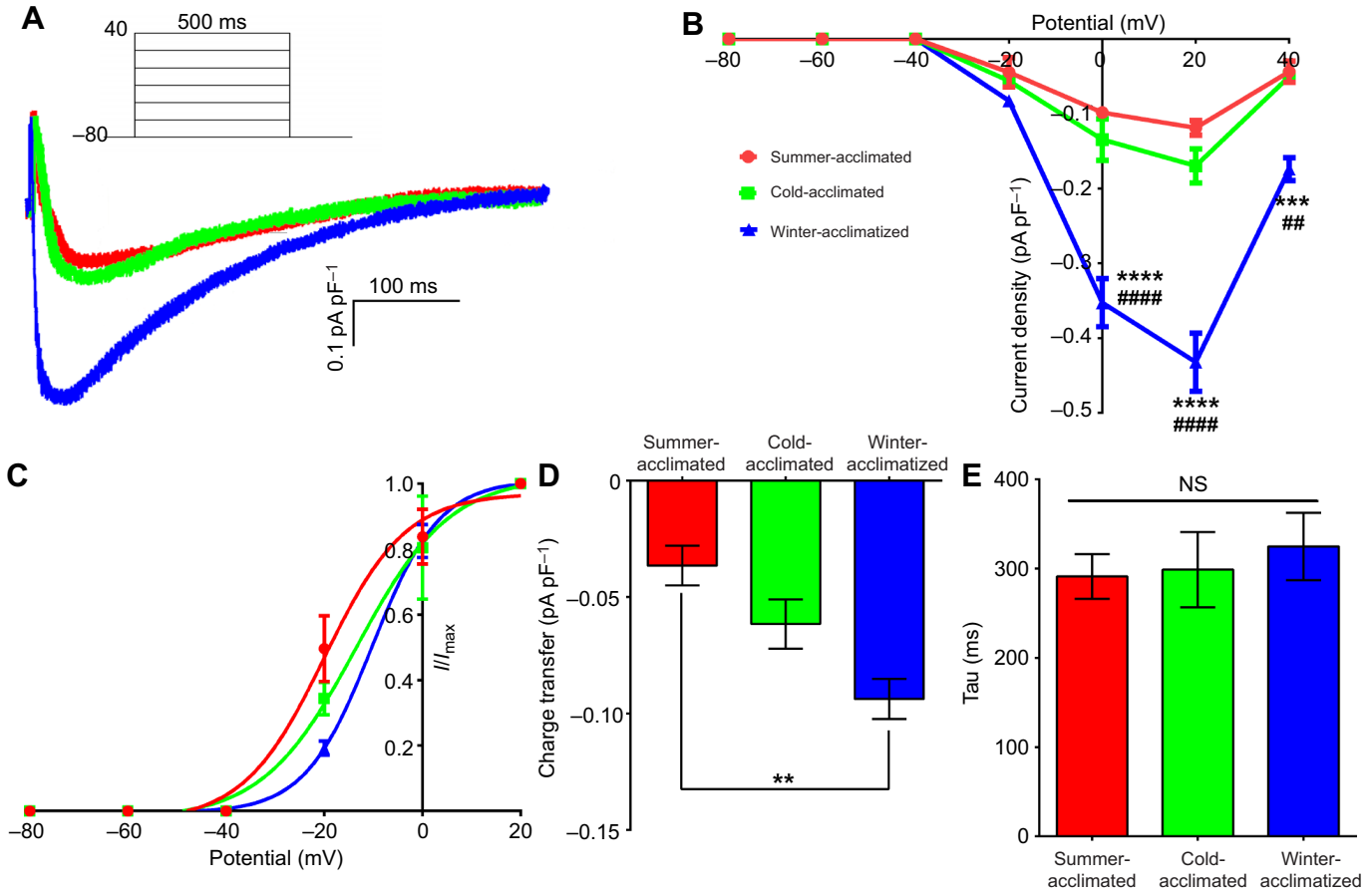

Fig. 6. Characteristics of I_{Ca} in sculpin atrium. (A) Representative recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of I_{Ca} current in isolated atrial myocytes from summer-acclimated (12°C, red, n=11 myocytes from 5 animals), cold-acclimated (3°C, green, n=10 myocytes from 5 animals) and winter-acclimatized sculpins (3°C, blue, n=9 myocytes from 6 animals). Statistically significant difference between: summer-acclimated and winter-acclimatized groups (**P<0.01, ***P<0.001, ****P<0.0001); winter-acclimatized groups and cold-acclimated groups ($^{\#\#}P$<0.01, $^{\#\#\#\#}P$<0.0001); NS, not significant.

