THERMAL TOLERANCE OF ELECTRICAL EXCITATION OF THE ROACH (*RUTILUS RUTILUS*) HEART

Ahmed Badr Mohamed Osman

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Author's address: Ahmed Badr Mohamed Osman
University of Eastern Finland
Depart. of Environmental and Biological Sciences
P.O. Box 111
80101, JOENSUU, FINLAND
email: ahmed.osman@uef.fi

Supervisors: Professor Matti Vornanen, Ph.D.
University of Eastern Finland
Depart. of Environmental and Biological Sciences
P.O. Box 111
80101, JOENSUU, FINLAND
email: matti.vornanen@uef.fi

Professor Mohamed Farag El-Sayed, Ph.D.
Sohag University
Depart. of Zoology
P.O. Box 82524, SOHAG, EGYPT
email: m_farag6000@yahoo.com

Professor El-Sabry Abu-Amra, Ph.D.
Sohag University
Depart. of Zoology
P.O. Box 82524, SOHAG, EGYPT
email: el-sabry hassainen@yahoo.com

Reviewers: Adj. Professor Katja Anttila, Ph.D.
University of Turku
Depart. of Biology
20014 TURKU, FINLAND
email: katja.anttila@utu.fi

Assoc. Professor Todd Gillis, Ph.D.
University of Guelph
Depart. of Integrative Biology
Summerlea Science Complex
50 Stone Road E
N1G 2W1, GUELPH, ONTARIO, CANADA
1-519-824-4120 ext 58786
email: tgillis@uoguelph.ca

Opponent: Reader Holly A. Shiels, Ph.D.
Division of Cardiovascular Sciences

Faculty of Biology, Medicine and Health
University of Manchester
3.15d Core Technology Facility
46 Grafton Street
M13 9NT, MANCHESTER, UK
email: holly.shiels@manchester.ac.uk

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ABSTRACT

Temperature has a profound effect on the physiology of ectothermal animals, since ectotherms cannot regulate their body temperature. At northern latitudes, fish experience large seasonal temperature variations, which they cope with physiological adjustments, collectively called acclimatization. Respiratory and cardiovascular functions are considered to play a central role in the thermal responses of fish as formulated in the hypothesis of oxygen- and capacity-limited thermal tolerance (OCLTT). Therefore, I studied seasonal acclimatization of electrical excitability of the heart in the roach (*Rutilus rutilus*), a eurythermal teleost fish species.

The experiments were conducted at four levels of biological organization: (1) in intact fish *in vivo* using electrocardiogram (ECG) recordings, (2) in excised hearts *in vitro* using the microelectrode technique, (3) in enzymatically isolated cardiac myocytes using the patch-clamp technique, and (4) by examining the molecular background of ion currents. The effects of seasonal acclimatization were much stronger *in vivo* in intact fish than at cellular or molecular level. At low temperatures, heart rate (f_H) *in vivo* was higher in winter- than in summer-acclimatized roach, while the situation was the opposite at higher temperatures. These findings indicate adjustments of cardiac function for active life at all seasonal temperatures. The heart's resilience against high temperatures was much better in summer than in winter roach: the break-point temperatures (T_{BP}) of the ECG variables were 6.9−10°C higher in summer- than winter-acclimatized roach. Similarly, the threshold temperature for benign cardiac arrhythmias was 12.4°C lower in winter than in summer roach. Among the measured ion currents, the T_{BP} of the sodium current (I_{Na}) shifted in a similar manner as the T_{BP} of the *in vivo* f_H (both were lower in winter fish), suggesting a causal relationship between them. Heat tolerance of L-type calcium (I_{CaL}) and inward rectifier potassium (I_{K1}) currents did not differ between winter and summer roach, while T_{BP} of rapid component of

delayed rectifier potassium current (I_{Kr}) current was lower in summer than in winter roach. I_{Na} was the most heat-sensitive cardiac ion current in roach. The parallel seasonal shift in thermal tolerance of f_H and I_{Na}, and the high heat sensitivity of I_{Na} suggest that electrical excitation of myocytes may limit f_H in roach.

Seasonal changes in ion current densities, in particular those of I_{Kr} and I_{Na}, were correlated with changes in transcript expression of the respective ion channels' genes, consistent with findings from other teleost fish species.

The present results are consistent with the "old truth" that temperature tolerance decreases with an increasing level of biological organization: the intact organism is most sensitive, and the molecules are most resistant. However, I_{Na} was an exception to this rule. The present findings are also consistent with the OCLTT hypothesis, and thermal sensitivity of electrical excitation may provide the mechanical explanation for this.

Universal Decimal Classification: *591.112.1, 591.112.2, 591.412, 591.543.1, 597.551.21*

***CAB Thesaurus**: acclimatization; environmental temperature; seasonal variation; tolerance; fishes; Rutilus rutilus; cardiovascular system; heart; electrical activity; electrophysiology; electrocardiography; heart rate; arrhythmia; ion transport; sodium channels; calcium channels; potassium channels.*

***Yleinen suomalainen asiasanasto:** akklimatisaatio; sopeutuminen; lämpötila; vaihtelu; vuodenajat; kalat; särki; verenkiertoelimet; sydän; elektrofysiologia; EKG; syke; rytmihäiriöt; ionit; natrium; kalsium; kalium.*

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Joensuu, 14th of March 2018
Ahmed Badr Mohamed Osman

LIST OF ABBREVIATIONS

$[K^+]_o$	Extracellular potassium ion concentration
$+V_{max}$	Maximum rate of upstroke velocity
AP	Action potential
APD	Action potential duration
APD_{10}	Action potential duration at 10% repolarization level
APD_{20}	Action potential duration at 20% repolarization level
APD_{50}	Action potential duration at 50% repolarization level
AV	atrioventricular
bpm	heartbeat per minute
CD	Critical depolarization
CO	Cardiac output
EC coupling	Excitation-contraction coupling
ECG	Electrocardiogram
EE	Electrical excitation
f_H	Heart rate
HP	Holding potential
I_{CaL}	L-type calcium current
I_{CaT}	T-type calcium current
I_f	Hyperpolarization-activated funny current
I_{K1}	Inward rectifier K^+ current
I_{KAch}	Acetylcholine-activated inward rectifier potassium current
I_{Kr}	Rapid component of delayed rectifier K^+ current
I_{Ks}	Slow component of delayed rectifier K^+ current
I_{Na}	Sodium current
I_{to}	Transient outward potassium current
OCLTT	Oxygen- and capacity-limited thermal tolerance hypothesis
OS	Overshoot of action potential
P	Atrial depolarization
PQ interval	Duration of impulse transmission from atrium to ventricle
Q_{10}	Thermal sensitivity over a 10°C change in temperature.
QRS	Ventricular depolarization
QT interval	Average duration of ventricular action potential
RMP	Resting membrane potential
SDNN	Standard deviation of successive interbeat intervals
SL	Sarcolemma
SR	Sarcoplasmic reticulum
SV	Stroke volume
T	Ventricular repolarization
T_{ARR}	Arrhythmia temperature
T_{BP}	Break-point temperature

TDEE	Temperature-dependent deterioration of electrical excitability hypothesis
TP	Threshold potential
TTX	Tetrodotoxin
$-V_{max}$	Maximum rate of AP repolarization (AP decay)

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman Numerals I-IV.

I **Badr, A.**, El-Sayed, M.F. & Vornanen, M. 2016. Effects of seasonal acclimatization on temperature dependence of cardiac excitability in the roach, *Rutilus rutilus*. *Journal of Experimental Biology*. 219(10): 1495-1504.

II **Badr, A.**, Hassinen, M., El-Sayed, M.F. & Vornanen, M. 2017. Effects of seasonal acclimatization on action potentials and sarcolemmal K^+ currents in roach (*Rutilus rutilus*) cardiac myocytes. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology*. 205: 15-27.

III **Badr, A.**, Korajoki, H., Abu-Amra, E.S., El-Sayed, M.F. & Vornanen, M. 2018. Effects of seasonal acclimatization on thermal tolerance of inward currents in roach (*Rutilus rutilus*) cardiac myocytes. *Journal of Comparative Physiology B*. 188: 255-269.

IV **Badr, A.**, Abu-Amra, E.S., El-Sayed, M.F. & Vornanen, M. 2018. Electrical excitability of roach (*Rutilus rutilus*) ventricular myocytes: effects of extracellular K^+, temperature and pacing frequency. *American Journal of Physiology-Regulatory Integrative & Comparative Physiology* (Submitted).

The above publications have been included at the end of this thesis with their copyright holders' permission.

AUTHOR'S CONTRIBUTION

I) *Journal of Experimental Biology* 219(10): 1495-1504, 2016.
The study was designed by M.V. and A.B. A.B. performed the experiments of *in vivo* ECG and *in vitro* microelectrode recording of APs, and analyzed the data. A.B. drafted the manuscript and finalized it together with M.V. A.B. processed the manuscripts in collaboration with the co-authors.

II) *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology* 205: 15-27, 2017.
The study was designed by M.V. and A.B. A.B. performed all electrophysiological experiments and analyzed the data. Molecular experiments were performed by M.H. A.B. drafted the manuscript and finalized it together with M.V. and M.H. A.B. processed the manuscripts in collaboration with the co-authors.

III) *Journal of Comparative Physiology B* 188:255-269, 2018.
The study was designed by M.V. and A.B. A.B. performed all electrophysiological experiments and analyzed the data. Molecular studies were done by H.K. A.B. drafted the manuscript and finalized it together with M.V. and H.K. A.B. processed the manuscripts in collaboration with the co-authors.

IV) *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology* (Submitted), 2018.
The study was designed by M.V. and A.B. A.B. performed electrophysiological experiments and analyzed the data. A.B. drafted the manuscript and finalized it together with M.V. A.B. processed the manuscripts in collaboration with the co-authors.

Ahmed Badr, A.B.; Minna Hassinen, M.H.; Hanna Korajoki, H.K.; Matti Vornanen, M.V.

CONTENTS

1 INTRODUCTION

1.1. ROACH ARE EURYTHERMAL FISH

Fish have adapted to different aquatic habitats, which extend from freshwater to marine water, from cold polar seas to warm tropical reefs, and from shallow surface waters to the deepest ocean waters. Fish represent the most variable and largest vertebrate group, including currently about 33000 species (**HTTP://FISHBASE.ORG/HOME.HTM**; Powers, 1989; Axelsson et al., 1992; Johnston et al., 1994). With the exception of some 30 partially endothermic species (Dickson and Graham, 2004), all fish are ectotherms, i.e. their body temperature is dependent on and in equilibrium with the ambient water temperature. The adaptation of fish to temperature involves different life styles. Some fish are active in winter and relatively inactive in summer (e.g. burbot, *Lota lota*) (Edsall et al., 1993; Carl, 1995; Pääkkönen and Marjomäki, 2000), others are active in summer and dormant in winter (e.g. crucian carp, *Carassius carassius*) (Holopainen et al., 1997), and still others are active throughout the year (e.g. rainbow trout, *Oncorhynchus mykiss*).

According to the thermal tolerance limits, fish can be classified into 3 groups: stenothermal, mesothermal and eurythermal. Stenothermal fish have a narrow range of temperature tolerance, such as the Antarctic fish, which live under constant cold and resist only a very limited range of temperatures, usually less than 10°C (Verde et al., 2006; Franklin et al., 2007). By contrast, the most eurythermal fish tolerate temperature changes between 0°C and 40°C (Bennett and Beitinger, 1997; Rantin et al., 1998; Beitinger and Bennett, 2000). Salmonid fish are sometimes called mesothermal, since their thermal tolerance range (from 0°C to 22−28°C) is between those of the most stenothermal and the most eurythermal fish species (Elliott and Elliott, 2010).

Roach (*Rutilus rutilus*; Linnaeus, 1758) is a cyprinid fish, which inhabits fresh and brackish waters in most of Europe, except for the Mediterranean zone and western Asia. In Finland, roach are the most widely distributed fish after perch (*Perca fluviatilis*) and pike (*Esox lucius*). They inhabit freshwaters throughout the country with the exception of northernmost Lapland. Roach favor eutrophic water bodies, and their numbers have risen with the increasing nutrient levels of lakes (**HTTP://WWW.LUONTOPORTTI.COM/SUOMI/EN/KALAT/ROACH**). Roach therefore play an important role in the energy flow and nutrient cycling of eutrophic lakes (Riemann et al., 1986). Roach are eurythermal fish tolerating a wide range of temperatures, from the lower critical temperature of about 0°C to the ultimate upper lethal temperature of 33.5°C (Cocking, 1959).

