© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

RESEARCH ARTICLE

Effects of seasonal acclimatization on temperature dependence of cardiac excitability in the roach, *Rutilus rutilus*

A. Badr[1,2,*], M. F. El-Sayed[2] and M. Vornanen[1]

ABSTRACT

Temperature sensitivity of electrical excitability is a potential limiting factor for performance level and thermal tolerance of excitable tissues in ectothermic animals. To test whether the rate and rhythm of the heart acclimatize to seasonal temperature changes, thermal sensitivity of cardiac excitation in a eurythermal teleost, the roach (*Rutilus rutilus*), was examined. Excitability of the heart was determined from *in vivo* electrocardiograms and *in vitro* microelectrode recordings of action potentials (APs) from winter and summer roach acclimatized to 4 and 18°C, respectively. Under heat ramps (3°C h^{-1}), starting from the acclimatization temperatures of the fish, heart rate increased to maximum values of 78±5 beats min^{-1} (at 19.8±0.5°C) and 150±7 beats min^{-1} (at 28.1±0.5°C) for winter and summer roach, respectively, and then declined in both groups. Below 20°C, heart rate was significantly higher in winter than in summer roach (P<0.05), indicating positive thermal compensation. Cardiac arrhythmias appeared with rising temperature as missing QRS complexes, increase in variability of heart rate, episodes of atrial tachycardia, ventricular bradycardia and complete cessation of the heartbeat (asystole) in both winter and summer roach. Unlike winter roach, atrial APs of summer roach had a distinct early repolarization phase, which appeared as shorter durations of atrial AP at 10% and 20% repolarization levels in comparison to winter roach (P<0.05). In contrast, seasonal acclimatization had only subtle effects on ventricular AP characteristics. Plasticity of cardiac excitation appears to be necessary for seasonal improvements in performance level and thermal resilience of the roach heart.

KEY WORDS: Electrocardiogram, Action potential, Eurythermal fish, Cardiac arrhythmias

INTRODUCTION

Temperature has profound effects on the physiology of ectothermic animals at all levels of body organization from the whole-body metabolic rate down to the molecular functions (Precht et al., 1955). Most fishes are ectotherms and their body temperature is practically identical to the ambient water temperature; therefore, each organ of the fish will experience all temperature variations of the habitat (Hazel and Prosser, 1974). Acute increases and decreases of water temperature directly affect the contractility of the fish heart and the rate of blood circulation. At northern temperate latitudes, seasonal temperature changes can be more than 20°C in amplitude, and so large changes in temperature would strongly affect cardiac contractility, unless physiological adjustments of cardiac function were available for the fish. It is currently clear that several fish species can change the cardiac phenotype on seasonal basis via thermal acclimatization (Anttila et al., 2014a,b; Bowler and Tirri, 1990; Driedzic et al., 1996; Matikainen and Vornanen, 1992; Morita and Tsukuda, 1994). This is often associated with reversible changes in gene expression, which give rise to compensatory changes in structure, metabolism, rate of enzyme activities, membrane composition and contractile function of the heart (Aho and Vornanen, 1999; Anttila et al., 2014a,b; Bowler and Tirri, 1990; Gracey et al., 2004; Graham and Farrell, 1990; Hazel and Prosser, 1974; Jayasundara et al., 2015; Keen et al., 1994; Klaiman et al., 2011; Shiels et al., 2011; Vornanen et al., 2002a,b, 2005).

Rate, rhythm and orderly spread of contraction in the vertebrate heart is determined by electrical excitation of cardiac myocytes (Fozzard, 1977). For ectothermic fishes, temperature-dependent regulation of cardiac excitation is vital for proper cardiac function under varying temperature conditions. The electrophysiological phenotype of the fish heart should be such that cardiac chambers remain electrically excitable at all temperatures that the fish may encounter in its habitat. In contrast, the heart should retain electrical stability to avoid cardiac arrhythmias, whatever the temperature might be. Excitability and stability are conflicting properties of cardiac cell membrane, and proper balance between them should prevail under acute and chronic temperature changes. Even though much work has been done on the temperature dependence of cardiac contractility (Gamperl and Farrell, 2004; Vornanen et al., 2002a,b), our knowledge on the electrical excitability of the fish heart is relatively meager, especially concerning physiological plasticity of cardiac excitation and temperature-related cardiac arrhythmias.

For optimal function of the heart, electrical excitability should be sensitive to temperature changes to produce temperature-dependent acceleration and deceleration of heart rate (f_H) and coordinated changes in the conduction rate of action potentials (APs) over the heart. However, it is well known that acute exposure to high temperatures results in depression of cardiac output (Gollock et al., 2006; Sandblom and Axelsson, 2007; Steinhausen et al., 2008), possibly due to temperature-related changes in electrical excitability. Since stroke volume is fairly insensitive to temperature increases, modulation of cardiac output is achieved by temperature-induced changes in f_H. Depression of f_H and cardiac output at high temperatures implicates limitations either in the rate of impulse generation and/or in impulse conduction, possibly involving cardiac arrhythmias (Vornanen et al., 2014). To elucidate the thermal plasticity of electrical excitation of fish hearts and to locate the possible sites of thermal limitation, chronic temperature exposures can be used to modify electrophysiological phenotype of the heart. The generated cardiac phenotypes can then be put under acute temperature tests to find out which steps of excitability are altered by acclimatization and where in the sequence of events thermal failures might occur. To this end we used a eurythermal teleost species, the

[1]Department of Environmental and Biological Sciences, Faculty of Science and Forestry, University of Eastern Finland, 80101 Joensuu, Finland. [2]Department of Zoology, Faculty of Science, Sohag University, 82524 Sohag, Egypt.

*Author for correspondence (ahmed.osman@uef.fi)

Received 28 January 2016; Accepted 3 March 2016

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

List of symbols and abbreviations

AP	action potential
APD	duration of action potential
C	cycle length of single cardiac beat
D_d	duration of ventricular diastole
D_s	duration of ventricular systole
ECG	electrocardiogram
f_H	heart rate
P	atrial depolarization
PQ interval	impulse transmission from atrium to ventricle
QRS	ventricular depolarization
QT interval	average duration of ventricular action potential
SDNN	standard deviation of interval between beats
T	ventricular repolarization
T_{ABP}	Arrhenius break point temperature
T_{ARR}	arrhythmic temperature
T_{BP}	break point temperature
V_{max}	maximum upstroke velocity of the action potential
V_{rm}	resting membrane potential

roach [*Rutilus rutilus* (Linnaeus 1758)]. Furthermore, we used seasonally acclimatized fish, as acclimatization in the wild was thought to cause stronger responses than thermal acclimation under laboratory conditions (Abramochkin and Vornanen, 2015). We hypothesized that seasonal acclimatization would shift high and low temperature tolerance limits of electrical excitation in order to accommodate the heart to seasonal temperature conditions.

MATERIALS AND METHODS

Animals

Roach were caught from Lake Pyhäselkä, Finland (62°35′ N, 21°34′ E), and reared at the aquarium facilities of the University of Eastern Finland in temperature-controlled 500 liter stainless steel tanks with a continuous supply of aerated groundwater. Winter acclimatized roach (96.6±4.1 g, n=53) were caught in February and maintained for a minimum of 2 weeks in the laboratory at water temperature of 4±1°C until used in the experiments. Summer acclimatized roach (58.2±4.6 g, n=41) were caught in June–September and maintained in the laboratory at water temperature of 18±1°C for a minimum of 2 weeks before starting the experiments. Both groups were fed two to three times per week with trout fodder (EWOS, Turku, Finland). All experiments were authorized by the National Animal Experimental Board in Finland (permission ESAVI/2832/04.10.07/2015).