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 Scombriformes (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 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_k 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 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

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

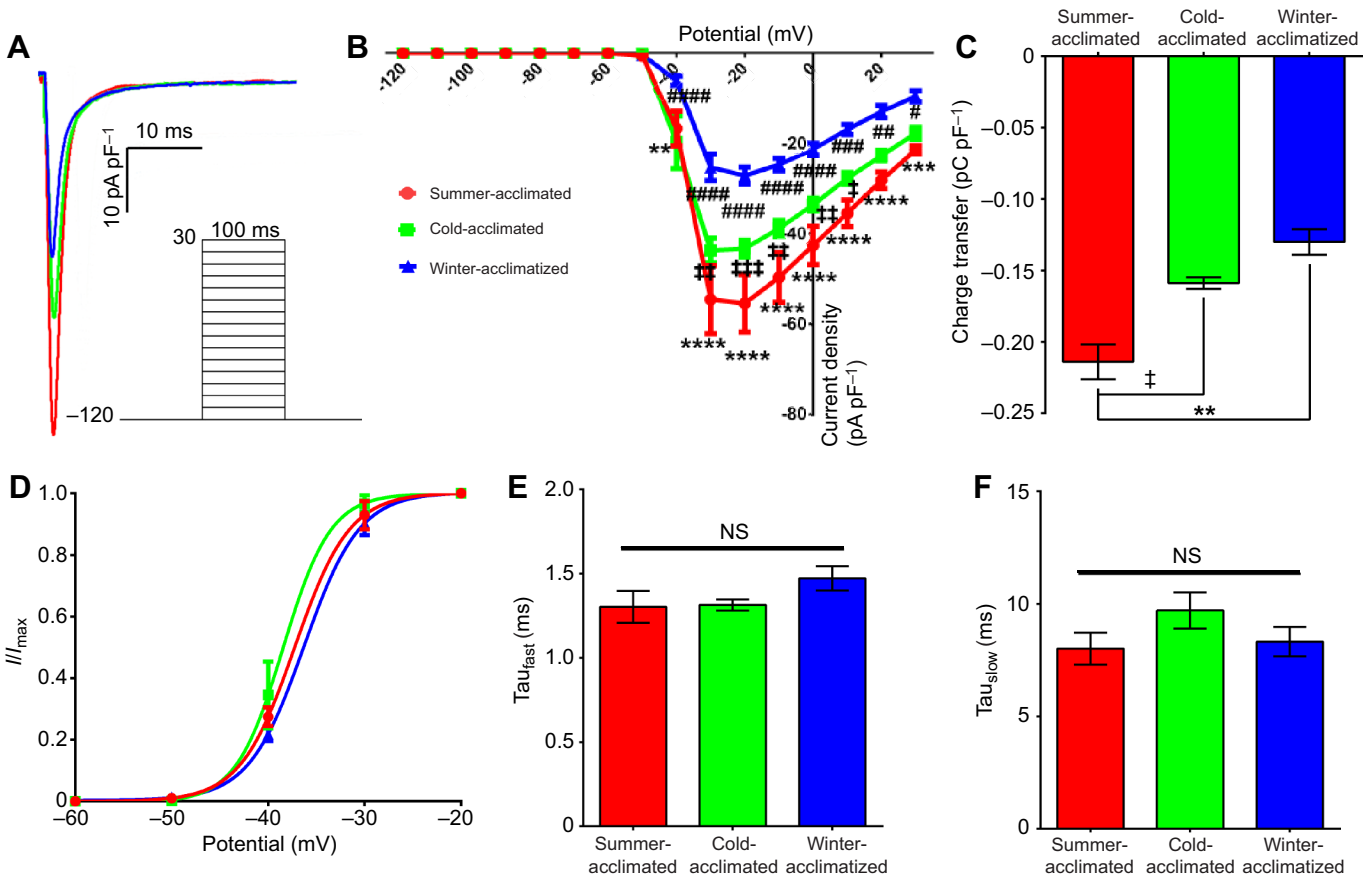

Fig. 7. Characteristics of I_{Na} in sculpin ventricle. (A) Representative recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of I_{Na} current in isolated ventricular myocytes from summer-acclimated (12°C, red, n=10 myocytes from 5 animals), cold-acclimated (3°C, green, n=6 myocytes from 5 animals) and winter-acclimatized sculpins (3°C, blue, n=13 myocytes from 7 animals). Statistically significant difference between: summer-acclimated and winter-acclimatized (**P<0.01, ***P<0.001, ****P<0.0001); winter-acclimatized groups and cold-acclimated groups ($^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001, $^{\#\#\#\#}P$<0.0001); summer-acclimated and cold-acclimated groups ($^{\ddagger}P$<0.05, $^{\ddagger\ddagger}P$<0.01, $^{\ddagger\ddagger\ddagger}P$<0.001); NS, not significant.