1.2. HEART AND CARDIOVASCULAR SYSTEM OF FISH

The heart is the muscular pump of the vertebrate cardiovascular system. It provides the necessary power for blood circulation, which delivers oxygen and nutrients to different tissues and removes cellular metabolites and CO_2 from the body (Olson and Farrell, 2006). Traditionally, the fish heart is considered to be composed of 4 chambers arranged in series: *sinus venosus*, atrium, ventricle and *bulbus arteriosus* (or *conus arteriosus* for the basal fish) within the pericardium. More recently, it has been put forward that the fish heart consists of 6 sequential chambers within the pericardium: *sinus venosus*, atrium, atrioventricular (AV) segment, ventricle, *conus arteriosus* and *bulbus arteriosus*. In the latter scheme, the contractile *conus arteriosus* resides between the ventricle and the non-contractile *bulbus arteriosus*. *Conus arteriosus* and *bulbus arteriosus* are collectively called the outflow tract (Icardo, 2006; Icardo and Colvee, 2011). In teleost fish, *sinus venosus* and *bulbus arteriosus* mainly consist of connective tissue and are non-contractile, while atrium and ventricle form the main muscular pump (Santer, 1985; Satchell, 1991).

Like all vertebrates, teleost fish have a closed cardiovascular system. The paired ducts of Cuvier and hepatic veins pass the deoxygenated venous blood into the thin-walled *sinus venosus*, which functions as a drainage pool for the venous system before the blood enters the atrium (Santer, 1985; Satchell, 1991). Between the atrium and the ventricle is the AV segment, a ring of cardiac tissue, which plays an essential role in regulating the conduction of action potential (AP) to the ventricle and supports the AV valves (Icardo and Colvee, 2011). Atrial contraction pushes the blood to the ventricle, the thick-walled main force-generating muscular chamber of the fish heart. The ventricle contracts and propels the blood to the outflow tract, which leads into the ventral aorta and directs blood to the gills for oxygenation and further to the tissues of the body (Santer, 1985; Olson and Farrell, 2006; Genge et al., 2012). Sino-atrial, atrio-ventricular and bulbo-ventricular valves prevent the recurcitation of blood when blood pressure varies during the cardiac cycle.

1.3. ELECTRICAL EXCITATION AND CONTRACTION OF FISH HEART

The pump function of the heart is based on regular and sequential contractions of atrium and ventricle. Each contraction of the heart is initiated by electrical excitation (EE) of the sarcolemma (SL) of cardiac myocytes, called cardiac AP, which spreads throughout the heart and activates myofilaments via increases in the intracellular free Ca^{2+} concentration ($[Ca]_i$) (Coraboeuf, 1978; Bers, 2000; Vornanen et al., 2002b). The sequence of events starting from the depolarization of SL to force production by myofilaments is called excitation-contraction (EC) coupling (Fabiato, 1983; Tibbits et al., 1992; Bers, 2002; Vornanen et al., 2002b; Shiels and Galli, 2014).

EE determines the rate and rhythm of cardiac contraction under varying physiological states of the animal and is under the control of the autonomic nervous system. EE is dependent on the electrochemical gradients of Na^+, K^+ and Ca^{2+} ions across the SL. These cations are unequally distributed across the SL and provide the driving force for entry or exit of ions through ion channels, which are gated to open by changes in membrane potential or by binding of external ligands to membrane receptors (Bezanilla, 2005; Vornanen, 2017). Carefully orchestrated opening and closing of ion channels result in generation of chamber-specific APs that travel from the site of origin throughout the heart, causing sequential contractions of the atrial and ventricular chambers (Coraboeuf, 1978; Noble, 1984; Maltsev et al., 2006). Na^+-, K^+- and Ca^{2+}-specific ion channels, integral membrane proteins or protein assemblies of the cardiac SL are vital entities for EE. Each of the nodal, atrial, and ventricular tissues has special electrophysiological characteristics, and therefore, different ion channel compositions.

The EE of the fish heart originates from an aggregation of spontaneously active pacemaker cells located as a ring-shaped tissue in the border area between *sinus venosus* and the atrium (Santer, 1985; Olson and Farrell, 2006; Haverinen and Vornanen, 2007). A complicated interaction between several ion channels and transporters generates diastolic depolarization of SL in pacemaker cells, the steepness of which determines f_H (Irisawa et al., 1993; Haverinen and Vornanen, 2007). The pacemaker AP slowly proceeds to the atrium, and then quickly spreads through the atrial wall to the atrioventricular canal, where it slows down again in order to allow sufficient time for ventricular filling. The velocity of AP propagation is rapid again in the endocardial trabeculae of the heart, when AP propagates to the apex of the ventricle. From there AP returns quickly epicardially back to the base of the ventricle (Randall, 1968; Sedmera et al., 2003; Chi et al., 2008; Icardo and Colvee, 2011; Poon and Brand, 2013).

1.4. CARDIAC ACTION POTENTIAL

Cardiac AP is the trigger of cardiac contraction (Bers, 2001). Ion current densities and ion channel compositions reveal regional specialization in the heart, i.e. each cardiac chamber has functionally distinct AP. Pacemaker APs are characterized by a slow diastolic depolarization and a slow upstroke of AP, while atrial and ventricular APs have stable negative resting membrane potential (RMP) and rapid depolarization. Furthermore, there is a striking difference between atrial and ventricular AP duration, in that the atrial AP is much shorter than the ventricular AP (Saito and Tenma, 1976; Haverinen and Vornanen, 2009; Lin et al., 2014; Vornanen, 2016).

As in other vertebrates, fish cardiac AP can be divided into 5 phases (0−4) (Fozzard, 1977; Roden et al., 2002; Vornanen, 2016). Resting atrial and ventricular cardiomyocytes have a stable RMP of -70 to -90 mV relative to the extracellular

fluid (Phase 4), which is maintained by an efflux of K^+ ions through SL K^+ channels. The resting state is broken by voltage spread from neighboring myocytes (depolarization or Phase 0), which depolarizes membrane potential to the AP threshold. This is the voltage level where Na^+ influx exceeds K^+ efflux, generating a rapid upstroke of the AP. Within a few ms membrane potential swings from the threshold potential (TP) across the zero level to +10 – +40 mV. Phase 0 is produced by Na^+ influx into the myocyte through SL Na^+ channels. Na^+ influx is necessary for unidirectional spread of EE between cells, and its amplitude is the major determinator for the rate of AP propagation through the heart. AP upstroke is followed by rapid repolarization (Phase 1), which is generated by transient efflux of K^+ ions. In contrast to mammalian cardiac APs, Phase 1 is often inconspicuous or absent in fish cardiac myocytes (Nemtsas et al., 2010; Vornanen and Hassinen, 2016). The long isoelectric phase of cardiac AP (Phase 2) is called a "plateau", when the close balance between influx of Ca^{2+} and efflux of K^+ current keeps membrane voltage almost unchanged. Phase 2 is essential for SL Ca^{2+} influx and cardiac contraction, and prevents the heart from beating prematurely by delaying the recovery of Na^+ channels from inactivation. The fast restoration of negative RMP starts at the end of the plateau phase (Phase 3), which is generated by various outward K^+ currents. Restoration of RMP brings the heart to diastole and allows blood to fill the atrium and the ventricle. Atrial AP has basically the same 5 phases as ventricular AP, but it is much shorter in duration (Fig. 1) (Saito and Tenma, 1976; Haverinen and Vornanen, 2009; Lin et al., 2014). In contrast to atrial and ventricular APs, pacemaker APs show a gradual and slow diastolic depolarization towards the TP, and have a smaller AP amplitude and duration and a slower rate of AP upstroke and repolarization. Pacemaker APs depolarize atrial myocytes with the resultant spread of EE through the heart (Harper et al., 1995; Haverinen and Vornanen, 2007; Tessadori et al., 2012).

Propagation of AP through the heart can be recorded *in vivo* as electrocardiograms (ECG). The P-wave of ECG represents the depolarization of atrial myocytes, QRS complex depolarization of the ventricle and T-wave repolarization of the ventricle. The P-wave is much smaller than the QRS complex, because the atrium is smaller than the ventricle. Repolarization of atrial AP cannot be seen in ECG, since it is buried beneath the large QRS-complex. In the European river lamprey (*Lampetra fluviatilis*), the relatively slow velocity of AP propagation in the atrial wall makes atrial repolarization visible as a Pt wave between the P wave and the QRS complex; it is not concealed under the large QRS complex as happens in the teleost ECG (Haverinen et al., 2014). The duration of QRS complex is the time taken by the ventricular depolarization to spread over the ventricle. The Q-T interval is the time between depolarization and repolarization of ventricular myocytes and represents the average duration of the ventricular AP.

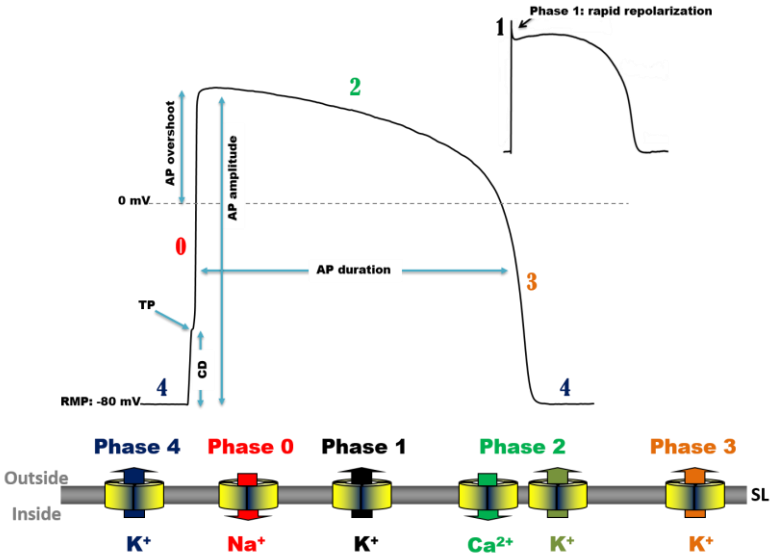

Figure 1. Ventricular AP of the roach heart indicating the measured AP parameters: RMP, resting membrane potential; CD, critical depolarization (=TP-RMP); TP, threshold potential; AP overshoot; AP amplitude and AP duration. AP begins with the inward Na^+ current (I_{Na}), which produces fast depolarization (Phase 0). In mammalian hearts, the rapid depolarization is followed by Phase 1 repolarization, which is poorly developed or absent in the fish ventricle (indicated by an arrow in the inset (Hassinen, 2010)). The plateau phase (Phase 2) is maintained by the balance between influx of Ca^{2+} (I_{Ca}) and efflux of K^+ ions (I_K). The final rapid repolarization (Phase 3) is achieved by three of the outward K^+ currents, I_{Kr}, I_{Ks} and I_{K1}. The resting membrane potential (Phase 4) is maintained by the I_{K1} current.

1.5. SARCOLEMMAL ION CHANNELS AND CURRENTS

Ion channels are integral membrane proteins or protein assemblies that form aqueous water-filled pores in the lipid membrane and allow ions to rapidly cross the membrane (Bers, 2001). There are several families of ion channels, each including several members with distinct ion specificities, voltage dependences, and opening and closing rates. Ions flow passively through ion channels down the electrochemical gradient, generating channel-specific ion currents. The ion flow through the channel is regulated by opening and closing of the channel pore, which is achieved via voltage-dependent or ligand-activated conformational changes of the protein. This is called ion channel gating. Closing and opening of the ion channel may also occur by blocking/unblocking of the channel pore by intracellular ions or small molecules such as Mg^{2+} and polyamines. Cardiac AP is generated by harmonious co-operation between depolarizing (inward) and repolarizing (outward) currents. Depolarization is mainly achieved by the inward flow of Na^+ and Ca^{2+} ions, while repolarization is achieved by the outward flow of K^+ ions (Hodgkin and Huxley, 1952; Opie, 1998; Bers, 2001).

1.5.1. Inward currents

Three physiologically essential inward currents exit in vertebrate cardiac myocytes: Na^+ current (I_{Na}), Ca^{2+} current (I_{Ca}) and hyperpolarization-activated "funny" current (I_f). Here I will only discuss the main inward currents of atrial and ventricular myocytes, I_{Na} and I_{Ca}.

1.5.1.1. Sodium current (I_{Na})

Voltage-gated Na^+ channels consist of the pore-forming α-subunits and auxiliary β-subunits (Catterall, 1992). The α-subunit is composed of 4 homologous domains (I–IV), each domain having six transmembrane spans (S_1–S_6) with the pore loop between S_5 and S_6 (Fig. 2A) (Catterall, 1992; Bers, 2001).