Recording of electrocardiogram

Electrocardiogram (ECG) recordings were made essentially as previously described (Campbell et al., 2004; Vornanen et al., 2014). Roach were anesthetized in neutralized tricaine methanesulfonate (MS-222, 0.3 mg l^{-1}, Sigma, St Louis, MO, USA) and placed ventral side up on an operating table. Gills were irrigated with tap water during the operation. Two recording electrodes (7-strand Teflon-coated wire, length 40 cm, diameter 0.23 mm; A-M Systems, Carlsborg, WA, USA) were hooked into the end of a 24-G hypodermic needle and obliquely inserted from the ventral surface at the level of the pectoral fins forward, close to the pericardium. The trailing wires were secured by a suture to the belly of the fish and by a second suture in the front of the dorsal fin. To restrain movements, the fish was placed into a respiratory chamber (1 liter, O_2 content ~9 mg l^{-1}) that was immersed in a large (250 liter) temperature-regulated (at the acclimation temperature of the fish) stainless steel tank. The ground electrode was immersed in the tank. The three electrodes were connected to a bioamplifier (ML 136, ADInstruments, Colorado Springs, CO, USA) and connected to the digital recording system (PowerLab, ADInstruments) and further to the computer. The temperature of the tank was controlled by a computer to generated heating or cooling ramps at the rate of 3°C h^{-1}. Recording of ECG was started immediately after the fish was placed into the recording chamber. The fish was allowed to fully recover from the operation for 1–2 days. Recovery was considered to be complete when a clear and steady f_H variability appeared in the ECG. ECGs were considered to be of sufficiently good quality when P, QRS and T waves (atrial depolarization, ventricular depolarization and ventricular repolarization, respectively) could be clearly recognized (Fig. 1). Six fish with noisy ECGs were omitted from experiments.

Controlled rising or falling temperature ramps were obtained by the computer-controlled temperature regulation system. Fish tanks at our facilities are integrated to the central cooling system of the department, and temperature change in each tank can be separately controlled (Computec Technologies, Joensuu, Finland). All tanks have a radiator through which the coolant flows at the rate set by computer-regulated valves (Belimo Holding AG, Switzerland) on the basis of temperature feedback from the tank water. The tanks also receive a flow-through of warm water from separate temperature-regulated heating tanks. The rate of temperature change in the tank is set by the rates of coolant flow in the radiator and the flow rate of warm water through the tank. This system provides very precisely and automatically controlled rising and falling heat ramps to the predefined final temperature. Temperature challenge always started from the acclimatization temperature of the fish (4±1°C for winter and 18±1°C for summer roach). Temperature and ECG were recorded throughout the experiment on a computer for off-line analyses in LabChart 7.1 (ADInstruments). For winter roach, temperature was raised from the acclimatization temperature at a rate of 3°C h^{-1} until a clear depression of f_H was noticed in the ECG. Two separate groups of fishes were needed for summer roach experiments. To measure the high temperature tolerance of summer roach, temperature was raised from the acclimatization temperature (18±1°C) at a rate of 3°C h^{-1}

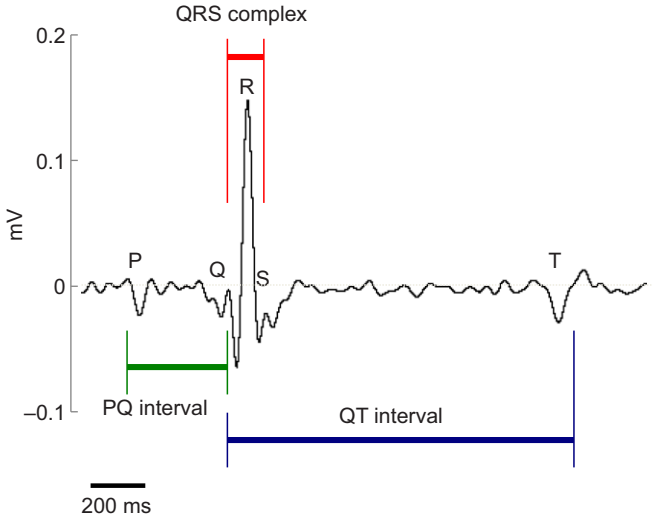

Fig. 1. A representative *in vivo* electrocardiogram tracing of the roach. The recording shows different waves (P, QRS and T) and wave intervals. P, atrial depolarization; QRS, ventricular depolarization; T, ventricular repolarization; PQ interval, impulse transmission from atrium to ventricle; QT interval, average duration of ventricular action potential.

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

until a clear depression of f_H was noticed in the ECG. To measure the low temperature tolerance of summer roach, water temperature was lowered from the acclimatization temperature (18±1°C) at a rate of 3°C h^{-1} until a clear sign of thermal intolerance (missing beats) appeared in the ECG. In this way, the fish were exposed to either a rising or a falling temperature ramp, but not to both. The results of the two summer roach groups were merged into the same graphs.

Besides f_H, several other parameters were determined from ECGs. Standard deviation of successive interbeat intervals (SDNN) was determined as a measure of the short-term variability of f_H. SDNN is expected to vary in a similar manner to NN (the time distance between two successive RR peaks in the ECG), i.e. with shortening of inter-beat intervals, the variability of inter-beat intervals decreases. SDNN was determined by manual identification of components from 20 to 30 consecutive beats. PQ interval represents the time for impulse propagation from atrium to ventricle, QRS duration is the time taken for impulse conduction over the ventricle and QT interval is representative of the average duration of ventricular AP. Because of their dependence on heart size, amplitude of QRS complex, QRS duration and PQ interval are presented as normalized values. QRS amplitude was normalized to the value at the acclimatization temperature, and QRS duration and PQ interval to their maximum value.

The break point temperature (T_{BP}) for f_H, QRS duration, PQ interval and QT interval under heat ramps were defined as the temperature after which steady increase/decrease of the parameters turned into continuous decrease/increase. The Arrhenius break point temperature (T_{ABP}) is the cross-point of the two straight lines fitted to Arrhenius plots of the parameters. Arrhythmia temperature (T_{ARR}) is the temperature of the heart ramp where QRS complexes were missing for the first time from the otherwise rhythmic ECG recording.

The ratio between the durations of diastole and systole was obtained from the ECG recordings. The cycle length (C) of single cardiac beat is obtained from f_H (60 s/beat min^{-1}). The QT interval is taken as the duration of ventricular systole (D_s). The duration of ventricular diastole (D_d) is then $C–D_s$.

Microelectrode recordings of action potentials

Each roach was stunned by a blow to the head and killed by cutting the spine. The excised whole heart was gently fixed with insect pins on the Sylgard-coated bottom of the 10 ml recording chamber filled with continuous oxygenated (100% O_2) physiological saline solution, containing (in mmol l^{-1}): 150 NaCl, 3 KCl, 1.8 $CaCl_2$, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 10 glucose and 10 HEPES, with pH adjusted to 7.6 with NaOH (all chemicals from Sigma). APs were recorded with sharp microelectrodes from spontaneously beating hearts (Haverinen and Vornanen, 2009). Pipettes were fabricated from borosilicate glass (OD: 1.5 mm, ID: 0.75 mm) using a P-97 model micropipette puller from Sutter Instruments (Novato, CA, USA). Electrode resistance was 10–20 MΩ when filled with 3 mol l^{-1} KCl. Temperature of the saline was adjusted to the desired value (4, 13 and 21°C for winter and 13, 21 and 26°C for summer roach) using a circulating water bath. Temperature and APs were continuously recorded via PowerLab 8/30 (ADInstruments) on a computer for off-line analysis with LabChart 7.1 (ADInstruments). Resting membrane potential (V_{rm}), AP overshoot, AP amplitude, AP durations at 0, 10, 20, 50 and 90% of repolarization levels (APD0, APD10, APD20, APD50 and APD90, respectively) and V_{max} (maximum upstroke velocity of AP) were determined.

Statistical analysis

Results are presented as means±s.e.m. Statistically significant differences (P<0.05) between means of winter and summer roach variables were assessed with one-way ANOVA. The fitting of two straight lines to Arrhenius plots was carried out in SigmaPlot (piecewise, two-segment linear analysis).