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_{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_{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 ∼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

Journal of Experimental Biology (2019) 222, jeb202242. doi:10.1242/jeb.202242

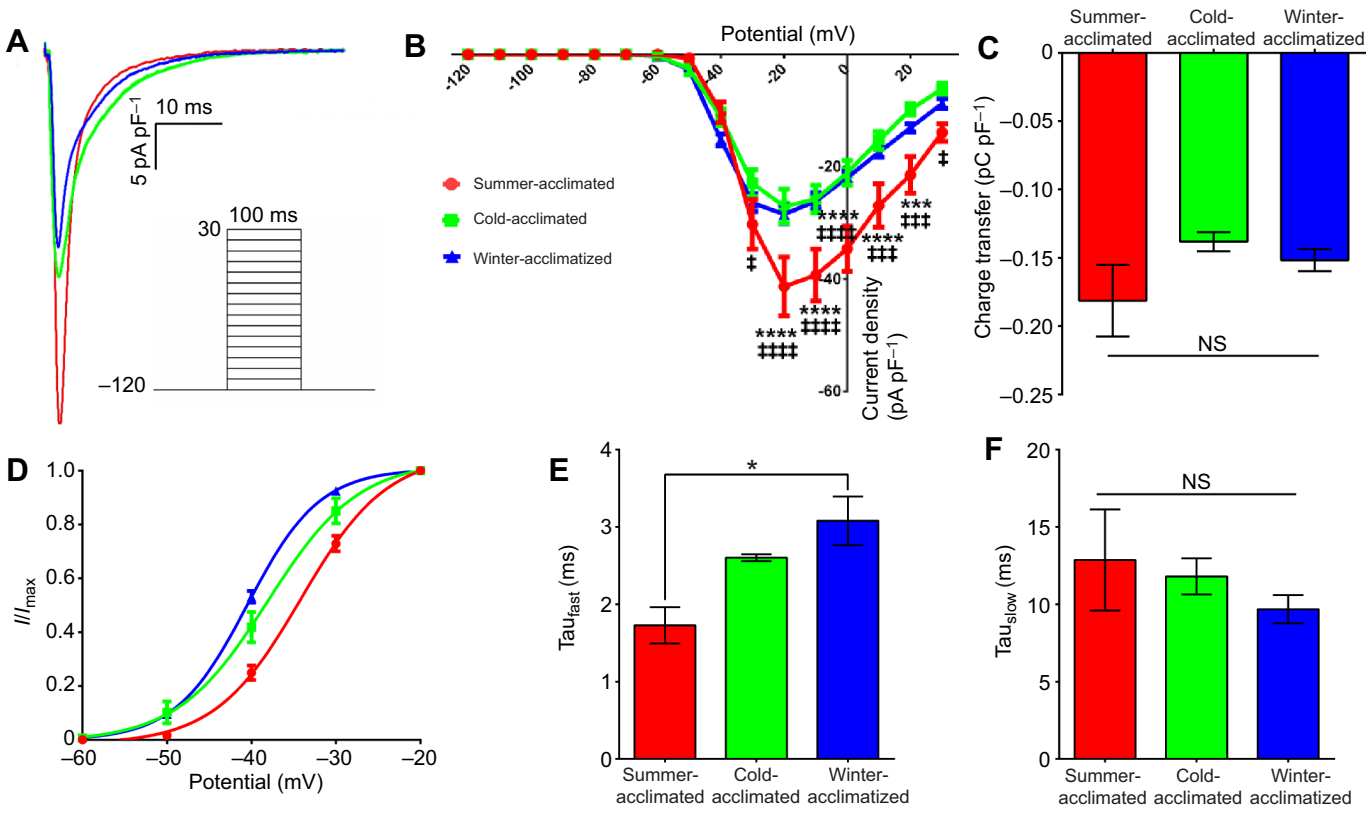

Fig. 8. Characteristics of I_{Na} in sculpin atrium. (A) Representative recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of I_{Na} current in isolated atrial myocytes from summer-acclimated (12°C, red, *n*=9 myocytes from 5 animals), cold-acclimated (3°C, green, *n*=10 myocytes from 7 animals) and winter-acclimatized sculpins (3°C, blue, *n*=15 myocytes from 8 animals). Statistically significant difference between: summer-acclimated and winter-acclimatized groups (**P*<0.05, ****P*<0.001, *****P*<0.0001); summer-acclimated and cold-acclimated groups (‡*P*<0.05, ‡‡‡*P*<0.001, ‡‡‡‡*P*<0.0001); NS, not significant.