Na^+ channels open very quickly, within a few ms, at the TP of AP and close spontaneously by the mechanism of fast inactivation. The opening of the Na^+ channel generates the rapid inward Na^+ current (I_{Na}). I_{Na} is the first activated current in the EE of atrial and ventricular myocytes (Phase 0), and produces the fast upstroke and overshoot of cardiac AP (Fozzard and Hanck, 1996). The density of I_{Na} determines the rate of the AP upstroke and the propagation velocity of AP over the whole heart. It provides the necessary charge to depolarize the cell membrane and activate other ion channels in the production of AP (Schram et al., 2002; Kleber and Rudy, 2004). K^+ currents, which maintain RMP, have an indirect effect on I_{Na} by regulating the number of available Na^+ channels: hyperpolarization and depolarization of SL increases and reduces the number of Na^+ channels, respectively, which are available for opening (Golod et al., 1998; Maltsev and Undrovinas, 1998). I_{Na} density is similar in atrial and ventricular myocytes of the fish (rainbow trout and zebrafish, *Danio rerio*) heart. However, the voltage dependence of steady-state activation is more negative in atrial than in ventricular myocytes (Warren et al., 2001; Haverinen and Vornanen, 2006). The relatively negative voltage dependence of I_{Na} activation, together with the small size of the inward rectifier K^+ current (I_{K1}), make atrial myocytes easily excitable by the depolarization wave from the pacemaker cells.

Fish have eight genes that encode α-subunits of the voltage-gated Na^+ channels, while mammals have nine genes for voltage-gated Na^+ channels (Widmark et al., 2010). In mammalian hearts, I_{Na} is mainly produced by $Na_v1.5$ α-subunits, while in rainbow trout the main cardiac isoform is $Na_v1.4$, and only small amounts of $Na_v1.5$ and $Na_v1.6$ channels are expressed. In crucian carp and zebrafish, $Na_v1.5$ is the main cardiac isoform, although they express some $Na_v1.4$ channels (Vornanen et al., 2011) (Table 1). Na^+ channels are specifically blocked by tetrodotoxin (TTX). Fish cardiac I_{Na} is TTX-sensitive, in contrast to mammalian cardiac I_{Na}, which is about 1000 times less sensitive to this marine toxin (Vornanen et al., 2011).

1.5.1.2. Calcium current (I_{Ca})

Ca^{2+} channel α-subunits are structurally similar to the Na^{+} channels (Fig. 2B). They are responsible for the voltage-dependent entry of extracellular Ca^{2+} into the cardiac myocyte and thereby indispensable for the EC coupling of cardiac myocytes (McDonald et al., 1994). There are two types of Ca^{2+} channels in vertebrate cardiac myocytes: (1) T-type channels (I_{CaT}), which produce the transient Ca^{2+} currents and activate at more negative voltages (Bean, 1985; Nilius et al., 1986), and (2) L-type channels (I_{CaL}), which produce long-lasting currents and activate at more depolarized voltages (Trautwein et al., 1975; Isenberg and Klöckner, 1980).

1.5.1.2.1. T-type Ca^{2+} current (I_{CaT})

I_{CaT} is activated and inactivated at more negative voltages than I_{CaL}: it reaches the peak value at around -30 mV and is completely inactivated at the holding potential (HP) of about -40 mV. It is kinetically faster than I_{CaL} (Bean, 1985; Nilius et al., 1986). I_{CaT} is crucial for nodal APs in mammals, birds and frogs (Irisawa et al., 1993; Mangoni et al., 2006), but it does not significantly contribute to the AP plateau, Ca^{2+} loading of SR or contractile regulation in working atrial and ventricular myocytes (Jaleel et al., 2008). Although I_{CaT} is abundantly expressed in the atria and ventricles of perinatal mammals, it is often low or completely absent in the atrial and ventricular myocytes of adult mammals (Mitra and Morad, 1986). In fish, I_{CaT} is documented in the ventricular myocytes of zebrafish and shark (dogfish; *Squalus acanthias*) and in the atrial myocytes of the Siberian sturgeon (*Acipenser baerii*) (Maylie and Morad, 1995; Baker et al., 1997; Nemtsas et al., 2010; Haworth et al., 2014). In the Siberian sturgeon, atrial I_{CaT} is approximately 2 times higher than atrial I_{CaL} (Haworth et al., 2014). Interestingly, in the adult zebrafish, both atrial and ventricular myocytes have a high level of I_{CaT}, although not quite as high as I_{CaL} (Nemtsas et al., 2010).

In mammalian hearts, two T-type Ca^{2+} channel α-subunit proteins, α1G (Ca_v3.1) and α1H (Ca_v3.2), are functionally expressed (Vassort et al., 2006). α1G (Ca_v3.1) is assumed to be expressed in the zebrafish heart and has been documented for the crucian carp heart (Table 1) (Nemtsas et al., 2010; Tikkanen et al., 2017).

1.5.1.2.2. L-type Ca^{2+} current (I_{CaL})

I_{CaL} is essential in maintaining the long plateau phase of cardiac AP and the regulation of EC coupling (McDonald et al., 1994). HP for the activation of I_{CaL} is about -40 mV, and I_{CaL} reaches its peak value around 0 mV. Inactivation is slower than that of I_{CaT} (Vornanen, 1997; Hove-Madsen and Tort, 1998; Shiels et al., 2000). SL Ca^{2+} influx is considered to be the primary source of Ca^{2+} for the activation of contractile filaments in the hearts of most teleost fish. A major part of this process probably occurs through I_{CaL}. I_{CaL} may also trigger Ca^{2+} release from intracellular

stores of the sarcoplasmic reticulum (SR), and hence can increase the force of contraction (Vornanen, 1989; Tibbits et al., 1991; Keen et al., 1994; Shiels and Farrell, 1997; Vornanen, 1997; Hove-Madsen and Tort, 1998; Vornanen et al., 2002b; Shiels et al., 2006).

In all the studied fish species, I_{CaL} is higher in ventricular than in atrial myocytes. There appear also to be species-specific differences in the density of I_{CaL}. Ventricular I_{CaL} is higher in crucian carp and zebrafish − both cyprinid species − in comparison to rainbow trout and burbot (Vornanen, 1997; Vornanen, 1998; Shiels et al., 2006; Brette et al., 2008; Zhang et al., 2011). This may be associated with species-specific differences in cardiac EC coupling and cardiac performance; e.g. atrial I_{CaL} density in the bluefin tuna (*Thunnus thynnus*), a powerful swimmer, is about double the density of rainbow trout I_{CaL} under similar conditions (Hove-Madsen and Tort, 1998; Shiels et al., 2004).

In mammals, there are four α1-subunits for L-type Ca^{2+} channels (α1S, α1C, α1D, α1F or $Ca_v1.1$−4) (Benitah et al., 2010). The main cardiac isoform in the atrial and ventricular myocytes of mammals is α1C ($Ca_v1.2$), while sinoatrial and atrioventricular nodal cells also express α1D ($Ca_v1.3$). The molecular basis of I_{CaL} in fish hearts has not been examined. However, a lethal mutation of $Ca_v1.2$ in the zebrafish eliminates cardiac I_{CaL}, suggesting that orthologous genes encode I_{CaL} in both mammals and in fish (Rottbauer et al., 2001). In crucian carp ventricular myocytes, at least two isoforms are expressed: $Ca_v1.2$ and $Ca_v1.3$ (Table 1) (Tikkanen et al., 2017).

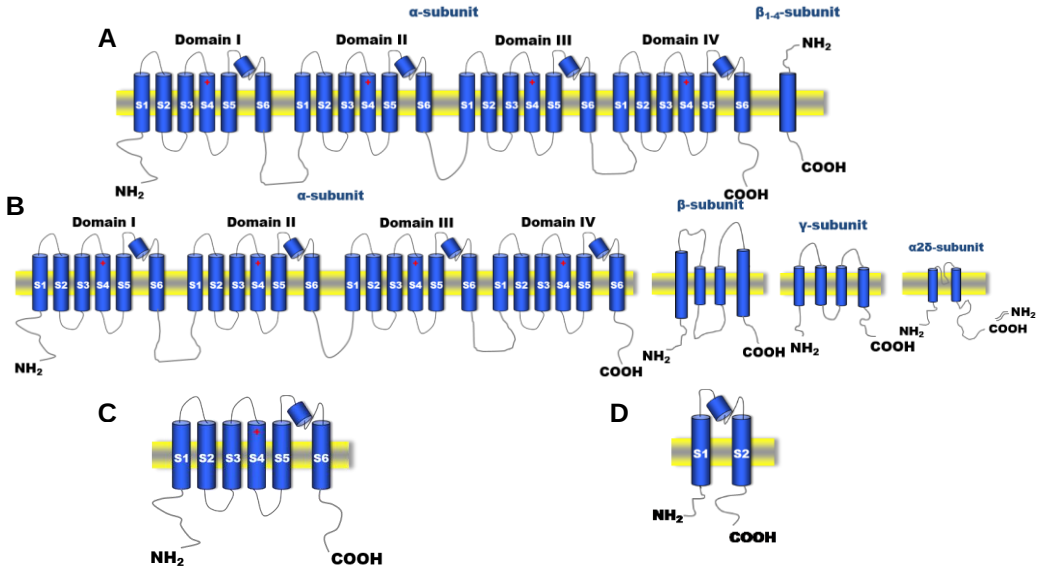

Figure 2. A graph showing the basic structure of cardiac ion channels. (A) Voltage-gated Na^+ channels, (B) voltage-gated Ca^{2+} channels, (C) voltage-gated K^+ channels and (D) inward rectifier K^+ channels. Na^+ channel α-subunits consist of 4 domains (I−IV) and an auxiliary β-subunit. Each domain has 6 transmembrane spans (S1−S6). The pore locates between S5 and S6, while S4 acts as the voltage sensor. Ca^{2+} channels have a similar α-subunit structure to that of Na^+ channels. However, the channel assembly also includes β, γ and α2δ subunits. Voltage-gated K^+-channels are tetramers of 4 identical α-subunits, each having 6 transmembrane spans similar to the domains of the Na^+ channel. Inward-rectifying K^+-channels are tetramers of molecules, which consist of 2 transmembrane spans and the pore.

1.5.2. Outward currents

Physiologically important K^+ currents are outwardly directed, i.e. in the normal voltage range of cardiac AP, they pass K^+ efflux. Their function is to maintain negative RMP and to repolarize AP. Therefore, K^+ currents are significant regulators for AP duration in atrial and ventricular myocytes (Vornanen et al., 2002a). They can be divided into voltage-gated K^+ currents and inward rectifier K^+ currents.

1.5.2.1. Voltage-gated K^+ currents

Voltage-gated K^+ channels have the same overall structure as Na^+ and Ca^{2+} channels, with the exception that each of the four domains is a separate protein (Fig. 2C) (Bers, 2001). Cardiac voltage-gated K^+ currents include: 1) transient outward current (I_{to}), 2) rapid (I_{Kr}), and 3) slow (I_{Ks}) components of the delayed rectifier K^+ current.

1.5.2.1.1. Transient outward K^+ current (I_{to})

In mammalian hearts, two functionally different transient outward currents, I_{to1} and I_{to2}, are expressed. They generate the rapid phase-1 repolarization of the cardiac AP. I_{to1} is carried by K^+ current and can be blocked by 4-aminopyridine (4-AP), while I_{to2} is a chloride (Cl^-) current and insensitive to 4-AP (Nerbonne and Kass, 2005). It is supposed that I_{to1} is formed by K_v 4.2 and/or K_v 4.3 α-subunits, while I_{to2} is produced by K_v1.4 α-subunits (Roden et al., 2002; Patel and Campbell, 2005). Rapid repolarization (Phase 1) is minor or completely absent in fish cardiac myocytes, where no I_{to} has been recorded (Nemtsas et al., 2010; Alday et al., 2014; Vornanen and Hassinen, 2016).

1.5.2.1.2. Rapid component of the delayed rectifier K^+ current (I_{Kr})

I_{Kr} has a significant role in the regulation of the AP duration and refractoriness of the fish heart, and is probably involved in the cardiac pacemaker mechanism (Langheinrich et al., 2003; Haverinen and Vornanen, 2007). Until recently, I_{Kr} has been documented and recorded in atrial, ventricular and pacemaker cells of all the studied fish species (Haverinen and Vornanen, 2007; Galli et al., 2009; Haverinen and Vornanen, 2009; Haverinen et al., 2014; Haworth et al., 2014; Vornanen et al., 2014; Abramochkin and Vornanen, 2015). Generally, the density of I_{Kr} in atrial myocytes is higher than that in ventricular myocytes.