RESULTS

Similar to the ECG of other teleost fishes, the roach ECG is characterized by P, QRS and T waves (Fig. 1). Acute increases in temperature increased f_H in both winter and summer roach, but with some marked seasonal differences. The break point temperature (T_{BP}) was 19.8±0.5°C for winter and 28.1±0.5°C for summer roach (P<0.05; Table 1). In the low temperature range between 6 and 17°C, f_H was higher in winter than in summer roach (P<0.05), whereas at temperatures above 22°C f_H was much higher in summer roach (P<0.05; Fig. 2A). The minimum and maximum f_H values of winter roach were 20±1 beats min^{-1} (at 3°C) and 78±5 beats min^{-1} (at 19.8±0.5°C), respectively. The f_H of summer roach at the acclimatization temperature (18°C) was 61±5 beats min^{-1}, while the maximum f_H of 150±7 beats min^{-1} was attained at 28.1±0.5°C. When temperature was decreased, f_H of summer roach declined to 22±3 beats min^{-1} at 6°C, which was the lowest temperature that the fish tolerated. The Q_{10} value for f_H in winter roach between 3 and 19.8°C was 2.24, while in summer roach the Q_{10} value between 18 and 28.1°C was 2.47 (P>0.05).

Short-term variability of f_H (SDNN) declined in a curvilinear manner with increasing temperature in both acclimatization groups (Fig. 2B). In the low temperature range of 6–20°C, the variability was larger for summer than winter roach because of the lower f_H of the summer-acclimatized fish in this temperature range (Fig. 2A). Notably, in winter roach, the smooth decline of SDNN turned to an abrupt increase at temperatures above 12°C, i.e. at the Arrhenius break point temperature (T_{ABP}; 12°C), but much below the T_{BP} of f_H. No such change was apparent in summer roach with T_{ABP} of 28.5°C (Fig. 2C, Table 1).

Duration of QRS complex (time required for impulse transmission over the ventricle) slightly declined with increasing temperature in both summer and winter roach (P<0.05; Fig. 3A). In winter roach, the minimum value was attained at 21.2±0.7°C, followed by a small but statistically insignificant increase of QRS duration at higher temperatures (24°C, P>0.05; Table 1). In summer roach, the minimum QRS duration occurred at 28.1±0.9°C, above which QRS duration clearly increased at 33°C (P<0.05; Table 1). Amplitude of the QRS complex remained steady almost throughout the whole temperature range in both acclimation groups (Fig. 3B). However, in winter roach, QRS amplitude declined at approximately 23°C, and similar depressions were evident in summer roach close to the lower (7°C) and upper (30°C) thermal tolerance limits. Duration of the PQ interval (atrioventricular conduction time) decreased in an exponential manner as a function of temperature in both acclimation groups (P<0.05; Fig. 3C). The minimum duration of the PQ interval was attained at 20.8±0.5°C and 30.8±0.5°C for winter and summer roach, respectively (Table 1). At higher temperatures, the PQ interval slightly increased in both acclimatization groups, indicating depression in the rate of impulse conduction (P>0.05).

Duration of the QT interval (average duration of ventricular AP) decreased in an exponential manner with increasing temperature in both acclimatization groups (P<0.05; Fig. 4A). When plotted on double logarithmic scale, there was a close linear correlation between f_H and QT interval over most of the temperature range. However, in winter roach, the correlation suddenly broke at

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

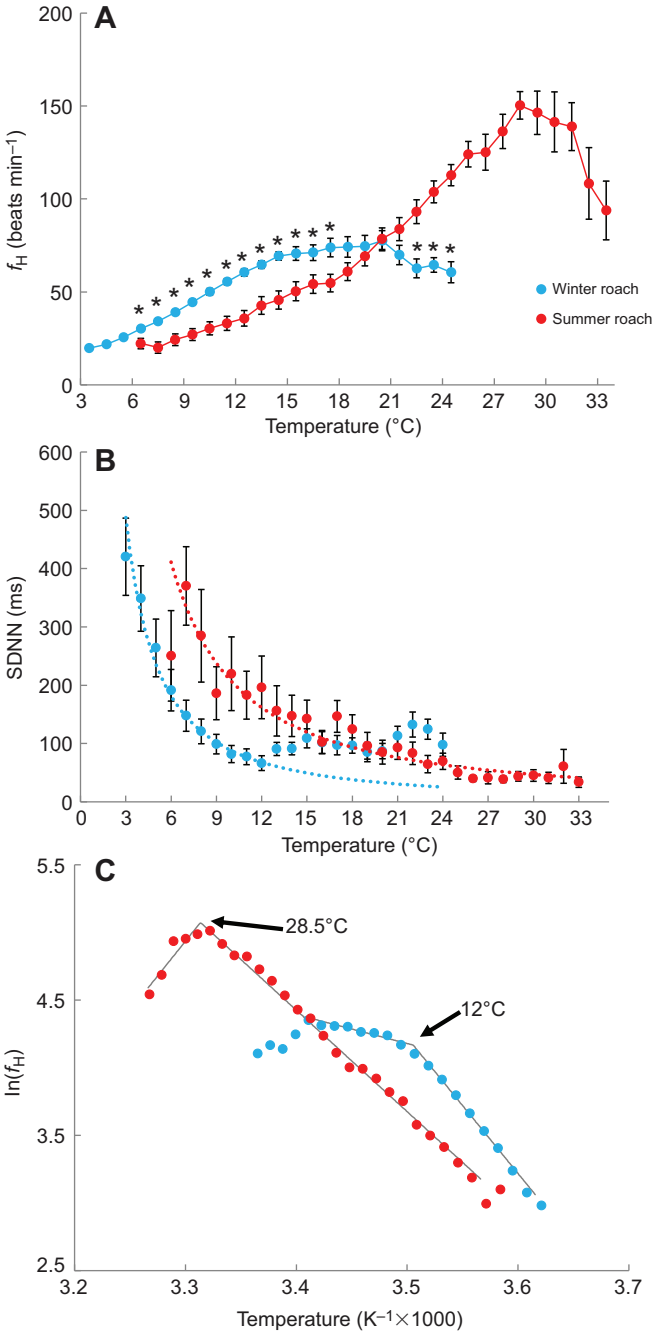

Fig. 2. Effects of temperature on heart rate of seasonally acclimatized roach. (A) Heart rate *in vivo*. Results are means±s.e.m. from 10 and 20 fish for winter- and summer-acclimatized roach, respectively. (B) Standard deviation of interval between beats (SDNN) in winter and summer roach. (C) Arrhenius plot of heart rate, the arrows marking the Arrhenius break point temperature (T_{ABP}). Asterisks indicate statistically significant differences (*P<0.05) between the mean values of winter and summer roach.