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 in rainbow trout (Tiitu 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; Stehlik, 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; Tiitu 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

Conceptualization: D.V.A., H.A.S.; Methodology: T.S.F., D.V.A.; Validation: H.A.S.; Formal analysis: T.S.F.; Investigation: T.S.F.; Data curation: T.S.F., D.V.A.; Writing - original draft: T.S.F., H.A.S.; Writing - review & editing: T.S.F., D.V.A., H.A.S.; Visualization: T.S.F., H.A.S.; Supervision: D.V.A.; Project administration: D.V.A., H.A.S.; Funding acquisition: D.V.A.

Funding

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

References

Abramochkin, D. V. and Vornanen, M. (2015). Seasonal acclimatization of the cardiac potassium currents (IK1 and IKr) in an arctic marine teleost, the navaga cod (*Eleginus navaga*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **185**, 883-890. doi:10.1007/s00360-015-0925-5

Abramochkin, D. V. and Vornanen, M. (2017). Seasonal changes of cholinergic response in the atrium of Arctic navaga cod (*Eleginus navaga*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **187**, 329-338. doi:10.1007/s00360-016-1032-y

Abramochkin, D. V., Haverinen, J., Mitenkov, Y. A. and Vornanen, M. (2019). Temperature and external K+ dependence of electrical excitation in ventricular myocytes of cod-like fishes. *J. Exp. Biol.* **222**, jeb193607. doi:10.1242/jeb.193607

Aho, E. and Vornanen, M. (1998). Ca2+-ATPase activity and Ca2+ uptake by sarcoplasmic reticulum in fish heart: effects of thermal acclimation. *J. Exp. Biol.* **201**, 525-532.

Aho, E. and Vornanen, M. (1999). Contractile properties of atrial and ventricular myocardium of the heart of rainbow trout *Oncorhynchus mykiss*: effects of thermal acclimation. *J. Exp. Biol.* **202**, 2663-2677.

Aho, E. and Vornanen, M. (2001). Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **171**, 173-179. doi:10.1007/s003600000171

Arnold, W., Ruf, T., Frey-Roos, F. and Bruns, U. (2011). Diet-independent remodeling of cellular membranes precedes seasonally changing body temperature in a hibernator. *PLoS ONE* **6**, e18641. doi:10.1371/journal.pone.0018641

Badr, A., Hassinen, M., El-Sayed, M. F. and Vornanen, M. (2017). Effects of seasonal acclimatization on action potentials and sarcolemmal K+currents in roach (*Rutilus rutilus*) cardiac myocytes. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **205**, 15-27. doi:10.1016/j.cbpa.2016.12.017

Bidoggia, H., Maciel, J. P., Capalozza, N., Mosca, S., Blaksley, E. J., Valverde, E., Bertran, G., Arini, P., Biagetti, M. O. and Quinteiro, R. A. (2000). Sex-dependent electrocardiographic pattern of cardiac repolarization. *Am. Heart J.* **140**, 430-436. doi:10.1067/mhj.2000.108510

Bowler, K. and Tirri, R. (1990). Temperature dependence of the heart isolated from the cold or warm acclimated perch (*Perca fluviatilis*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **96**, 177-180. doi:10.1016/0300-9629(90)90061-V

Buda, C., Dey, I., Balogh, N., Horvath, L. I., Maderspach, K., Juhasz, M., Yeo, Y. K. and Farkas, T. (2006). Structural order of membranes and composition of phospholipids in fish brain cells during thermal acclimatization. *Proc. Natl. Acad. Sci. USA* **91**, 8234-8238. doi:10.1073/pnas.91.17.8234

Cardinale, M. (2000). Ontogenetic diet shifts of bull-rout, *Myoxocephalus scorpius* (L.), in the south-western Baltic Sea. *J. Appl. Ichthyol.* **16**, 231-239. doi:10.1046/j.1439-0426.2000.00231.x

Chung, D. J., Bryant, H. J. and Schulte, P. M. (2017). Thermal acclimation and subspecies-specific effects on heart and brain mitochondrial performance in a eurythermal teleost (*Fundulus heteroclitus*). *J. Exp. Biol.* **220**, 1459-1471. doi:10.1242/jeb.151217