In mammals, I_{Kr} channels are encoded by three genes (Erg1−3 or *Kcnh2*, *Kcnh6*, *Kcnh7*) (Warmke and Ganetzky, 1994). There are at least 4 Erg gene products in the zebrafish heart (*kcnh2a*, *kcnh2b*, *kcnh6*, *kcnh7*) (Vornanen and Hassinen, 2016). In mammalian hearts, Erg1 (*Kcnh2*) is the main isoform, while Erg2 (*kcnh6*) is the main isoform in zebrafish, rainbow trout and crucian carp (Table 1) (Hassinen et al., 2008a; Hassinen et al., 2014; Hassinen et al., 2015b; Vornanen and Hassinen, 2016).

1.5.2.1.3. Slow component of delayed rectifier K^+ current (I_{Ks})

In mammals, I_{Ks} has a crucial role in controlling repolarization of cardiac AP. Under normal physiological conditions it may be concealed, but activates when additional repolarizing current is needed. In situations such as exercise, increased adrenergic tone and high f_H, the impact of I_{Ks} on cardiac AP readily appears (Roden and Yang, 2005; Schmitt et al., 2014). Until now, I_{Ks} has been documented for only one fish species, the crucian carp, where the density of I_{Ks} is higher in atrial than in ventricular myocytes (Hassinen et al., 2011). It is suggested that I_{Ks} is absent in zebrafish cardiomyocytes (Nemtsas et al., 2010; Alday et al., 2014).

In mammalian hearts, I_{Ks} current is produced by K_v7.1 α-subunits (KCNQ1 gene), together with the auxiliary β-subunit MinK (Barhanin et al., 1996; Sanguinetti et al., 1996). Surprisingly, the expression of MinK is very low in the crucian carp

heart, where I_{Ks} is suggested to be generated solely by the $K_v7.1$ α-subunits, without the MinK β-subunit (Table 1) (Hassinen et al., 2011).

1.5.2.2. Inward rectifier K^+ currents

Inward rectifier K^+ currents of the vertebrate heart play a crucial role in maintaining negative RMP (Phase 4) and increasing the rate of AP repolarization (Phase 3) (Hibino et al., 2010). Inward rectified K^+ channels have a simpler structure than the voltage-gated K^+ channels. They consist of two transmembrane domains, resembling S_5 and S_6 of the voltage-gated K^+ channels (Bers, 2001) (Fig. 2D). The cardiac inward rectified K^+ currents include: 1) the background inward rectifier (I_{K1}), 2) the acetylcholine-activated inward rectifier (I_{KAch}), and 3) the ATP-sensitive inward rectifier current (I_{KATP}). I will discuss here only the background I_{K1} and I_{KAch}.

1.5.2.2.1. The background inward rectifier K^+ currents (I_{K1})

I_{K1} maintains negative RMP and accelerates the rate of AP repolarization (Vornanen et al., 2002a; Vornanen, 2016). In contrast to I_{Kr}, the density of I_{K1} is higher in ventricular than in atrial myocytes in all the studied fish species (Galli et al., 2009; Haverinen and Vornanen, 2009). The density of I_{K1} is species-specific, e.g. in the roach and crucian carp ventricle, I_{K1} is higher than in the rainbow trout or burbot ventricle (Vornanen et al., 2002a; Paajanen and Vornanen, 2004; Haverinen and Vornanen, 2009). I_{K1} is generated by the Kir2 subfamily inward rectifier K^+ channels (Ehrlich, 2008). Three major genes are expressed in the mammalian heart, Kir2.1, Kir2.2 and Kir2.3, Kir2.1 being the main isoform (Wang et al., 1998; Schram et al., 2003; Hibino et al., 2010). Six Kir2 gene products are expressed in zebrafish cardiomyocytes (Kir2.1a, Kir2.1b, Kir2.2a, Kir2.2b, Kir 2.3 and Kir2.4) (Hassinen et al., 2015a). Kir2.4 seems to be the main cardiac isoform in most fish species (Hassinen et al., 2007; Hassinen et al., 2015a). Also, Kir2.1, Kir2.2a and Kir2.2b are expressed to some extent in fish hearts (Hassinen et al., 2007; Hassinen et al., 2008b; Hassinen et al., 2014) (Table 1).

1.5.2.2.2. Acetylcholine-activated inward rectifier K^+ current (I_{KAch})

Acetylcholine (Ach) activates atrial I_{KAch} through the muscarinic cholinergic receptors under the parasympathetic tone. Increasing the parasympathetic tone increases I_{KAch}, which in turn strongly reduces f_H and the duration of the atrial AP. Indeed, fish atrium can become completely in excitable in the presence of Ach (Abramochkin and Vornanen, 2017). Inward rectification of I_{KAch} is much weaker than that of I_{K1}, and therefore it has very strong effect on atrial AP duration. I_{KAch} can be also activated via cardiac adenosine receptors (Belardinelli and Isenberg, 1983; Aho and Vornanen, 2002). I_{KAch} has been recorded from atrial myocytes of rainbow trout, crucian carp and navaga cod (*Eleginus navaga*). It seems to be absent

from the ventricular myocytes of fish (Molina et al., 2007; Vornanen et al., 2010; Abramochkin et al., 2014). In some fish species, atrial I_{K1} may be so low, that it cannot maintain negative RMP (Vornanen et al., 2002a; Haverinen and Vornanen, 2009). Thus, I_{KAch} may contribute to the maintenance of RMP in the atrial myocytes of these species (Molina et al., 2007). I_{KAch} is generated by Kir3 subfamily inward rectifier K^+ channels (Ehrlich, 2008). In mammalian hearts, I_{KAch} is produced by Kir3.1 and Kir3.4 channels (Dobrzynski et al., 2001), while there are no studies on the molecular basis of fish cardiac I_{KAch}.

Table 1. Major cardiac ion currents, channels, blockers and functions in the fish heart.

Current	Channel	Blocker	Function
Sodium current (I_{Na})	$Na_v1.4$, $Na_v1.5$, $Na_v1.6$	Tetrodotoxin [a]	Produces fast upstroke and overshoot of AP (Phase 0)
L-type calcium current (I_{CaL})	$Ca_v1.2$, $Ca_v1.3$	Nifedipine [b] Verapamil	Maintains long plateau phase of cardiac AP (Phase 2); regulates EC coupling
T-type calcium current (I_{CaT})	$Ca_v3.1$	Ni^{2+} [c]	Important for AP generation in nodal tissues
Inward rectifier K^+ current (I_{K1})	Kir2.1, Kir2.2 Kir2.4	Ba^{2+} [d]	Maintains negative RMP (Phase 4) and contributes to Phase 3 repolarization
Rapid component of delayed rectifier K^+ current (I_{Kr})	$K_v11.1$ (Erg 1) $K_v11.2$ (Erg 2)	E-4031 [e] Astemizole	Regulates duration and refractoriness of AP (Phases 2 and 3)
Slow component of delayed rectifier K^+ current (I_{Ks})	$K_v7.1$	Chromanol 239B [f]	Controls repolarization of AP (Phases 2 and 3)

a: Vornanen et al., 2011; **b**: McDonald et al., 1994; **c**: Lee et al., 1999; **d**: Haverinen and Vornanen, 2009; **e**: Haverinen and Vornanen, 2009; Zhang, 2006; **f**: Bett et al., 2006

1.6. EFFECTS OF TEMPERATURE ON FISH HEART FUNCTION

Temperature is a powerful environmental factor, which has prominent effects on the growth, nutrition, reproduction, distribution and behavior of fish (Brett, 1971). Temperature determines the rate of biochemical reactions and metabolism, and thereby sets demands on blood circulation and cardiac function. Therefore, it is understandable that temperature has a strong effect on cardiac structure and function. Thermal acclimation alters the amount and quality of the myocardial connective tissue and changes the function of cardiac myofilaments in rainbow trout and zebrafish (Klaiman et al., 2011; Klaiman et al., 2014; Johnson et al., 2014; Keen et al., 2017). Also, temperature affects the EE and EC coupling of fish hearts (Vornanen et al., 2002b; Shiels and Galli, 2014; Vornanen, 2016)

1.6.1. Significance of f_H in thermal responses of the fish heart

The volume of blood circulated through the heart in one minute is called the cardiac output (CO). It is the product of f_H and stroke volume (SV). In exercising fish, changes in cardiac output are achieved by increases in both SV and f_H, while f_H is the main factor in the regulation of CO during acute temperature changes (Brett, 1971; Cech Jr et al., 1976; Randall, 1982; Pörtner, 2001; Vornanen et al., 2002b; Gamperl and Farrell, 2004; Gollock et al., 2006; Steinhausen et al., 2008; Mendonça and Gamperl, 2010; Farrell and Smith, 2017). This is why f_H is considered to be one of the key physiological variables in the environmental adaptation and acclimation of aquatic vertebrates.

The rate and rhythm of the fish heart originate from the sinoatrial pacemaker. An increase in temperature increases the discharge rate of pacemaker APs by an unknown mechanism: f_H varies from a few heartbeats per minute (bpm) at near zero temperatures of the cold-adapted teleosts to a maximum of about 300 bpm in tropical fish species (Rantin et al., 1998; Lillywhite et al., 1999; Gollock et al., 2006; Mendonça and Gamperl, 2010; Lin et al., 2014; Sidhu et al., 2014; Vornanen and Hassinen, 2016).

Chronic changes in water temperature induce compensatory changes in f_H (Vornanen, 2016). In seasonally acclimatized plaice (*Pleuronectes platessa*) and thermally acclimated rainbow trout, physiological adjustment to low temperatures induces increases in f_H by shortening the duration of pacemaker AP, without any changes in the steepness of diastolic depolarization (Harper et al., 1995; Haverinen and Vornanen, 2007). In the yellowfin tuna (*Thunnus albacares*) and Nile tilapia (*Oreochromis niloticus*), f_H is higher in warm-acclimated fish than in cold-acclimated fish (Maricondi-Massari et al., 1998; Blank et al., 2002). By contrast, in the cold-acclimated Pacu (*Piaractus mesopotamicus*), goldfish (*Carassius auratus*) and crucian carp f_H is higher than that of the warm-acclimated species (Matikainen and Vornanen, 1992; Morita and Tsukuda, 1994; Aguiar et al., 2002). These are examples of so-called reverse thermal compensation, and are probably associated with the dormant life style of these species in the cold season. Evidently, temperature-induced changes in f_H are not uniform among teleosts, and may be dependent on the species-specific adaptation "strategy" of the fish to its habitat. Positive thermal compensation improves cardiac output in the cold by opposing the direct depressive effect of temperature on f_H. Inverse thermal compensation may reduce cardiac output in cold conditions, but it also reduces the energy consumption of the heart, and thereby is part of the whole-body metabolic depression (Vornanen et al., 2009).

The physiological significance of f_H in the thermal responses of the fish heart become evident when f_H is depressed or the normal rhythm is lost. Acute temperature changes can cause different types of arrhythmias in fish hearts, which

appear when the temperature approaches or exceeds the upper critical temperature of the fish. Cardiac arrhythmias reported to occur in fish hearts include missed beats, bradycardia, and bursts of rapid beating, as well as complete cessation of heartbeat (asystole) (Casselman et al., 2012; Anttila et al., 2013; Verhille et al., 2013; Ferreira et al., 2014; Vornanen et al., 2014).

1.6.2 Effect of temperature on cardiac AP

Acute temperature changes significantly change the shape of pacemaker, atrial and ventricular APs of fish hearts by altering the flow of inward and outward currents through SL ion channels (Harper et al., 1995; Vornanen et al., 2002a; Haverinen and Vornanen, 2007; Haverinen and Vornanen, 2009; Ballesta et al., 2012; Hassinen et al., 2014; Lin et al., 2014; Vornanen et al., 2014; Shiels et al., 2015). The duration of AP must inversely correlate with f_H to allow sufficient time for systole and diastole; i.e., when f_H is high, AP must become shorter to allow enough time for diastolic filling of the heart with blood (Shiels et al., 2002). Therefore, exercising fish, active fish species, such as tunas and tropical fish (e.g. zebrafish) living at high temperatures have higher f_H and shorter AP in comparison to cold-dormant fish, such as crucian carp or fish that live in cold polar waters (Antarctic fish) (Galli et al., 2009; Haverinen and Vornanen, 2009; Hassinen et al., 2014; Abramochkin and Vornanen, 2015; Vornanen and Hassinen, 2016).