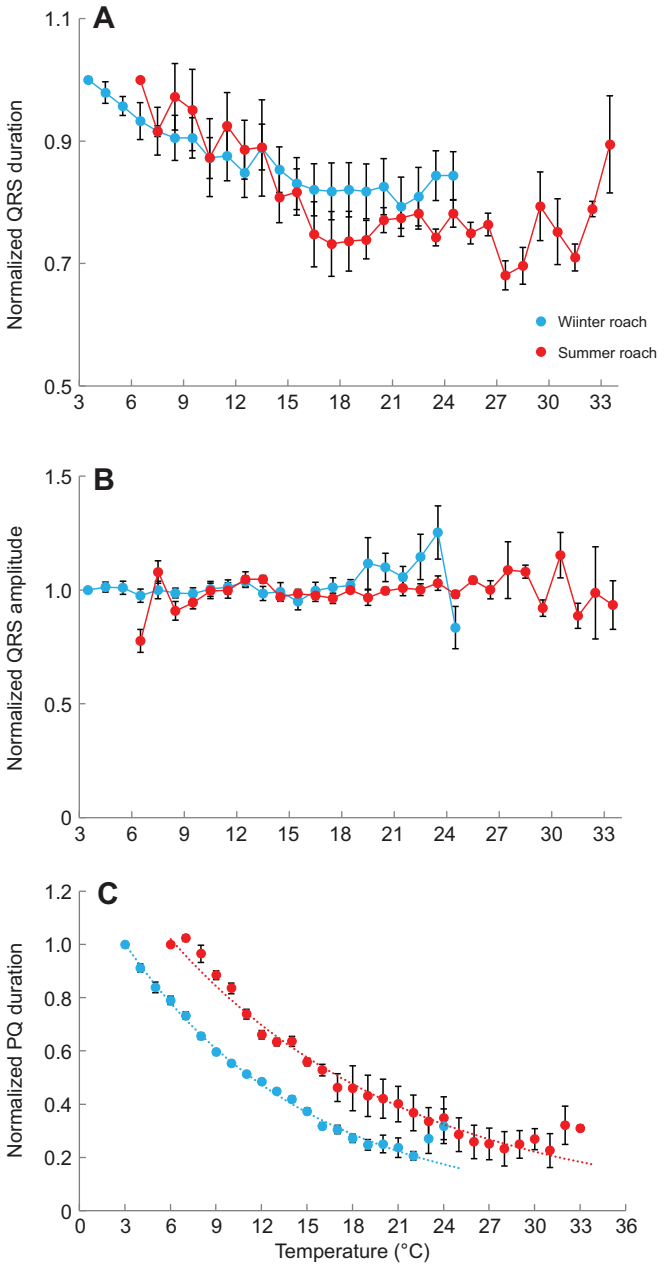

Fig. 3. Effects of temperature on electrocardiograms of seasonally acclimatized roach. (A) QRS duration, (B) QRS amplitude and (C) PQ interval in winter- and summer-acclimatized roach. QRS duration and PQ interval were normalized to the value at the lowest experimental temperature, whereas the amplitude of QRS complex was normalized to the value at the acclimatization temperature of the fish.

approximately 14°C, because the QT interval became short relative to f_H (Fig. 4B). The ratio between diastolic and systolic duration slightly declined with increasing temperature in winter roach up to +14°C and then increased at higher temperatures. In summer roach, this ratio steadily decreased with increasing temperature (Fig. 4C).

Cardiac arrhythmias

Several types of cardiac arrhythmias appeared under acute thermal stress in both seasonal acclimatization groups. The first signs of arrhythmia under heat ramps were missing QRS complexes, indicating atrioventricular block of impulse transmission (Fig. 5B,E). The number of missing beats increased gradually with temperature to the maximum around T_{BP} of f_H in both acclimation groups (Fig. 5F,G). When using missing QRS complexes as the index of cardiac arrhythmia, the arrhythmia temperature (T_{ARR}) was 14.0±0.5°C and 26.4±1.4°C in winter and summer roach, respectively. T_{ARR} was similar to the T_{ABP} in both groups (12 and 28.5°C for winter and summer roach, respectively; Fig. 2C, Table 1). Furthermore, in winter roach, T_{ARR} and T_{ABP} agree with the temperatures at which the QT interval–f_H plot

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

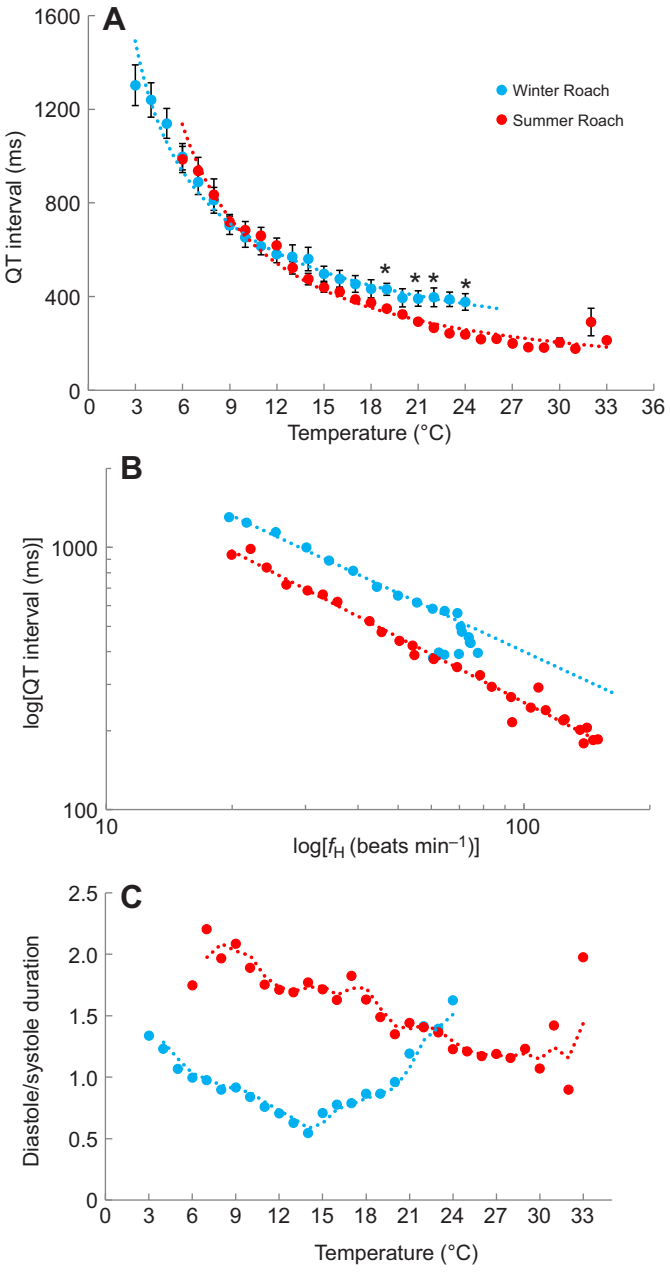

Fig. 4. Effects of temperature on systole and diastole of the seasonally acclimatized roach heart. (A) Effects of temperature on QT interval. (B) Correlation between QT interval and heart rate on a double logarithmic plot. (C) Effects of temperature on diastole/systole duration. The dotted lines represent two-point moving averages. Asterisks indicate statistically significant differences (*P<0.05) between mean values of winter and summer roach.

deviates from a linear relationship (Fig. 4B) and the diastole/systole ratio increases (Fig. 4C). At higher temperatures, close to and above the T_{BP} of f_H, an additional type of arrhythmia appeared as short episodes of atrial tachycardia (Fig. 5C). The most consistent type of arrhythmia was ventricular bradycardia (T_{BP} of f_H), which quickly developed into complete cessation of the heartbeat (asystole).

Atrial and ventricular action potentials

AP characteristics were determined at three temperatures for both acclimatization groups. As in other vertebrates, APD is longer in the ventricle than in the atrium because of the more pronounced plateau phase of the ventricle around 0 mV (Figs 6A, 7A). The maximum upstroke velocity of AP (V_{max}) was faster in the atrium than in the ventricle in both winter and summer roach hearts (P<0.05). Higher temperatures caused prominent declines in APD and increases in the rate of AP upstroke in both cardiac chambers and in both seasonally acclimatized groups (Figs 6, 7, Table 2).

There were only subtle differences in ventricular AP characteristics between winter and summer roach hearts. At 13°C AP amplitude, AP overshoot, V_{rm} and APD50 and ADP90 were smaller in summer than in winter roach (P<0.05; Table 2). A more prominent difference was evident in the shape of the atrial AP between winter and summer roach hearts (Fig. 6A). The most striking difference was in the early repolarization of the atrial AP, which was prominent in summer roach hearts but practically absent in winter roach hearts (Fig. 6A). Consequently, APD10 and ADP20 were significantly shorter in summer than in winter roach atria (P<0.05; Fig. 6D,E). At 21°C, V_{rm}, AP amplitude, APD0 and APD90 were also smaller in summer than in winter roach (P<0.05; Table 2).