Corcoran, M. P., Lamon-Fava, S. and Fielding, R. A. (2007). Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. *Am. J. Clin. Nutr.* **85**, 662-677. doi:10.1093/ajcn/85.3.662

Cottrell, M. T. and Kirchman, D. L. (2009). Photoheterotrophic microbes in the Arctic Ocean in summer and winter. *Appl. Environ. Microbiol.* **75**, 4958-4966. doi:10.1128/AEM.00117-09

Driedzic, W. R. and Gesser, H. (1994). Energy-metabolism and contractility in ectothermic vertebrate hearts-hypoxia, acidosis, and low-temperature. *Physiol. Rev.* **74**, 221-258. doi:10.1152/physrev.1994.74.1.221

Driedzic, W. R., Bailey, J. R. and Sephton, D. H. (1996). Cardiac adaptations to low temperature in non-polar teleost fish. *J. Exp. Zool. A Ecol. Genet. Physiol.* **275**, 186-195. doi:10.1002/(SICI)1097-010X(19960601/15)275:2/3<186::AID-JEZ10>3.0.CO;2-I

Ennis, G. P. (1970a). Age, growth, and sexual maturity of the shorthorn sculpin, *Myoxocephalus scorpius*, in Newfoundland waters. *J. Fish. Res. Board Canada* **27**, 2155-2158. doi:10.1139/f70-244

Ennis, G. P. (1970b). Reproduction and associated behaviour in the shorthorn sculpin, *Myoxocephalus scorpius* in Newfoundland waters. *J. Fish. Res. Board Canada* **27**, 2037-2045. doi:10.1139/f70-227

Farrar, R. S., Battipolu, P. K., Pierson, N. S. and Rodnick, K. J. (2006). Steroid-induced cardiac contractility requires exogenous glucose, glycolysis and the sarcoplasmic reticulum in rainbow trout. *J. Exp. Biol.* **209**, 2114-2128. doi:10.1242/jeb.02241

Farrell, A. P., Hammons, A. M., Graham, M. S. and Tibbits, G. F. (1988). Cardiac growth in rainbow trout, *Salmo gairdneri*. *Can. J. Zool.* **66**, 2368-2373. doi:10.1139/z88-351

Farrell, A. P., Altimiras, J., Franklin, C. E. and Axelsson, M. (2013). Niche expansion of the shorthorn sculpin (*Myoxocephalus scorpius*) to Arctic waters is supported by a thermal independence of cardiac performance at low temperature. *Nrc. Reserach Press* **91**, 573-580. doi:10.1139/cjz-2013-0038

Franklin, C. E., Farrell, A. P., Altimiras, J. and Axelsson, M. (2013). Thermal dependence of cardiac function in arctic fish: implications of a warming world. *J. Exp. Biol.* **216**, 4251-4255. doi:10.1242/jeb.087130

Galli, G. L. J., Shiels, H. A. and Brill, R. W. (2009a). Temperature sensitivity of cardiac function in pelagic fishes with different vertical mobilities: yellowfin tuna (*Thunnus albacares*), bigeye tuna (*Thunnus obesus*), mahimahi (*Coryphaena hippurus*), and swordfish (*Xiphias gladius*). *Physiol. Biochem. Zool.* **82**, 280-290. doi:10.1086/597484

Galli, G. L. J., Lipnick, M. S. and Block, B. A. (2009b). Effect of thermal acclimation on action potentials and sarcolemmal K+ channels from Pacific bluefin tuna cardiomyocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R502-R509. doi:10.1152/ajpregu.90810.2008

Graham, M. S. and Farrell, A. P. (1989). The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* **62**, 38-61. doi:10.1086/physzool.62.1.30159997

Hanson, K. C., Hasler, C. T., Cooke, S. J., Suski, C. D. and Philipp, D. P. (2008). Intersexual variation in the seasonal behaviour and depth distribution of a freshwater temperate fish, the largemouth bass. *Can. J. Zool.* **86**, 801-811. doi:10.1139/Z08-057

Hassinen, M., Paajanen, V., Haverinen, J., Eronen, H. and Vornanen, M. (2007). Cloning and expression of cardiac Kir2.1 and Kir2.2 channels in thermally acclimated rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R2328-R2339. doi:10.1152/ajpregu.00354.2006

Hassinen, M., Haverinen, J. and Vornanen, M. (2008). Electrophysiological properties and expression of the delayed rectifier potassium (ERG) channels in the heart of thermally acclimated rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R297-R308. doi:10.1152/ajpregu.00612.2007