The shape and duration of cardiac AP, and the underlying ion currents, are highly sensitive to chronic temperature changes, and they are crucial in the acclimation or acclimatization of both freshwater and marine teleosts to seasonal temperature regimes. In many fish species, e.g. in rainbow trout, pike and navaga, acclimation to cold induces a compensatory decrease in AP duration, which makes room for the cold-induced increase in f_H (Haverinen and Vornanen, 2009; Abramochkin and Vornanen, 2015).

1.6.3 Effects of temperature on cardiac ion currents and channels

Cardiac ion currents and the shape of cardiac AP are modulated by acute and chronic temperature changes. The diffusion of ion through the pore of the ion channel is weakly temperature-dependent (Q_{10} about 1.3), while the opening and closing of ion channels are more strongly dependent on temperature (Q_{10}>2.0) (Vornanen, 2016). Acute increases in temperature increases the density and kinetics of ion currents up to T_{BP}, where the function of the channels starts to deteriorate. The inward rectifier K^+ channels (Kir2) are "gated" by voltage-dependent blocking and unblocking of the channel pore by intracellular Mg^{2+} and polyamines, and therefore I_{K1} is only weakly dependent on temperature. By contrast, the genuinely voltage-gated K^+, Na^+ and Ca^{2+} ion channels are more strongly temperature-dependent, similarly to the catalytic activity of enzymes. (Shiels et al., 2000; Paajanen and Vornanen, 2004; Shiels et al., 2006; Galli et al., 2009; Haverinen and

Vornanen, 2009; Galli et al., 2011; Shiels and Galli, 2014; Vornanen et al., 2014; Abramochkin and Vornanen, 2015; Kubly and Stecyk, 2015).

Thermal acclimation or acclimatization also affect the density of ion currents and AP shape (Haverinen and Vornanen, 2009). I_{K1}, I_{Kr}, I_{Ca} and I_{Na} are all modified by thermal acclimation (Haverinen and Vornanen, 2004; Hassinen et al., 2007; Hassinen et al., 2008a,b; Galli et al., 2009; Haverinen and Vornanen, 2009; Abramochkin and Vornanen, 2015). The most consistent response to thermal acclimation is noted for I_{Kr}. In most fish, I_{Kr} is upregulated by cold-acclimation, i.e. the density of I_{Kr} is higher in cold-acclimated fish than in warm-acclimated fish (Galli et al., 2009; Haverinen and Vornanen, 2009; Hassinen et al., 2014; Abramochkin and Vornanen, 2015). Moreover, there is a close correlation between the density of I_{Kr} and f_H in cold- and warm-acclimated fish (Vornanen, 2016), indicating the importance of this current in adjusting AP duration to f_H.

Acclimation response of I_{K1} is species- and chamber-specific. In some fish it is increased by cold-acclimation as in crucian carp and roach, while in others it is depressed by cold-acclimation, as in rainbow trout, and in others such as pike, burbot, perch and bluefin tuna there is no response to chronic temperature changes (Vornanen et al., 2002a; Galli, et al., 2009; Haverinen and Vornanen, 2009). Also, there are sometimes differences in thermal response between atrial and ventricular I_{K1} in the same fish. For example, the density of I_{K1} is higher in the atrial myocytes of warm-acclimated burbot and bluefin tuna than in the cold-acclimated species, while the opposite is true for ventricular myocytes (Galli et al., 2009; Haverinen and Vornanen, 2009). Since I_{K1} regulates RMP, increases or decreases in the density of I_{K1} will reduce or enhance excitability, respectively.

The response of I_{Na} to thermal acclimation is also species-specific. I_{Na} is up-regulated in the cold-active rainbow trout and down-regulated in the cold-dormant crucian carp. Increases or decreases in I_{Na} density enhance or depress cardiac excitability, respectively. Thus, the species-specific responses of I_{Na} can be adaptive, considering the different life styles of the two species (Haverinen and Vornanen, 2004).

In contrast to other voltage-dependent ion currents, I_{CaL} seem to be more resistant to chronic temperature changes (Vornanen et al., 2014). The absence of acclimatory response in I_{CaL} density to temperature may be related to Ca^{2+} influx through Ca^{2+} channels, which is relatively independent of temperature (Kim et al., 2000; Shiels et al., 2000; Shiels et al., 2006). However, seasonal acclimatization changes the density of I_{Ca} in the ventricular myocytes of crucian carp; I_{CaL} was higher in summer than in winter fish (Vornanen and Paajanen, 2004).

Temperature-induced changes (or constancy) of ion current densities are often associated with increased expression of the respective ion channel gene transcripts. This is consistent with the finding that the density of cardiac ion currents is mainly regulated at the transcriptional level (Rosati and McKinnon, 2004). This conclusion applies to fish cardiac Erg (I_{Kr}), Kir2 (I_{K1}), Na_v1 (I_{Na}) and $K_v7.1$ (I_{Ks}) channels.

(Hassinen et al., 2007; Haverinen and Vornanen, 2007; Hassinen et al., 2008a,b; Hassinen et al., 2014; Vornanen and Hassinen, 2016; Tikkanen et al., 2017).

1.7 ROLE OF EXTRACELLULAR K^+ IN ELECTRICAL EXCITATION

The concentration of K^+ ions in the blood plasma of roach caught in the wild in different seasons of the year varies between 0.2 and 6.9 mmol L^{-1} (Martem'yanov, 2001). The lowest extracellular K^+ concentrations ($[K^+]_o$) were measured in the breeding time in spring. Exercise and handling stress in fish can cause a marked rise of $[K^+]_o$ up to 20 mmol L^{-1} (Wells et al., 1986). Furthermore, it has been found that the rise of $[K^+]_o$ is dependent on temperature and the thermal history of the fish (Jain and Farrell, 2003; Gale et al., 2013; Danylchuk et al., 2014). Since K^+ currents are important for maintaining RMP and repolarization of AP in excitable tissues, changes in $[K^+]_o$ are probably physiologically important in the regulation of cardiac and neural functions. Indeed, early electrophysiological findings demonstrated that $[K^+]_o$ has a strong effect on the EE of single Ranvier nodes of the frog (Belyaev, 1964). Later on, $[K^+]_o$ has been shown also to affect EE of the mammalian heart (Kolb, 1990; Lindinger, 1995). However, relatively little is known about the effects of $[K^+]_o$ on cardiac function in fish (Hove-Madsen and Gesser, 1989; Nielsen and Gesser, 2001).

1.8. HYPOTHESES ABOUT THERMAL TOLERANCE OF FISH

It has already been known for some time that the survival of fish at different temperatures is dependent on their ability to increase the aerobic metabolic rate over the standard level (Fry, 1947; Brett, 1971). This is necessary in order to maintain life-supporting activities, such as locomotion, growth, foraging and reproduction (Fry, 1947). Aerobic metabolic scope is dependent on the function of the respiratory and cardiovascular systems, which deliver oxygen to active tissue and thereby maintain the aerobic performance of the fish. Based on these early findings, Hans-Otto Pörtner formulated the hypothesis of oxygen- and capacity-limited thermal tolerance (OCLTT) (Pörtner, 2001). The central idea of the OCLTT hypothesis is that physiological performance and thermal tolerance of ectotherms are determined by the oxygen transport capacity of the respiratory and cardiovascular systems (Pörtner, 2010). Indeed, several findings suggest that deterioration of heart function could set the upper thermal tolerance limit of fish (Lannig et al., 2004; Gollock et al., 2006; Eliason et al., 2011). In particular, f_H is known to collapse at high temperatures, providing a putative organ-level explanation for the OCLTT concept (Gollock et al., 2006; Sandblom and Axelsson, 2007; Clark et al., 2008; Steinhausen et al., 2008). The OCLTT hypothesis has been for several years the major, and basically, the only hypothesis regarding the thermal tolerance of ectotherms. However, more recent findings suggest that the

OCLTT hypothesis might be valid for only about half of the studied aquatic species (Nilsson et al., 2009; Lefevre, 2016). Indeed, the latest versions of the OCLTT hypothesis are severely criticized, e.g. for their ambiguity and confusing use of terms (Jutfelt et al., 2018; Pörtner et al., 2018). Furthermore, little is known about the cellular and molecular mechanisms, which may be involved in the temperature resistance of fish cardiac function. Some studies suggest that the failure of aerobic ATP production in the mitochondria of cardiac myocytes might explain the deterioration of cardiac function at high temperatures (Iftikar and Hickey, 2013; Iftikar et al., 2014). It has also been suggested that cardiac function is restricted by limitations in the oxygen supply to the heart itself (Lannig et al., 2004; Clark et al., 2008; Farrell, 2009). Recently, a hypothesis based on EE of cardiac myocytes has been suggested to explain thermal deterioration of the heart rate in fish (Vornanen, 2016).

1.9. OBJECTIVES OF THE STUDY

Temperature directly affects the body functions of fish and other ectotherms. The prevailing hypothesis on high temperature tolerance of aquatic ectotherms suggests that the ability of circulatory and respiratory systems to deliver oxygen to the body tissues is crucial in setting thermal limits for animal life (Pörtner, 2001). According to the OCLTT hypothesis, aerobic performance level (aerobic scope) is reduced at extremes of high temperature due to impaired function of the circulatory and respiratory systems, in particular the function of the heart (Pörtner, 2001; Pörtner and Farrell, 2008). Recently, Vornanen presented a more mechanistically orientated hypothesis, which tries to explain thermal tolerance of cardiac function, starting from the EE of cardiac myocytes as found in the studies with 12°C-acclimated brown trout (*Salmo trutta fario*) (Vornanen et al., 2014; Vornanen, 2016). The hypothesis on "temperature dependence of electrical excitability" (TDEE) is based on the antagonism of Na^+ and K^+ ion currents across the cell membrane, which at high temperatures become unbalanced and therefore prevent impulse generation or conduction in the heart (Vornanen, 2016). Interestingly, the TDEE hypothesis might not only explain the upper thermal tolerance of heart function, but it could be applicable to all electrically excitable cells, including nerve, muscle and gland cells. My thesis examines the physiological plasticity of electrical excitation of the roach heart in order to better understand the role of the heart in the thermal tolerance of fish.

The main objectives of my thesis were to test the predictions of the TDEE hypothesis by using the seasonally-acclimatized (winter and summer) roach as an experimental animal. To this end, I examined how the function of the roach heart adjusts itself to large seasonal temperature changes that the fish face in the wild. Roach are eurythermal fish with a wide thermal tolerance range and are suitable for

testing the temperature tolerance of excitable cells. The thesis aims to test the following main research hypotheses (H).

H1: Seasonal acclimatization will shift high and low temperature tolerance limits of electrical excitation in order to accommodate cardiac function to seasonal temperature conditions.

H2: The upper thermal tolerance limit of f_H is higher in summer- than in winter-acclimatized roach.

H3: High temperature induces cardiac arrhythmias in roach.

H4: Consistent with the TDEE hypothesis, high temperature tolerance of I_{Na} is lower than that of I_{K1}, with a resultant decrease in EE and depression of f_H at high temperatures.

H5: High extracellular K^+ concentration depolarizes the membrane potential of cardiac myocytes and depresses EE.

2 MATERIAL AND METHODS

Experiments were conducted on seasonally acclimatized roach, which were maintained in the animal facilities of the University of Eastern Finland at appropriate seasonal temperatures. The activity of the roach heart and its responses to acute changes of temperature were measured at different levels of biological organization, starting from *in vivo* recordings of heart function in living fish (**Paper I**) down to organ, cell and molecule level in *in vitro* experiments (**Papers II, III and IV**). Heart function in living fish was determined from electrocardiograms (ECG), while spontaneously beating whole hearts were used to measure intracellular APs with microelectrodes (**Paper I**). Single enzymatically isolated cardiac myocytes were used to measure K^+, Ca^{2+} and Na^+ ion currents through specific ion channels (**Papers II and III**). APs were measured from single ventricular myocytes (**Papers II and IV**).

The electrophysiological studies were supplemented by molecular studies to reveal the genetic and molecular basis of electrical excitability. The molecular methods involved gene cloning and sequencing, as well as quantitative PCR to measure expression of different ion channel genes at the transcript level (**Papers II and III**).

Collectively, experiments at 4 different levels of biological organization (organismal, organ, cell and molecule) provide complementary data that form a powerful combination in revealing the effects of seasonal acclimatization and acute temperature changes on EE and EC coupling of the roach heart. In the following pages, the main material and methods will be briefly outlined.