DISCUSSION

Thermal acclimation of f_H

Traditionally, thermal acclimation is divided into resistance acclimation and capacity acclimation (Precht et al., 1955). Acclimation to new thermal conditions could appear as widening of thermal tolerance limits and quantitative changes in physiological performance in order to restore body functions towards their original levels (Hazel and Prosser, 1974). In fact, this happens to be the case for thermal acclimatization of the roach heart, as there were clear, seasonally induced changes in thermal resistance limits of f_H and absolute levels of beating rate at low and high temperatures. Resistance acclimation appears as an 8.3°C increase in the T_{BP} of f_H in summer roach and in improved cold tolerance of f_H in winter roach. Improved performance is manifested in higher f_H values of winter roach at low temperatures in comparison to summer roach and in the higher maximum f_H of the summer roach. These findings indicate that roach possess marked physiological plasticity of heart function under seasonally changing temperature regimes. It should be noted, however, that thermal compensation in f_H was only partial, because f_H in the winter roach at 3°C was only 20 beats min^{-1} in comparison to 65 beats min^{-1} in the summer roach at 18°C.

f_H in teleost fishes is determined by the intrinsic rate of pacemaker cells and its extrinsic control by nervous and humoral factors (Laurent et al., 1983). In sole (*Solea vulgaris*) and eel (*Anguilla anguilla*), higher f_H of cold-acclimated in comparison to warm-acclimated fish is attributable to weaker inhibitory cholinergic tone of the cold-acclimated fish (Seibert, 1979; Sureau et al., 1989). Experiments on rainbow trout (*Oncorhynchus mykiss*) heart have shown that acclimation-related changes in f_H are at least partly inherent to the electrophysiological properties of the pacemaker myocytes (Haverinen and Vornanen, 2007). In trout, higher f_H of cold-acclimated (4°C) fish is associated with shorter duration of the pacemaker AP and higher density of the fast component of the delayed rectifier K^+ current, I_{Kr}, in comparison to the warm-acclimated (18°C) species mates. Similar ionic mechanisms may be underlying the seasonal acclimatization of f_H in the marine plaice (*Pleuronectes platessa*) (Harper et al., 1995). The general mechanistic principles in thermal acclimation of fish f_H remain to be shown. It would be informative to know the mechanisms by which the maximum f_H in summer roach is almost double in comparison to winter roach. Patch-clamp experiments on enzymatically isolated pacemaker cells are needed to determine the electrophysiological basis of f_H response in roach. Furthermore, contribution of the autonomic nervous regulation to thermal

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

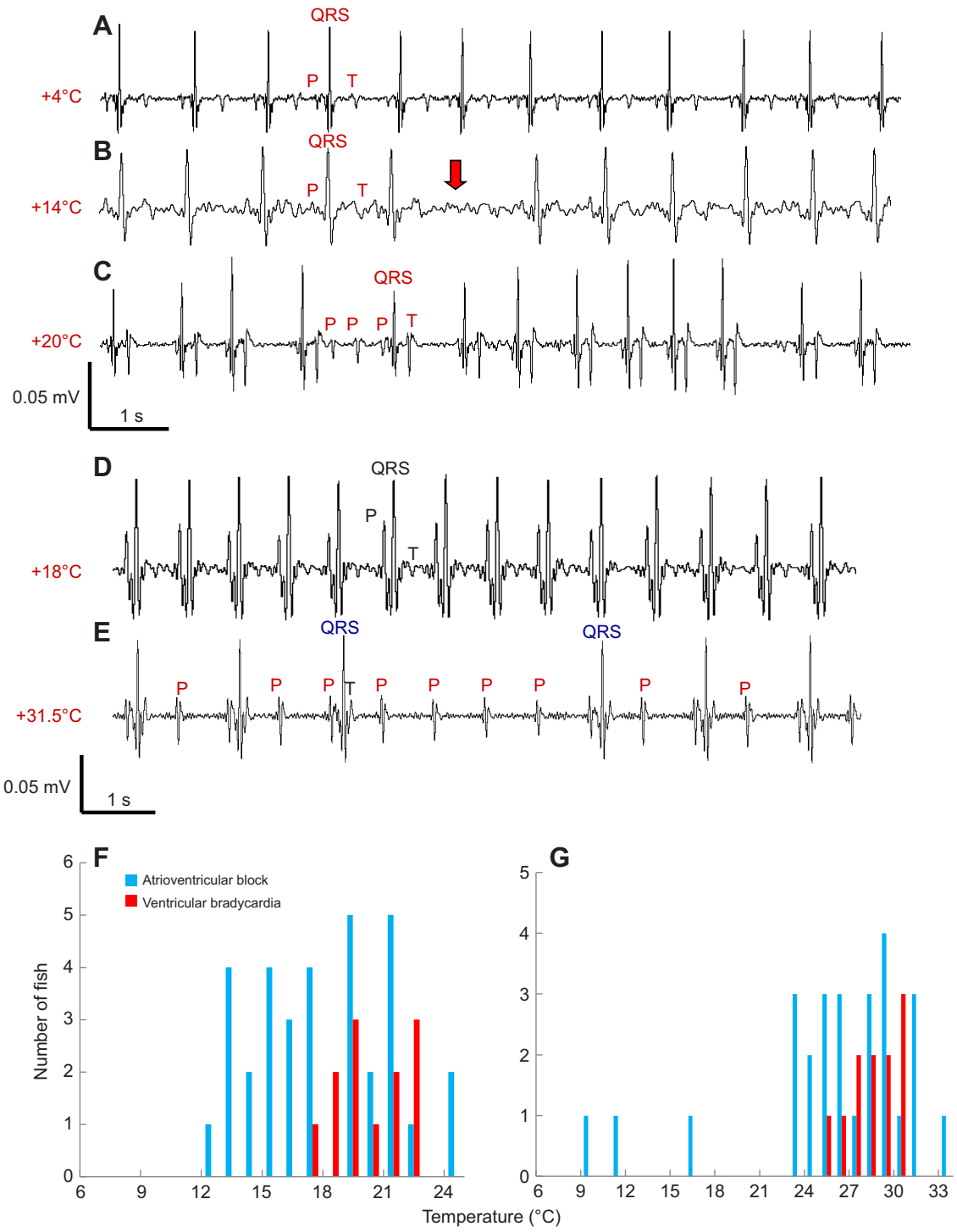

Fig. 5. Electrocardiogram tracings of winter- and summer-acclimatized roach showing various types of cardiac arrhythmias. (A) Normal rhythmic heartbeat of winter roach at +4°C with distinct P, T and QRS waves. (B) A missing QRS complex (arrow) in the middle of normal heart rhythm of winter roach at +14°C. (C) A short episode of atrial tachycardia and more variable QRS amplitudes and RR intervals at +20°C. (D) Normal rhythmic heartbeat of summer roach at +18°C with clear P, T and QRS waves. (E) Arrhythmic heartbeat of summer roach at +31.5°C with three episodes of missing QRS complexes (atrioventricular block). (F,G) Frequency histograms for the occurrence of missing QRS complexes (atrioventricular block) and ventricular bradycardia in winter (F) and summer roach (G) under heat ramps.

acclimatization of f_H needs to be examined (Robinson et al., 2011; Seibert, 1979; Sureau et al., 1989).

Heat tolerance and cardiac arrhythmias

Roach are eurythermal fish with a thermal tolerance window that extends from freezing temperatures to an upper thermal tolerance limit of 33.5°C (Cocking, 1959). Important questions in regard to thermal tolerance of heart function in roach and other fishes are: (1) how does deterioration of heart function appear in the ECG, (2) is it possible to determine the exact temperature for cardiac arrhythmias, and (3) what are the ionic and molecular mechanisms that determine thermal limits of electrical excitation? Answers to these questions