Hassinen, M., Abramochkin, D. V. and Vornanen, M. (2014). Seasonal acclimatization of the cardiac action potential in the Arctic navaga cod (*Eleginus navaga*, Gadidae). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **184**, 319-327. doi:10.1007/s00360-013-0797-5

Haverinen, J. and Vornanen, M. (2004). Temperature acclimation modifies Na+ current in fish cardiac myocytes. *J. Exp. Biol.* **207**, 2823-2833. doi:10.1242/jeb.01103

Haverinen, J. and Vornanen, M. (2007). Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *Am. J. Physiol. Integr. Comp. Physiol.* **292**, R1023-R1032. doi:10.1152/ajpregu.00432.2006
Haverinen, J. and Vornanen, M. (2009). Responses of action potential and K+ currents to temperature acclimation in fish hearts: phylogeny or thermal preferences? *Physiol. Biochem. Zool.* **82**, 468-482. doi:10.1086/590223
Hsieh, S. L. and Kuo, C.-M. (2005). Stearoyl-CoA desaturase expression and fatty acid composition in milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon idella*) during cold acclimation. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **141**, 95-101. doi:10.1016/j.cbpc.2005.02.001
Hyvärinen, H., Holopainen, I. J. and Piironen, J. (1985). Anaerobic wintering of crucian carp (*Carassius carassius*). I. Annual dynamics of glycogen reserves in nature. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **82**, 797-803. doi:10.1016/0300-9629(85)90485-2
Johansen, I. B., Lunde, I. G., Rosjo, H., Christensen, G., Nilsson, G. E., Bakken, M. and Overli, O. (2011). Cortisol response to stress is associated with myocardial remodeling in salmonid fishes. *J. Exp. Biol.* **214**, 1313-1321. doi:10.1242/jeb.053058
Johansen, I. B., Sandblom, E., Skov, P. V., Gräns, A., Ekström, A., Lunde, I. G., Vindas, M. A., Zhang, L., Höglund, E., Frisk, M. et al. (2017). Bigger is not better: cortisol-induced cardiac growth and dysfunction in salmonids. *J. Exp. Biol.* **220**, 2545-2553. doi:10.1242/jeb.135046
Keen, J. E., Vianzon, D. M., Farrell, A. P. and Tibbits, G. F. (1993). Thermal-acclimation alters both adrenergic sensitivity and adrenoceptor density in cardiac tissue of rainbow-trout. *J. Exp. Biol.* **181**, 27-47.
Keen, A. N., Fenna, A. J., McConnell, J. C., Sherratt, M. J., Gardner, P. and Shiels, H. A. (2016). The dynamic nature of hypertrophic and fibrotic remodeling of the fish ventricle. *Front. Physiol.* **6**, 427. doi:10.3389/fphys.2015.00427
Keen, A. N., Klaiman, J. M., Shiels, H. A. and Gillis, T. E. (2017). Temperature-induced cardiac remodelling in fish. *J. Exp. Biol.* **220**, 147-160. doi:10.1242/jeb.128496
Keen, A. N., Fenna, A. J., McConnell, J. C., Sherratt, M. J., Gardner, P. and Shiels, H. A. (2018). Macro- and micromechanical remodelling in the fish atrium is associated with regulation of collagen 1 alpha 3 chain expression. *Pflugers Arch. Eur. J. Physiol.* **470**, 1205-1219. doi:10.1007/s00424-018-2140-1
Kent, J., Koban, M. and Prosser, C. L. (1988). Cold-acclimation-induced protein hypertrophy in channel catfish and green sunfish. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **158**, 185-198. doi:10.1007/BF01075832
Klaiman, J. M., Fenna, A. J., Shiels, H. A., Macri, J. and Gillis, T. E. (2011). Cardiac remodeling in fish: Strategies to maintain heart function during temperature change. *PLoS ONE* **6**, e24464. doi:10.1371/journal.pone.0024464
Klaiman, J. M., Pyle, W. G. and Gillis, T. E. (2014). Cold acclimation increases cardiac myofilament function and ventricular pressure generation in trout. *J. Exp. Biol.* **217**, 4132-4140. doi:10.1242/jeb.109041
Korajoki, H. and Vornanen, M. (2012). Expression of SERCA and phospholamban in rainbow trout (*Oncorhynchus mykiss*) heart: comparison of atrial and ventricular tissue and effects of thermal acclimation. *J. Exp. Biol.* **215**, 1162-1169. doi:10.1242/jeb.065102