2.1 ANIMALS

Seasonally acclimatized roach, a teleost fish species of the family Cyprinidae, were captured in winter and summer from Lake Pyhäselkä in Central Finland (62°35'N, 21°34'E). The winter-acclimatized roach were caught in February-March (water temperature 0−4°C) and the summer-acclimatized roach were caught in June–September (water temperature 15−19°C). Both acclimatization groups were maintained in 500 L metal aquaria in the animal facilities of the University of Eastern Finland until used in the experiments. Water temperature was regulated at 4±1°C and 18±1°C for winter- and summer-acclimatized roach, respectively (Computec Technologies, Joensuu). Oxygen saturation was maintained by aeration with compressed air, and ground water was constantly flowing through the aquaria at the rate of about 200 L/day with a 12−12 h light-dark photoperiod. The fish were fed commercial trout fodder (EWOS, Turku, Finland) 3−5 times a week. All the

experiments were authorized by the national animal experimental board in Finland (permission ESAVI/2832/04.10.07/2015).

2.2 RECORDING OF ELECTROCARDIOGRAM (PAPER I)

Electrocardiogram (ECG) recording is a simple and useful way to examine the cardiac function of fish *in vivo* (Tort et al., 1987; Endo et al., 1988; Campbell et al., 2006). During the *in vivo* recordings of ECG, seasonally acclimatized fish were exposed to acute temperature changes in order to provide variables of electrical excitability of the heart (**Paper I**). The ECG recordings were made essentially as previously described (Campbell et al., 2004; Vornanen et al., 2014). Thin trailing wires were inserted from the ventral side of the fish close to the heart and connected to appropriate amplifiers for continuous data acquisition on computer. Fish preparation and the insertion of wires are described in detail in **Paper I** (Fig. 3). The fish were allowed to fully recover from the operation for 1–2 days. Recovery was considered complete, when a clear and steady f_H variability appeared in ECG. The recordings were considered to be of sufficiently good quality when P, QRS and T waves could be clearly recognized (Fig. 4). Temperature challenges, heating or cooling ramps, were applied at a rate of 3°C h^{-1}. Fish with noisy ECGs were omitted from the analyses.

Effects of temperature on *in vivo* ECG variables were analyzed off-line using the LabChart 7.1 software (ADInstruments, Colorado Springs, CO, USA). f_H, standard deviation of successive interbeat intervals (SDNN), PQ interval, amplitude and duration of QRS complex, QT interval and the arrhythmia temperature (T_{ARR}) were determined. Also, T_{BP}, the temperature after which steady increase/decrease of the parameters turned into continuous decrease/increase, for f_H, QRS duration, PQ interval and QT interval was measured.

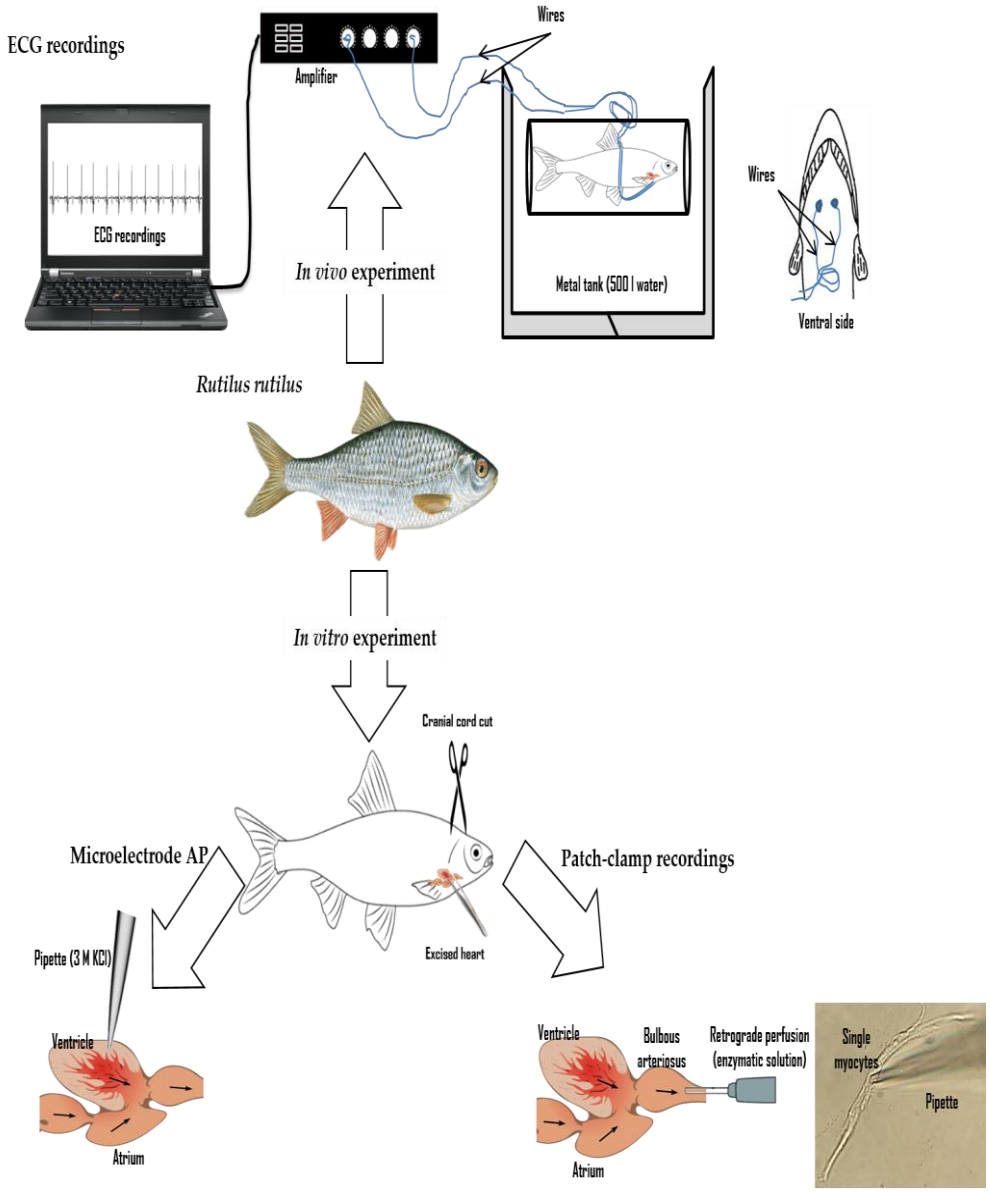

Figure 3. A Diagram for *in vivo* (ECG recordings) and *in vitro* (microelectrode and whole-cell patch-clamp) experiments.

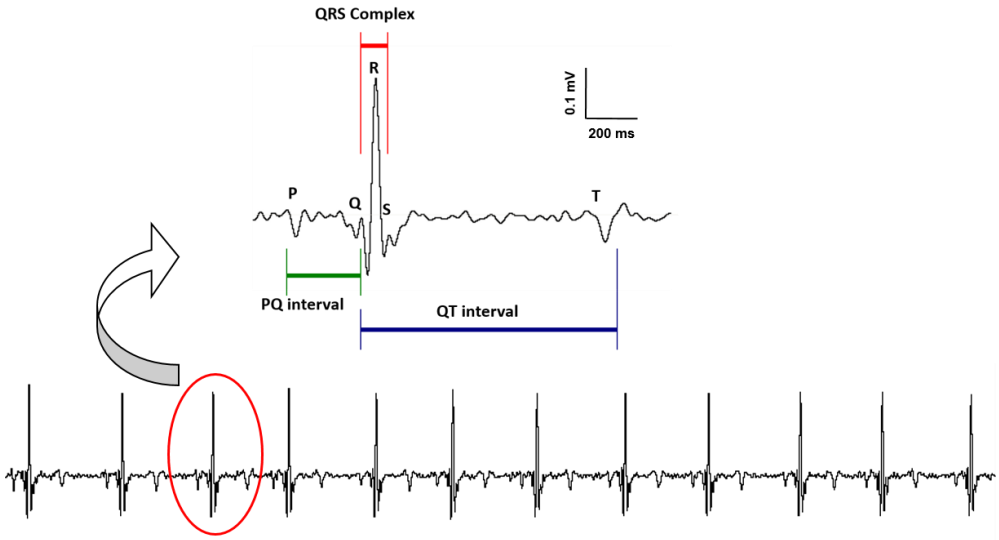

Figure 4. A representative *in vivo* electrocardiogram tracing of the roach. The recording shows different waves (P, QRS and T) and wave intervals. P, atrial depolarization; QRS, ventricular depolarization; T, ventricular repolarization; PQ interval, impulse transmission from atrium to ventricle; QT interval, average duration of ventricular action potential.

2.3 MICROELECTRODE RECORDINGS OF APS (PAPER I)

Atrial and ventricular APs were recorded from spontaneously beating whole hearts of roach *in vitro* by using sharp microelectrodes (Fig. 3) (Haverinen and Vornanen, 2009). The excised whole heart was gently fixed with small pins to the bottom of the 10 ml recording chamber filled with continuously oxygenated (100% O_2) physiological saline solution. Electrode resistance was 10–20 MΩ when filled with 3 mol l^{-1} KCl. The temperature of the saline solution was adjusted to the test temperatures (4, 13 and 21°C for winter roach and 13, 21 and 26°C for summer roach). Temperature and APs were continuously recorded via PowerLab 8/30 (ADInstruments) on a computer for off-line analysis with LabChart 7.1 software (ADInstruments). RMP, AP overshoot, AP amplitude, AP durations at 0, 10, 20, 50 and 90% of repolarization levels (APD_0, APD_{10}, APD_{20}, APD_{50} and APD_{90}, respectively) and $+V_{max}$ (maximum upstroke velocity of AP) were determined.

2.4 PATCH-CLAMP RECORDINGS (PAPERS II, III AND IV)

2.4.1 Myocytes isolation

Atrial and ventricular cardiac myocytes were enzymatically isolated using the previously described methods (Vornanen, 1997). The heart was cannulated via *bulbous arteriosus* for retrograde perfusion with oxygenated saline solutions. The perfusion consisted of two different treatments. First, the heart was perfused for 7

min with a Ca^{2+}-free low Na^{+} solution to wash the blood out and to lower the concentration of Ca^{2+} ions in the extracellular space of the heart. Second, the intercellular connective tissue was digested using proteolytic enzymes: the Ca^{2+}-free low Na^{+} solution was supplemented with collagenase (Type IA; 0.75 mg ml^{-1}), trypsin (Type IX; 0.5 mg ml^{-1}) and fatty acid-free bovine serum albumin (0.75 mg ml^{-1}). The enzyme perfusion was continued for 15 min. Both solutions were oxygenated with 100% O_2. After enzymatic digestion, softened atrial and ventricular muscles were separately placed in a small volume of low Na^{+} solution, chopped into small pieces with scissors, and single myocytes were released by gentle agitation of muscle pieces through the opening of a Pasteur pipette. Isolated myocytes were stored at 5°C in a Ca^{2+}-free low Na^{+} solution and used within 8 h of isolation.

2.4.2 Whole-cell patch-clamp

Ventricular APs, and atrial and ventricular ion currents were measured from enzymatically isolated cardiac myocytes. Isolated myocytes were allowed to adhere to the bottom of a 150 µl chamber that was mounted on the stage of an inverted microscope (Fig. 3). The myocytes were continuously superfused with the external solution at the rate of 1.5–2 ml min^{-1} and internally perfused with the pipette solution (Table 2). After gaining a giga ohm seal, the membrane was ruptured by a short voltage pulse (zap) to gain access to the myocyte, transients resulting from series resistance and pipette capacitance were cancelled out using the compensation circuits of the amplifier, allowing determination of the capacitive cell size. Ion current densities are expressed as pA pF^{-1}. The recordings were performed either in current-clamp or voltage-clamp modes, depending on whether APs or ion currents were measured, respectively.

2.4.2.1. Current-clamp protocols

Ventricular APs (current-clamp mode of patch-clamp) were recorded to study the temperature-dependence of AP characteristics (**Paper II**) and the electrical excitability of ventricular myocytes at different temperatures and external K^{+} ion concentrations ($[K^{+}]_o$) (**Paper IV**). APs were triggered with short (4 ms) square wave pulses of constant strength (800 pA) (**Paper II**) at a frequency of 0.25 Hz, or with stepwisely increasing pulse strength until the excitation threshold was found (**Paper IV**). The initial stimulus strength was 300 pA and it was raised by 20 pA increments until an all-or-none AP was elicited (**Paper IV**). In the latter study, either constant pacing frequency (0.25 Hz) or temperature-specific frequencies of 0.37, 1.16 and 1.96 Hz were used at 4, 14 and 24°C, respectively (**Paper IV**). These frequencies correspond to the physiological heart rates of winter-acclimatized roach at the respective temperatures (**Paper I**).