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

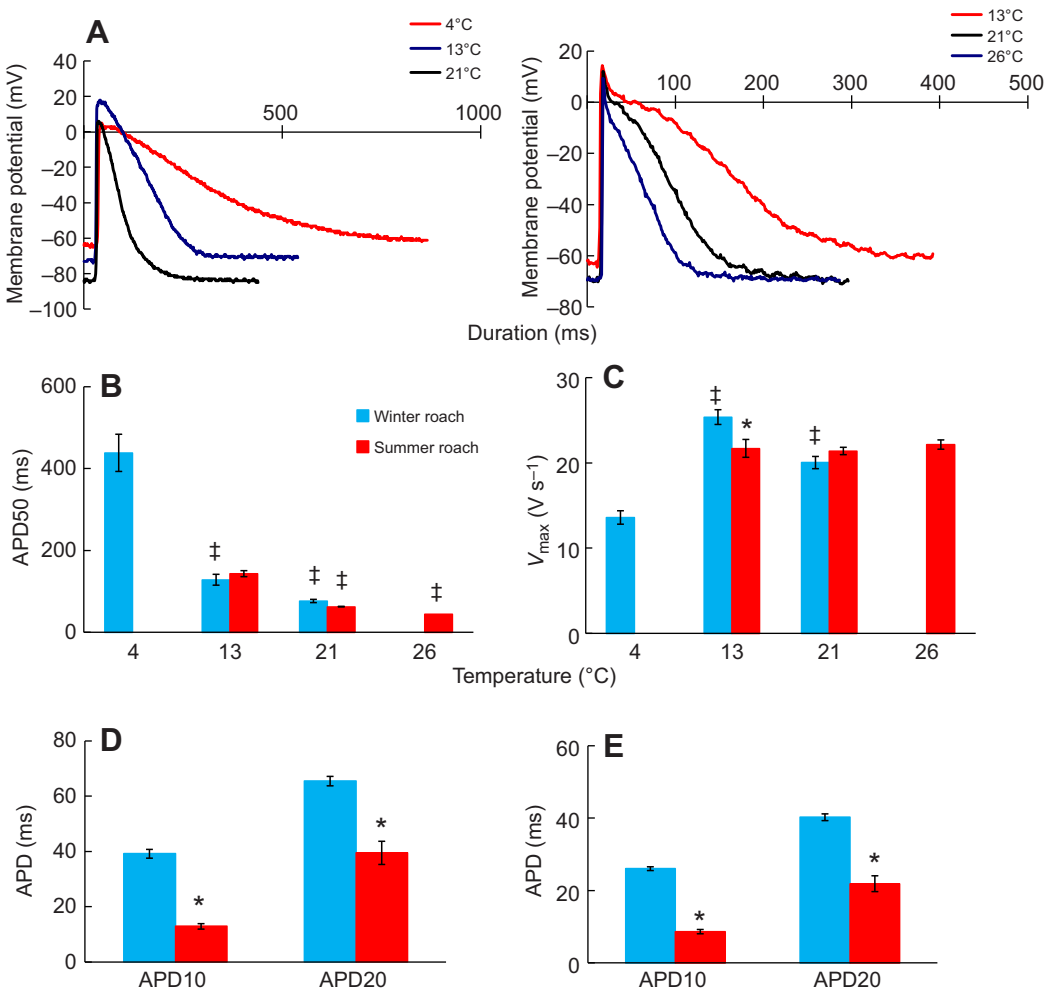

Fig. 6. Effects of temperature on atrial action potentials of the isolated roach heart. (A) Representative recordings of atrial action potentials at three experimental temperatures for winter-acclimatized (left) and summer-acclimatized (right) roach. Note the fast initial repolarization of the summer roach atrium. (B,C) Means±s.e.m. of the maximum upstroke velocity of the action potential (V_{max}; B) and action potential duration at the 50% repolarization level (APD50; C) at three experimental temperatures for both acclimatization groups. (D,E) Action potential durations at 10% and 20% repolarization levels (APD10, APD20), respectively, at the common experimental temperatures (13 and 21°C) of summer and winter roach. Asterisks indicate statistically significant differences (P<0.05) between winter and summer roach, and double daggers between experimental temperatures within an acclimatization group.

are important, as cardiac arrhythmias in fishes are poorly described and seldom defined, but are, however, sometimes used to make far-reaching ecological predictions about the fate and success of fishes in future climates (Munoz et al., 2014). The T_{BP} of *in vivo* f_H, determined as the temperature at which bradycardia started, for winter (19.8±0.5°C) and summer roach (28.1±0.5°C) are close to the upper thermal tolerance limits of 22 and 28°C determined by Cocking for 3°C- and 18°C-acclimated roach, respectively (Cocking, 1959). In addition, the lower thermal limit of summer roach heart activity (~6°C) is similar to the thermal tolerance of the 3°C-acclimated roach (~4°C; Cocking, 1959). These findings indicate that the acute heat death and heat-induced bradycardia are correlated in roach. However, bradycardia was not the first sign of cardiac arrhythmia in the roach.

Irregularities in the rhythm of heartbeat first appeared as occasional absence of QRS complexes, i.e. as a failure of impulse to conduct from the atrium to the ventricle or to excite the ventricle. Because atrial depolarization in the form of a P wave was present, it appears that nodal tissues are not totally compromised by high temperatures, thus suggesting that the ventricle is unresponsive to the stimulus. Missing beats appeared at approximately 10 and 8°C above the acclimatization temperature of the fish in winter and summer roach, respectively, indicating that there is some safety margin in regard to these relatively mild arrhythmias. Increased variability of interbeat intervals coincided with missing QRS complexes, suggesting that it was at least partly due to omission of ventricular contractions. Increased f_H variability under temperature stress has been previously noticed in cod (*Gadus morhua*) and brown trout (*Salmo trutta fario*) (Claireaux et al., 1995; Vornanen et al., 2014). The third type of cardiac arrhythmia in roach appeared as short episodes of atrial tachycardia, also previously noticed in the brown trout heart (Vornanen et al., 2014). Because missing QRS complexes and atrial tachycardia occurred in the midst of continuously increasing f_H, the effect of these arrhythmias on pump function of the heart could be considered to be of minor importance. Indeed, the roach (and brown trout) heart never showed chaotic ventricular tachycardia, similar to the torsades de pointes of the mammalian heart, which can completely compromise the pump function of the heart (Yap and Camm, 2003). The analysis of cardiac arrhythmia in the roach suggests that temperature-induced bradycardia (T_{BP} of f_H) demarcates best the severe deterioration of fish heart function, which is then soon followed by complete

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

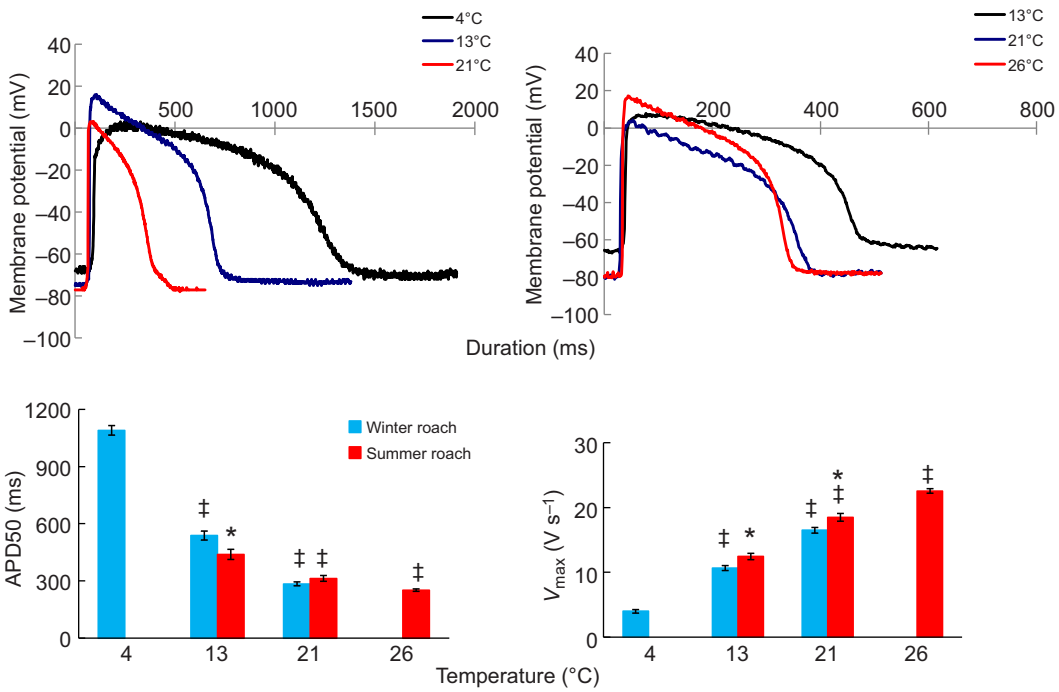

Fig. 7. Effects of temperature on ventricular action potentials of the isolated roach heart. Top panels (A) show representative recordings of ventricular action potentials at three experimental temperatures for winter-acclimatized (left) and summer-acclimatized (right) roach. Bar graphs indicate means±s.e.m. of V_{max} (B) and APD50 (C). Asterisks indicate statistically significant differences (P<0.05) between winter and summer roach, and double daggers between experimental temperatures within an acclimatization group.

cessation of the heartbeat. It remains to be shown whether the milder forms of cardiac arrhythmia have any debilitating effect on fish life under sustained elevation of temperature or whether thermal acclimation overrides them.