Lamp, F. (1966). Beitrage zur Biologie der Seeskorpione *Myoxocephalus scorpius* (L.) und *Taurulus bubalis* (Euphr.) in der Keilder Forde. *Kieler Meeresforsch* **22**, 98-120.
Leaf, A. (2006). Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. *Fundam. Clin. Pharmacol.* **98**, 355-377. doi:10.1111/j.1472-8206.2006.00438.x
Little, A. G. and Seebacher, F. (2014). The evolution of endothermy is explained by thyroid hormone-mediated responses to cold in early vertebrates. *J. Exp. Biol.* **217**, 1642-1648. doi:10.1242/jeb.088880
Liu, X. K., Katchman, A., Whitfield, B. H., Wan, G., Janowski, E. M., Woosley, R. L. and Ebert, S. N. (2003). In vivo androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. *Cardiovasc. Res.* **57**, 28-36. doi:10.1016/S0008-6363(02)00673-9
Luksenburg, J. A. and Pedersen, T. (2002). Sexual and geographical variation in life history parameters of the shorthorn sculpin. *J. Fish Biol.* **61**, 1453-1464. doi:10.1111/j.1095-8649.2002.tb02489.x
Luksenburg, J. A., Pedersen, T. and Falk-Petersen, I. B. (2004). Reproduction of the shorthorn sculpin *Myoxocephalus scorpius* in northern Norway. *J. Sea Res.* **51**, 157-166. doi:10.1016/j.seares.2003.09.001
Matikainen, N. and Vornanen, M. (1992). Effect of season and temperature acclimation on the function of crucian carp (*Carassius carassius*) heart. *J. Exp. Biol.* **167**, 203-220.
Pelouch, V. and Vornanen, M. (1996). Effects of thermal acclimation on ventricle size, protein composition, and contractile properties of crucian carp heart. *J. Therm. Biol.* **21**, 1-9. doi:10.1016/0306-4565(95)00013-5
Pomeroy, L. R. (1997). Primary production in the Arctic Ocean estimated from dissolved oxygen. *J. Mar. Syst.* **10**, 1-8. doi:10.1016/S0924-7963(96)00059-0
Raciborski, K. (1984). Migrations, reproduction, growth and feeding of *Myoxocephalus scorpius* (L.) in Gdansk Bay (South Baltic). *Polish Arch. Hydrobiol.* **31**, 109-118.
Sellner, P. A. and Hazel, J. R. (1982). Desaturation and elongation of unsaturated fatty acids in hepatocytes from thermally acclimated rainbow trout. *Arch. Biochem. Biophys.* **213**, 58-66. doi:10.1016/0003-9861(82)90439-8
Sephton, D. H. and Driedzic, W. R. (1991). Effect of acute and chronic temperature transition on enzymes of cardiac metabolism in white perch (*Morone americana*), yellow perch (*Perca flavescens*), and smallmouth bass (*Micropterus dolomieui*). *Can. J. Zool.* **69**, 258-262. doi:10.1139/z91-040
Shiels, H. A. and Farrell, A. P. (1997). The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca2+ to force development in isolated ventricular trabeculae from rainbow trout. *J. Exp. Biol.* **200**, 1607-1621.
Shiels, H. A., Vornanen, M. and Farrell, A. P. (2000). Temperature-dependence of L-type Ca(2+) channel current in atrial myocytes from rainbow trout. *J. Exp. Biol.* **203**, 2771-2780.
Shiels, H. A., Vornanen, M. and Farrell, A. P. (2002). Temperature dependence of cardiac sarcoplasmic reticulum function in rainbow trout myocytes. *J. Exp. Biol.* **205**, 3631-3639.
Shiels, H. A., Vornanen, M. and Farrell, A. P. (2003). Acute temperature change modulates the response of ICa to adrenergic stimulation in fish cardiomyocytes. *Physiol. Biochem. Zool.* **76**, 816-824. doi:10.1086/378918
Shiels, H. A., Di Maio, A., Thompson, S. and Block, B. A. (2011). Warm fish with cold hearts: Thermal plasticity of excitation-contraction coupling in bluefin tuna. *Proc. R. Soc. B Biol. Sci.* **278**, 18-27. doi:10.1098/rspb.2010.1274
Sollid, J., De Angelis, P., Gundersen, K. and Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *J. Exp. Biol.* **206**, 3667-3673. doi:10.1242/jeb.00594