2.4.2.2. Voltage-clamp protocols

Inward Na^+ and Ca^{2+} currents and outward K^+ currents were measured from atrial and ventricular myocytes (Fig. 5A). I_{K1} was elicited from the HP of -80 mV for every 10 s by 2 s repolarizing voltage ramps from +60 to -120 mV, and then 1 mmol l^{-1} Ba^{2+} was applied externally to block the current rapidly. Subtraction of the current in the presence of Ba^{2+} from the recording before Ba^{2+} application gives I_{K1} with inward and outward current components. I_{Kr} was determined using a two-step protocol. The channels were first activated to different degrees by using a series of depolarizing prepulses (4 s in duration; HP of -80 mV) to voltages between -80 and +80 mV (20 mV increments), and the voltage was then stepped up to -40 mV for 4 s to remove inactivation. I_{Kr} tail current, at the constant electrochemical driving force, was determined at -40 mV. I_{Ca} was elicited by using 300 ms depolarizing pulses with 10 mV increments for every 10 s in the voltage range of -80 to +60 and -50 to +60 mV. HPs of -80 and -50 mV were used to check whether both I_{CaT} and I_{CaL} were present, respectively. I_{Ca} was measured as the difference between the peak inward current and the current at the end of the 300 ms pulse. I_{Na} was elicited from the HP of -120 mV for every 2 s, and recorded in the voltage range of -100 to +70 mV (60 ms) with 10 mV increments (Haverinen and Vornanen, 2004). Approximately 80% of the series resistance was compensated in order to achieve good control of membrane voltage during activation of fast and large I_{Na}.

2.4.2.3. Thermal tolerance of APs and ion currents

Myocytes were exposed to "heat ramps" to examine the thermal tolerance of ventricular APs and sarcolemmal ion currents (Fig. 5B). Thermal tolerance of I_{K1} and I_{Ca} were measured from ventricular myocytes, while I_{Kr} and I_{Na} were measured from atrial myocytes. This selection of cells was based on the size of each current. The experiment was started at 4°C for winter roach and at 13°C for summer roach. The temperature was then gradually increased until APs could no longer be elicited or when the ion currents showed progressive deterioration. The last temperature recording preceding the initial and sustained deterioration of the measured variable was considered as T_{BP}. APs were triggered by using short (4 ms) square pulses at a frequency of 0.25 Hz. When a stable AP waveform was established, heat ramp was initiated. Thermal tolerances of the following AP variables were analyzed off-line: RMP, AP amplitude, APD_{50} and the maximum rate of AP repolarization ($-V_{max}$). I_{K1} was elicited from the holding potential of -80 mV for every 8 s by 2 s repolarizing voltage ramp (+60 − -120 mV), and temperature sensitivity was measured from the peak outward I_{K1} in ventricular myocytes. I_{Kr} was measured from the peak tail current at -40 mV after activating of the channels at +40 mV (4 s) for every 12 s in atrial myocytes. I_{Ca} was elicited from the HP of -80 to 0 mV at the interval of 8 s, and I_{CaL} was determined as the difference between the peak inward current and the current at the end of a 300 ms depolarizing pulse from ventricular myocytes. I_{Na}

was triggered in atrial myocytes by a short (20 ms) depolarizing voltage pulse from the HP of -120 to -50 mV for every 8 s. In heat ramps, temperature was raised at the rate of 2−4°C min^{-1}. This rate was slow enough to achieve thermal equilibrium in the cell (Pahapill and Schlichter, 1990; Chowdhury et al., 2014) and allowed determination of current and voltage responses in the same cell for the whole temperature range spanning 24−40°C (duration of the experiment 15−25 min).

Table 2. External and internal solutions used in the patch-clamp (current- and voltage-clamp modes) for measuring Ventricular AP and ion currents from enzymatically isolated cardiomyocytes, respectively.

Recordings	External (bath) solution (mmol l^{-1})	Internal (pipette) solution (mmol l^{-1})
AP	150 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 1.8 $CaCl_2$, 10 glucose, 10 HEPES; pH 7.6 (NaOH)	140 KCl, 4 Na_2ATP, 1 $MgCl_2$, 0.03Tris-GTP, 10 HEPES; pH 7.2 (KOH)
I_{K1}	150 NaCl, 5.4 KCl, 1.2 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 10 glucose; pH 7.6 (NaOH) Blockers (μM): 0.5 TTX (I_{Na}), 10 nifedipine (I_{CaL}), 4 E-4031 (I_{Kr})	140 KCl, 4 MgATP, 1 $MgCl_2$, 5 EGTA, 10 HEPES; pH 7.2 (KOH)
I_{Kr}	150 NaCl, 5.4 KCl, 1.2 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 10 glucose; pH 7.6 (NaOH) Blockers (μM): 0.5 TTX (I_{Na}), nifedipine (I_{CaL}), 200 Ba^{2+} (I_{K1})	
I_{Ca}	150 NaCl, 5.4 CsCl, 1.8 CaCl2, 1.2 $MgCl_2$, 10 HEPES, 10 glucose; pH 7.6 (CsOH) Blockers (μM): 0.5 TTX (I_{Na}), 2 astemizole (I_{Kr})	130 CsCl, 15 TEACl, 5 MgATP, 1 $MgCl_2$, 5 oxaloacetate, 10 HEPES, 5 EGTA; pH 7.2 (CsOH)
I_{Na}	20 NaCl, 120 CsCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose, 10 HEPES; pH 7.7 (CsOH) Blockers (μM): 10 nifedipine (I_{CaL})	5 NaCl, 130 CsCl, 1 $MgCl_2$, 5 EGTA, 5Mg_2ATP, 5 HEPES; pH 7.2 (CsOH)

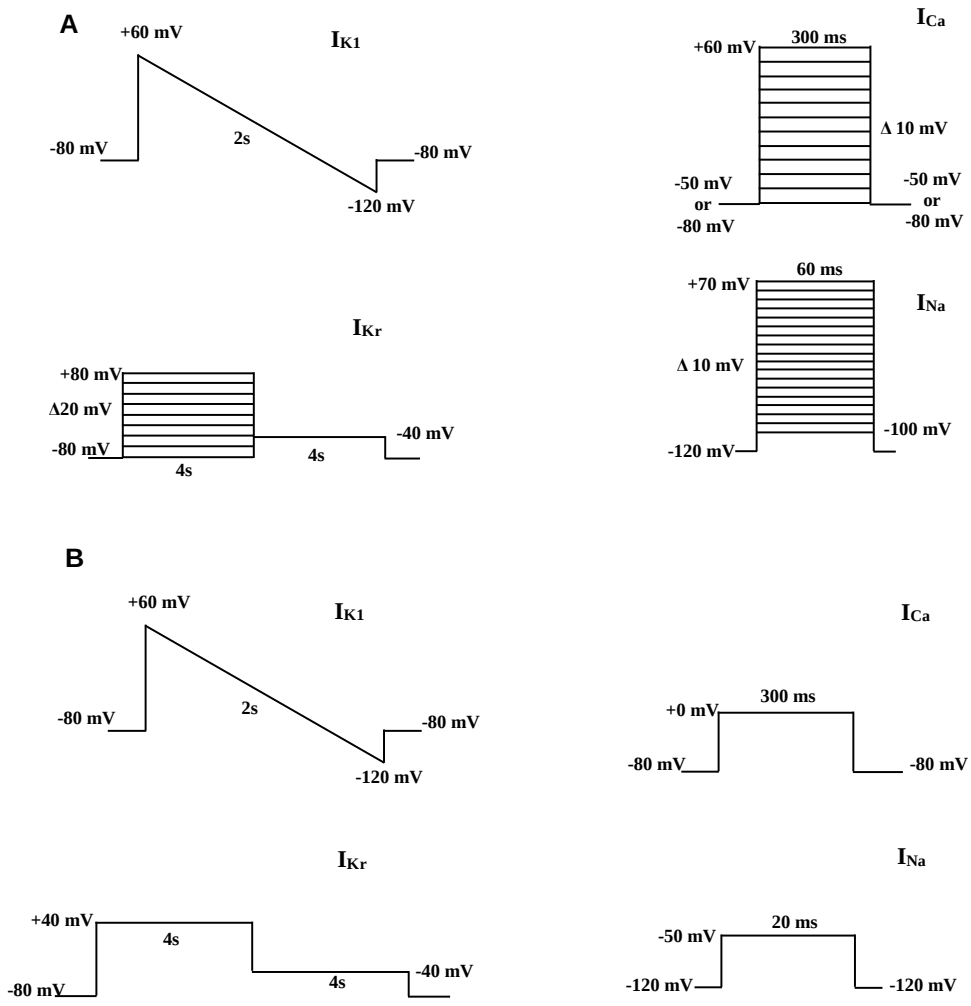

Figure 5. The voltage protocols for ion current recordings (A) and testing thermal tolerance of ion currents (B).

3 RESULTS

Acute temperature changes and temperature (seasonal) acclimatization had striking effects on the cardiac excitability at the 4 different levels of biological organization, from the intact animal down to the molecular level. Heat tolerance of cardiac excitability in enzymatically isolated cardiac myocytes *in vitro* was better than in the intact fish *in vivo*. Generally, the data from electrophysiological experiments were consistent with molecular studies and revealed the link between genes and the cardiac function which could set the upper thermal tolerance of EE in the roach heart. The main results are briefly described in the following pages.

3.1 THERMAL TOLERANCE OF CARDIAC EXCITABILITY IN THE INTACT ANIMAL (PAPER I)

Thermal tolerance of EE in the seasonally acclimatized roach heart was examined at the level of intact animal through ECG recordings. At a lower range of temperature (below 20°C), f_H was higher in winter- than in summer-acclimatized roach, while above 22°C the opposite was true. The T_{BP} of f_H was lower (19.8°C) in the winter-acclimatized roach than in the summer-acclimatized roach (28.1°C). As expected, QRS duration and PQ and QT intervals became shorter with increasing temperature. The T_{BP}s of f_H, QRS duration and PQ interval were 8.3°C, 6.9°C and 10.0°C, respectively, lower in winter- than in summer-acclimatized roach (Table 3). These findings are consistent with the hypotheses H1 and H2.

T_{ARR} was as much as 12.4°C lower in winter- than in summer-acclimatized roach. Arrhythmias became more prevalent with increasing temperature in both acclimatization groups (Fig. 6). Missing QRS complexes were the first sign of cardiac arrhythmia under heat stress. 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. Ventricular bradycardia was the most consistent type of cardiac arrhythmia, which quickly developed into complete cessation of the heartbeat (asystole) in both acclimatization groups. These findings are consistent with the hypothesis H3.

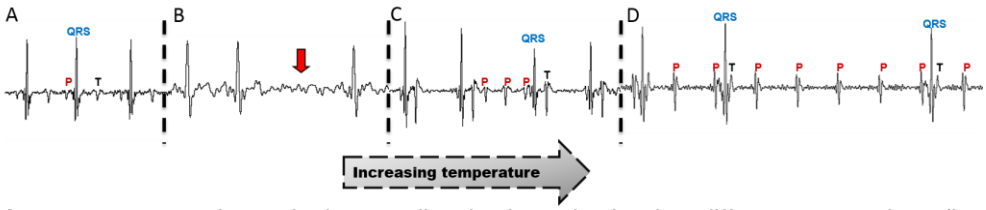

Figure 6. ECG tracings of winter-acclimatized roach showing different types of cardiac arrhythmias under increasing temperature. (A) Normal heartbeat of winter roach at 4°C. (B) A missing QRS complex (arrow) in the middle of an otherwise normal heartbeat as an indication for T_{ARR}. (C) A short episode of atrial tachycardia and more variability in QRS amplitudes and RR intervals. (D) Missing QRS complexes (atrioventricular block) became more prevalent close to or above the T_{BP} of f_H.

3.2 EFFECTS OF ACUTE TEMPERATURE CHANGES ON CARDIAC AP (PAPER I)

APs were recorded from whole heart preparations *in vitro* by the microelectrode technique. As in other vertebrates, AP duration was longer in the ventricle than in the atrium due to the more pronounced plateau phase around 0 mV. The maximum upstroke velocity of AP ($+V_{max}$) was faster in the atrium than in the ventricle in both acclimatization groups. Higher temperatures caused considerable declines in APD and increases in $+V_{max}$ in both cardiac chambers and in both acclimatization groups. The most striking difference in AP shape between winter and summer roach was apparent in the early repolarization of the atrial AP, which was prominent in summer roach but practically absent in winter roach. Therefore, APD_{10} and APD_{20} were significantly shorter in summer than in winter roach atria.