Thermal acclimation of cardiac action potentials

Considering the clear temperature-induced changes in f_H between winter and summer roach, it would be expected that there were also changes in AP shape, in particular in AP duration (Abramochkin and Vornanen, 2015; Harper et al., 1995; Haverinen and Vornanen, 2009; Talo and Tirri, 1991; Vornanen et al., 2002a,b). Acclimatization-induced changes in ventricular AP waveform were, however, minor. There was no compensatory shortening of ventricular AP duration in winter roach at 13°C, even though thermal compensation of f_H was prominent at this temperature. On the contrary, ventricular AP was slightly shorter in summer roach at 13°C. ECG recordings support the microelectrode data: QT intervals of winter and summer roach were similar, indicating that the average duration of ventricular AP *in vivo* does not differ between seasons. The results from seasonally acclimatized roach also conform to the findings from the laboratory-acclimated roach hearts in regard to AP duration (Haverinen and Vornanen, 2009). The conclusion from these findings is that neither seasonal acclimatization nor thermal acclimation change the duration of ventricular AP to any significant extent in roach. The absence of acclimatization effects on AP duration does not, however, exclude changes in sarcolemmal ion currents, because changes in depolarizing and repolarizing currents can cancel each other out at the AP plateau. The differences in the rate of AP upstroke and AP amplitude between the ventricles of winter and summer roach suggest that the density of the Na^+ current (I_{Na}), the main determinant of those variables, is higher in winter than in summer. This would equate as a faster propagation of AP over the ventricle in winter roach.

Table 1. Break point temperatures (T_{BP}) for different variables of *in vivo* ECG of summer- and winter-acclimatized roach

	T_{BP} (°C)		
Variable	Winter	Summer	*P* value
f_H (beats min^{-1})	19.8±0.5	28.1±0.5	<0.05
QRS duration (ms)	21.2±0.7	28.1±0.9	<0.05
PQ interval (ms)	20.8±0.5	30.8±0.5	<0.05
QT interval (ms)	–	29.1±0.7	
T_{ARR} (°C)	14.0±0.5	26.4±1.4	<0.05
T_{ABP} (°C)	12	28.5	

Results are means±s.e.m. of 13 and 20 fishes for winter and summer roach, respectively. f_H, heart rate; QRS duration, ventricular depolarization duration; PQ interval, impulse transmission from atrium to ventricle; QT interval, average duration of ventricular action potential; T_{ABP}, Arrhenius break point temperature; T_{ARR}, arrhythmic temperature.

Atrial APs of summer roach were characterized by a fast initial repolarization, which was not present in the winter roach atrium. In mammalian heart, the phase-1 repolarization is generated by the transient outward current (I_{to}), carried mainly by K^+ efflux through Kv4.2 and Kv4.3 voltage-gated K^+ channels (Dixon et al., 1996). However, until recently, I_{to} has not been demonstrated for fish hearts, and therefore it is possible that the ionic mechanism of the phase-1 repolarization differs from that of the mammalian heart. Irrespective of the ionic basis, the balance between repolarizing and depolarizing currents is changed in favor of repolarization in the summer roach atrium to bring about the change in AP waveform. AP amplitude at 21°C was larger and the rate of AP upstroke at 13°C faster in winter than in summer roach, suggesting that the density of

Journal of Experimental Biology (2016) 219, 1495-1504 doi:10.1242/jeb.138347

Table 2. Comparison of action potential parameters of winter- and summer-acclimatized roach from the microelectrode recordings

	Winter roach			Summer roach		
	4°C	13°C	21°C	13°C	21°C	26°C
Atrium						
V_{rm} (mV)	−65.92±1.33‡	−77.71±2.11	−79.64±0.95*	−75.53±2.71	−73.32±1.67	−72.78±0.62
Overshoot (mV)	14.67±1.43‡	10.00±1.28	9.21±1.16	7.96±0.87‡	7.47±1.84	10.63±0.84
Amplitude (mV)	80.58±1.90‡	87.71±2.67	88.86±1.37*	83.48±3.04	80.80±1.95	83.41±0.91
APD0 (ms)	188.17±29.39‡	29.93±5.76	17.36±1.86*	19.35±2.79‡	10.42±2.55	11.50±1.55
APD50 (ms)	438.42±45.44‡	128.43±13.26	76.50±3.97	143.24±13.51‡	62.75±7.36	44.29±0.71
APD90 (ms)	765.67±57.01‡	256.36±22.46	166.29±7.83*	262.35±18.51‡	132.58±11.33	115.93±5.39
V_{max} (V s^{-1})	13.61±0.79‡	25.39±0.86*	20.05±0.72	21.72±1.05	21.41±0.43	22.16±0.54
APD10 (ms)		39.14±1.59*	26.07±0.52*	12.88±0.98	8.67±0.59	
APD20 (ms)		65.43±1.71*	40.29±0.93*	39.47±4.19	21.92±2.19	
n	7 (12)	14 (14)	5 (14)	5 (17)	3 (12)	3 (14)
Ventricle						
V_{rm} (mV)	−63.33±1.48‡	−75.14±1.21*	−80.50±1.36	−64.68±0.75‡	−78.75±2.22	−77.55±0.47
Overshoot (mV)	6.42±1.13‡	13.57±1.90*	7.43±0.70	7.52±0.74‡	8.84±1.33	15.49±0.45
Amplitude (mV)	69.75±1.45‡	88.71±2.03*	87.93±1.27	72.18±0.92‡	87.59±3.10	92.96±0.70
APD0 (ms)	336.67±58.38‡	234.29±30.21	71.43±7.22	185.27±4.42‡	87.17±16.96	122.19±6.47
APD50 (ms)	1089.58±24.93‡	538.43±23.52*	285.14±10.26	439.55±26.54‡	313.83±15.25	252.25±6.28
APD90 (ms)	1282.08±33.59‡	637.93±29.54*	357.07±11.12	501.55±30.66‡	371.67±17.71	283.19±5.46
V_{max} (V s^{-1})	3.98±0.27‡	10.66±0.38*	16.50±0.44*	12.45±0.49‡	18.50±0.60	22.57±0.35
n	3 (12)	7 (14)	5 (14)	4 (11)	3 (12)	3 (16)

V_{rm}, resting membrane potential; APD0, APD10, APD20, APD50 and APD90, duration of action potential at 0, 10, 20, 50 and 90 mV of repolarization, respectively; V_{max}, the maximum upstroke velocity of the action potential; *n*, number of fishes (number of stably impaled cells). Asterisks indicate a statistically significant difference (P<0.05) between winter and summer roach at the same experimental temperatures (13 and 21°C); double daggers indicate a statistically significant difference (P<0.05) of action potential parameters between experimental temperatures 4, 13 and 21°C in winter roach and 13, 21 and 26°C in summer roach.

atrial I_{Na} is higher in winter than in summer. This is consistent with the shorter PQ interval of winter roach in the temperature range between 3 and 15°C.

Collectively, the *in vitro* AP recordings show that seasonal acclimatization has a stronger effect on atrial than ventricular AP. In both cardiac chambers, AP amplitude is larger and the rate of AP upstroke faster in winter than in summer roach, suggesting a partial thermal compensation in the rate AP propagation over the heart.