Soriguer, F. J., Tinahones, F. J., Monzón, A., Pareja, A., Rojo-Martínez, G., Moreno, F., Esteva, I. and Gómez-Zumaquero, J. M. (2000). Varying incorporation of fatty acids into phospholipids from muscle, adipose and pancreatic exocrine tissues and thymocytes in adult rats fed with diets rich in different fatty acids. *Eur. J. Epidemiol.* **16**, 585-594. doi:10.1023/A:1007684808188
Stehlik, L. L. (2009). Effects of seasonal change on activity rhythms and swimming behavior of age-0 bluefish (*Pomatomus saltatrix*) and a description of gliding behavior. *Fish. Bull.* **107**, 1-12.
Tiitu, V. and Vornanen, M. (2001). Cold adaptation suppresses the contractility of both atrial and ventricular muscle of the crucian carp heart. *J. Fish Biol.* **59**, 141-156. doi:10.1111/j.1095-8649.2001.tb02344.x
Tiitu, V. and Vornanen, M. (2003). Does different thyroid state effect on the contractility of the cardiac muscle of eurythermal fish species, rainbow trout (*Oncorhynchus mykiss*, Walbaum)? *J. Therm. Biol.* **28**, 35-42. doi:10.1016/S0306-4565(02)00033-5
Tillman, T. S. and Cascio, M. (2003). Effects of membrane lipids on ion channel structure and function. *Cell Biochem. Biophys.* **38**, 161-190. doi:10.1385/CBB:38:2:161
Valtonen, T. (1974). Seasonal and sex-bound variation in the carbohydrate metabolism of the liver of the whitefish. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **47**, 713-727. doi:10.1016/0300-9629(74)90032-2
Vornanen, M. (1994). Seasonal and temperature-induced changes in myosin heavy chain composition of crucian carp hearts. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **267**, R1567-R1573. doi:10.1152/ajpregu.1994.267.6.R1567
Vornanen, M. (1997). Sarcolemmal Ca influx through L-type Ca channels in ventricular myocytes of a teleost fish. *Am. J. Physiol. Integr. Comp. Physiol.* **272**, R1432-R1440. doi:10.1152/ajpregu.1997.272.5.R1432
Vornanen, M. (1998). L-type Ca2+ current in fish cardiac myocytes: effects of thermal acclimation and beta-adrenergic stimulation. *J. Exp. Biol.* **201**, 533-547.
Vornanen, M. and Paajanen, V. (2004). Seasonality of dihydropyridine receptor binding in the heart of an anoxia-tolerant vertebrate, the crucian carp (*Carassius carassius* L.). *Am. J. Physiol. Integr. Comp. Physiol.* **167**, 203-220. doi:10.1152/ajpregu.00317.2004
Vornanen, M. and Paajanen, V. (2006). Seasonal changes in glycogen content and Na+-K+-ATPase activity in the brain of crucian carp. *Am. J. Physiol. Integr. Comp. Physiol.* **291**, R1482-R1489. doi:10.1152/ajpregu.00172.2006
Vornanen, M., Stevens, E. D., Farrell, A. P. and Graham, J. B. (1998). L-type Ca^{2+} current in fish cardiac myocytes: effects of thermal acclimation and beta-adrenergic stimulation. *J. Exp. Biol.* **201**, 533-547.
Vornanen, M., Ryökkynen, A. and Nurmi, A. (2002). Temperature-dependent expression of sarcolemmal K+currents in rainbow trout atrial and ventricular myocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R1191-R1199. doi:10.1152/ajpregu.00349.2001
Vornanen, M., Hassinen, M., Koskinen, H. and Krasnov, A. (2005). Steady-state effects of temperature acclimation on the transcriptome of the rainbow trout heart. *Am. J. Physiol. Integr. Comp. Physiol.* **289**, R1177-R1184. doi:10.1152/ajpregu.00157.2005
Vornanen, M., Haverinen, J. and Egginton, S. (2014). Acute heat tolerance of cardiac excitation in the brown trout (Salmo trutta fario). *J. Exp. Biol.* **217**, 299-309. doi:10.1242/jeb.091272
West, J. L. and Driedzic, W. R. (1999). Mitochondrial protein synthesis in rainbow trout (*Oncorhynchus mykiss*) heart is enhanced in sexually mature males but impaired by low temperature. *J. Exp. Biol.* **202**, 2359-2369.
Wingfield, J. C. and Grimm, A. S. (1977). Seasonal changes in plasma cortisol, testosterone and oestradiol-17β in the plaice, *Pleuronectes platessa* L. *Gen. Comp. Endocrinol.* **31**, 1-11. doi:10.1016/0016-6480(77)90184-8