CONCLUSIONS

It is obvious that seasonal thermal acclimation of electrical excitation is vital for proper function of the heart under widely differing temperatures of winter and summer waters. The cardiac phenotype of the winter roach heart, with an inability to increase f_H at temperatures above 20°C and the first appearance of cardiac arrhythmias at 14°C, would be completely inadequate to support heart function at summer temperatures (up to 25°C at the study site). Similarly, the depression of f_H in summer roach at low temperatures and complete cessation of excitability at approximately 6°C would be fatal for winter roach living under ice in a temperature range of 0–4°C. Seasonal acclimatization of electrical excitability increases pumping capacity of the roach heart by maximizing f_H in both seasons, but without compromising the stability of cardiac excitation. Even though f_H is seasonally optimized, both winter and summer roach hearts retain the safety margin of approximately 10°C for cardiac arrhythmias. How the balance between sensitivity and stability of electrical excitation is achieved at the level of the excitable membrane could be resolved by studying the ionic and molecular mechanisms of cardiac excitation of cardiac myocytes.

Acknowledgements

We thank Anita Kervinen for assistance in taking care of the fish and preparing the solutions. Jaakko Haverinen is acknowledged for teaching A.B. the ECG recoding techniques.

Competing interests

The authors declare no competing or financial interests.

Author contributions

The study was designed by M.V., M.F.E.-S. and A.B. A.B. performed the experiments, analyzed the data and drafted the paper. M.V. finalized the manuscript.

Funding

A.B. was supported by a personal grant from the Ministry of Higher Education, Egypt (Cultural Affairs and Missions Sector). A research grant from Suomen Akatemia (Academy of Finland; no. 14955) to M.V. covered the material costs of the study.

References

Abramochkin, D. V. and Vornanen, M. (2015). Seasonal acclimatization of the cardiac potassium currents (I_{K1} and I_{Kr}) in an arctic marine teleost, the navaga cod (*Eleginus navaga*). *J. Comp. Physiol. B.* **185**, 883-890.

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.

Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E. and Farrell, A. P. (2014a). Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nat. Commun.* **5**, 4252.

Anttila, K., Eliason, E. J., Kaukinen, K. H., Miller, K. M. and Farrell, A. P. (2014b). Facing warm temperatures during migration: cardiac mRNA responses of two adult *Oncorhynchus nerka* populations to warming and swimming challenges. *J. Fish Biol.* **84**, 1439-1456.

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 Physiol.* **96**, 177-180.

Campbell, H. A., Taylor, E. W. and Egginton, S. (2004). The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin *Myoxocephalus scorpius*. *J. Exp. Biol.* **207**, 1969-1976.

Claireaux, G., Webber, D., Kerr, S. and Boutilier, R. (1995). Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating salinity and oxygenation conditions. *J. Exp. Biol.* **198**, 61-69.

Cocking, A. W. (1959). The effects of high temperatures on roach (*Rutilus rutilus*): I. The effects of constant high temperatures. *J. Exp. Biol.* **36**, 203-216.

Dixon, J. E., Shi, W., Wang, H.-S., McDonald, C., Yu, H., Wymore, R. S., Cohen, I. S. and McKinnon, D. (1996). Role of the Kv4.3 K^+ channel in ventricular muscle: a molecular correlate for the transient outward current. *Circ. Res.* **79**, 659-668.

Driedzic, W. R., Bailey, J. R. and Sephton, D. H. (1996). Cardiac adaptations to low temperature in non-polar teleost fish. *J. Exp. Zool.* **275**, 186-195.

Fozzard, H. A. (1977). Cardiac muscle: excitability and passive electrical properties. *Prog. Cardiovasc. Dis.* **19**, 343-359.

Gamperl, A. K. and Farrell, A. P. (2004). Cardiac plasticity in fishes: environmental influences and intraspecific differences. *J. Exp. Biol.* **207**, 2539-2550.

Gollock, M. J., Currie, S., Petersen, L. H. and Gamperl, A. K. (2006). Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. *J. Exp. Biol.* **209**, 2961-2970.

Gracey, A. Y., Fraser, E. J., Li, W., Fang, Y., Taylor, R. R., Rogers, J., Brass, A. and Cossins, A. R. (2004). Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proc. Natl. Acad. Sci. USA* **101**, 16970-16975.

Graham, M. S. and Farrell, A. P. (1990). Myocardial oxygen consumption in trout acclimated to 5°C and 15°C. *Physiol. Zool.* **63**, 536-554.

Harper, A. A., Newton, I. P. and Watt, P. W. (1995). The effect of temperature on spontaneous action potential discharge of the isolated sinus venosus from winter and summer plaice (*Pleuronectes platessa*). *J. Exp. Biol.* **198**, 137-140.

Haverinen, J. and Vornanen, M. (2007). Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *Am. J. Physiol.* **292**, R1023-R1032.

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.

Hazel, J. R. and Prosser, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620-677.

Jayasundara, N., Tomanek, L., Dowd, W. W. and Somero, G. N. (2015). Proteomic analysis of cardiac response to thermal acclimation in the eurythermal goby fish *Gillichthys mirabilis*. *J. Exp. Biol.* **218**, 1359-1372.

Keen, J. E., Vianzon, D.-M., Farrell, A. P. and Tibbits, G. F. (1994). Effect of temperature and temperature acclimation on the ryanodine sensitivity of the trout myocardium. *J. Comp. Physiol. B* **164**, 438-443.

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.

Laurent, P., Holmgren, S. and Nilsson, S. (1983). Nervous and humoral control of the fish heart: structure and function. *Comp. Biochem. Physiol. A Physiol.* **76**, 525-542.

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.

Morita, A. and Tsukuda, H. (1994). The effect of thermal acclimation on the electrocardiogram of goldfish. *J. Therm. Biol.* **19**, 343-348.

Munoz, N. J., Anttila, K., Chen, Z., Heath, J. W., Farrell, A. P. and Neff, B. D. (2014). Indirect genetic effects underlie oxygen-limited thermal tolerance within a coastal population of chinook salmon. *Proc. R. Soc. B Biol. Sci.* **281**, 20141082.

Precht, H., Christophersen, J. and Hensel, H. (1955). *Temperatur und Leben*, p. 514. Berlin: Springer-Verlag.

Robinson, E., Egginton, S. and Davison, W. (2011). Warm-induced bradycardia and cold-induced tachycardia: mechanisms of cardiac and ventilatory control in a warm-acclimated Antarctic fish. *Polar Biol.* **34**, 371-379.

Sandblom, E. and Axelsson, M. (2007). Venous hemodynamic responses to acute temperature increase in the rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol.* **292**, R2292-R2298.

Seibert, H. (1979). Thermal adaptation of heart rate and its parasympathetic control in the European eel *Anguilla anguilla*. *Comp. Biochem. Physiol.* **64C**, 275-278.

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.

Steinhausen, M. F., Sandblom, E., Eliason, E. J., Verhille, C. and Farrell, A. P. (2008). The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *J. Exp. Biol.* **211**, 3915-3926.

Sureau, D., Lagardere, J. P. and Pennec, J. P. (1989). Heart rate and its cholinergic control in the sole (*Solea vulgaris*), acclimatized to different temperatures. *Comp. Biochem. Physiol. A Physiol.* **92**, 49-51.

Talo, A. and Tirri, R. (1991). Temperature acclimation of the perch (*Perca fluviatilis* L.): changes in duration of cardiac action potential. *J. Therm. Biol.* **16**, 89-92.

Vornanen, M., Ryökkynen, A. and Nurmi, A. (2002a). Temperature-dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. *Am. J. Physiol.* **282**, R1191-R1199.

Vornanen, M., Shiels, H. A. and Farrell, A. P. (2002b). Plasticity of excitation-contraction coupling in fish cardiac myocytes. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **132**, 827-846.

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.* **289**, R1177-R1184.

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.

Yap, Y. G. and Camm, A. J. (2003). Drug induced QT prolongation and torsades de pointes. *Heart* **89**, 1363-1372.