3.3 THERMAL TOLERANCE OF VENTRICULAR AP (PAPER II)

APs were measured from enzymatically isolated ventricular myocytes (current-clamp mode of patch-clamp) under heat ramps. Acute increases in temperature made RMP more negative, AP amplitude larger, APD_{50} shorter, and the maximum rates of AP repolarization faster ($-V_{max}$). Each variable reached its characteristic T_{BP}, above which it − with the exception of APD_{50} − started to deteriorate (Fig. 7). The AP characteristics of winter roach were slightly less tolerant to high temperatures than the corresponding variables of summer roach APs. The T_{BP} of AP characteristics were 2.2–5.0°C lower in winter than summer roach (Table 3).

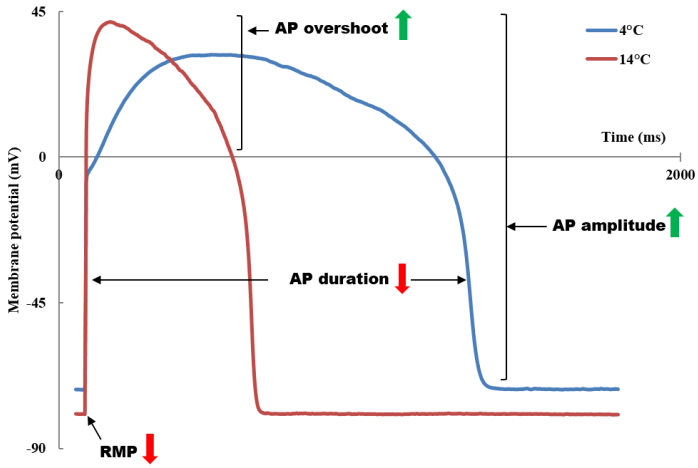

Figure 7. Representative ventricular APs from winter-acclimatized roach at the acclimatization temperature (4°C; blue line) and at 14°C (red line)**.** High temperature makes RMP more negative, AP amplitude larger, APD_{50} shorter, and $-V_{max}$ faster (indicated by arrows).

3.4 EFFECTS OF SEASONAL ACCLIMATIZATION AND ACUTE TEMPERATURE CHANGES ON SARCOLEMMAL K^+ CURRENTS (PAPER II)

3.4.1 Densities of I_{K1} and I_{Kr}

Fish cardiomyocytes have two major K^+ currents, I_{K1} and I_{Kr} (Vornanen et al., 2002a; Hassinen et al., 2007). Maximum densities of inward and outward I_{K1} and the peak outward tail current of I_{Kr} were measured at three different temperatures in atrial and ventricular myocytes. The density of I_{K1} was higher in ventricular than in atrial myocytes in both acclimatization groups. Acute increases in temperature enhanced I_{K1} and I_{Kr} in both cell types from both acclimatization groups. No significant differences between winter and summer roach appeared in the density of inward or outward I_{K1} in atrial myocytes. By contrast, the inward and outward I_{K1} of ventricular myocytes were significantly higher in summer than in winter roach at all experimental temperatures with the exception of outward I_{K1} at 21°C. I_{Kr} density was clearly higher in both cell types in winter than in summer roach. The thermal sensitivity (Q_{10} value) of I_{Kr} was higher than that of I_{K1} in both cell types from both acclimatization groups.

3.4.2 K^+ channel (Kir2 and Erg) expression

The genes encoding Kir2 (I_{K1}) and Erg (I_{Kr}) channels were partially cloned from roach. The transcript abundances of Kir2 and Erg genes were separately measured

from atrial and ventricular tissues. In atrium, Kir2.2a was the main Kir2 isoform in both acclimatization groups. By contrast, Kir2.4 was the main isoform in the ventricle of both winter and summer roach. No seasonal differences appeared between both groups in either atrial or ventricular Kir2 transcript abundances. Erg2 was the main Erg isoform in both cardiac chambers in both acclimatization groups. Erg2 expression was considerably higher in winter than in summer roach.

3.5 EFFECT OF SEASONAL ACCLIMATIZATION AND ACUTE TEMPERATURE CHANGES ON INWARD CURRENTS (PAPER III)

3.5.1 Densities of I_{Ca} and I_{Na}

The main sarcolemmal inward currents, I_{Ca} and I_{Na}, were measured in atrial and ventricular myocytes at 13°C. I_{Ca} was elicited from two HPs of -80 and -50 mV. The voltage dependence of atrial or ventricular I_{Ca} was similar in winter and in summer roach and was independent of HP, indicating a complete absence of I_{CaT} in roach atrial and ventricular myocytes. However, the density of I_{CaL} activated from -80 mV was significantly larger than if activated from -50 mV. The density of I_{CaL} was significantly higher in ventricular myocytes than in atrial myocytes for both acclimatization groups.

I_{Na} was also determined at the common experimental temperature of 13°C. Atrial I_{Na} was higher than ventricular I_{Na} in both acclimatization groups. In atrial and ventricular myocytes, I_{Na} density was higher in winter- than in summer-acclimatized roach by 38% and 48%, respectively.

3.5.2 Na^+ channel (*scn*) expression

Partial sequences of 3 Na^+ channel genes (*scn4ab*, *scn5a* and *scn8ab*) were cloned from the roach heart. Transcripts for the 3 Na^+ channel genes were found in both chambers of the roach heart. In the atrium, *scn4ab* and *scn5a* were almost equally expressed, while in the ventricle, *scn5a* was more strongly expressed than *scn4ab*. Expression of *scn8ab* was less than 3% of all Na^+ channel transcripts in both atrium and ventricle. Consistent with the I_{Na} density data, transcripts of all three Na^+ channel genes were upregulated in winter, with the exception of atrial *scn8ab*. However, the increase was more prominent for *scn4ab* than for *scn5a* or *scn8ab*. In both chambers of the roach heart, the total amount of the 3 gene products was higher in winter than in summer roach.

3.6 THERMAL TOLERANCE OF SARCOLEMMAL ION CURRENTS (PAPERS II AND III)

The acute heat resistances of I_{K1}, I_{Kr}, I_{Ca} and I_{Na} were determined by using heat ramps. The densities of all the ion currents initially increased with increasing temperature, reaching a characteristic T_{BP}, and then declined. I_{K1} was the most resilient current to high temperatures and I_{Na} the most heat-sensitive current in both acclimatization groups (Table 3; Fig. 8). I_{Na} was more heat sensitive in winter roach than in summer roach, while the opposite was true for I_{Kr}.

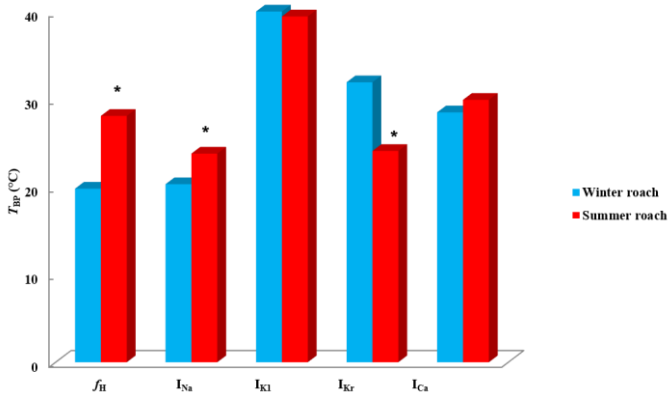

Figure 8. Break-point temperatures (T_{BP}) of heart rate (f_H) and sarcolemmal ion currents in winter- and summer-acclimatized roach. Notice the close correlation between thermal tolerance of f_H and I_{Na}, and the mismatch between the thermal tolerance of I_{Na} and I_{K1}. An asterisk (*) indicates a statistically significant differences between acclimatization groups ($p<0.05$).

Table 3. Break-point temperatures (T_{BP}) for different variables of *in vivo* and *in vitro* recordings of winter- and summer-acclimatized roach.

Variable	T_{BP} (°C) winter roach	T_{BP} (°C) summer roach	p value
f_H	19.8±0.5	28.1±0.5	<0.05
QRS duration	21.2±0.7	28.1±0.9	<0.05
PQ interval	20.8±0.5	30.8±0.5	<0.05
QT interval	--	29.1±0.7	--
T_{ARR}	14.0±0.5	26.4±1.4	<0.05
RMP (ventricle)	37.6 ± 0.4	41.0 ±1.0	<0.05
AP amplitude (ventricle)	28.0 ± 1.1	30.2 ±1.0	NS
Max. rate of AP repolarization (ventricle)	35.3 ± 0.8	40.3 ±0.6	<0.05
I_{K1} (ventricle)	40.0 ± 0.4	39.4 ±0.4	NS
I_{Kr} (atrium)	31.9 ± 0.8	24.1 ±0.5	<0.05
I_{Ca} (ventricle)	28.5 ± 0.9	29.9 ± 1.1	NS
I_{Na} (atrium)	20.3 ± 1.2	23.8 ± 0.7	<0.05

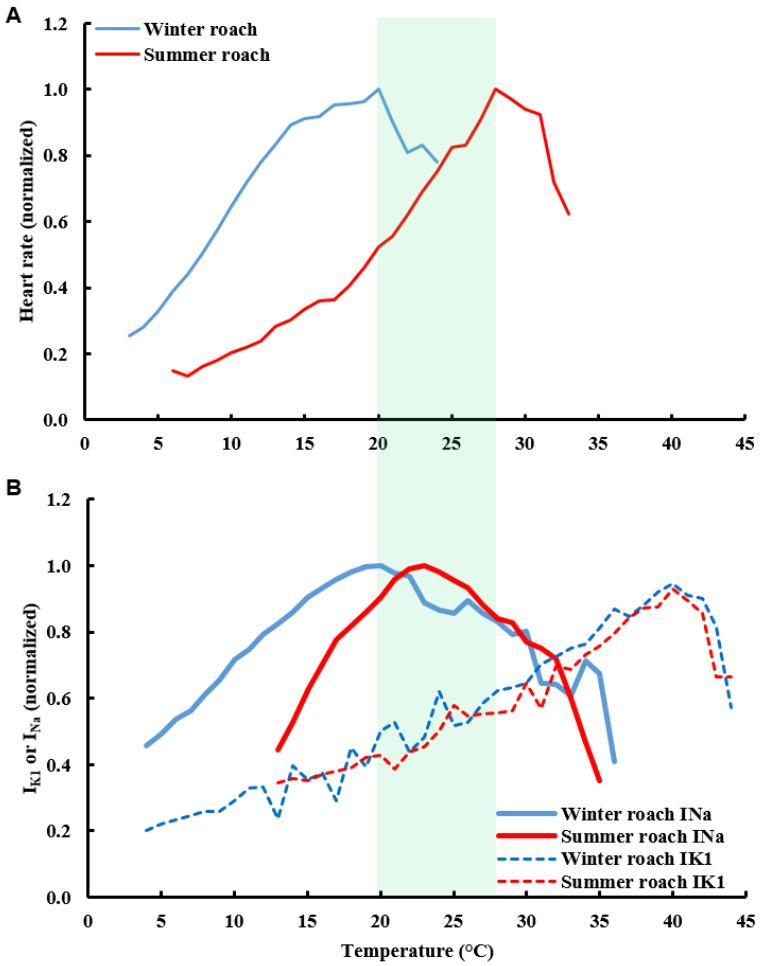

Figure 9. Effects of acute temperature increase on heart rate and densities of I_{Na} and I_{K1} in winter- and summer-acclimatized roach. (A) Effect of temperature on heart rate (f_H) *in vivo*. (B) Effect of temperature on density of I_{Na} and I_{K1} in atrial and ventricular myocytes, respectively. The green shaded area indicates the difference in thermal tolerance of f_H *in vivo* between winter and summer roach. Note that in the winter fish the depression of f_H and I_{Na} coincide at the same temperature, while in the summer fish the depression of I_{Na} precedes that of f_H. All variables were normalized to simplify comparisons. Data for Figure 9A are from Badr et al., 2016 (Paper I) and for Fig 9B from Badr et al., 2017 and Badr et al., 2018 (Papers II and III).

3.7 EFFECTS OF PACING RATE, TEMPERATURE AND EXTRACELLULAR K^+ ON EXCITABILTY OF ROACH VENTRICULAR MYOCYTES (PAPER IV)

The electrical excitability (AP) of ventricular myocytes in winter-acclimatized roach was measured by varying pacing rates, temperatures and extracellular K^+ concentrations ($[K^+]_o$). Pacing rate had no significant effect on excitability. The effects of temperature and high $[K^+]_o$ on the excitability of roach ventricular myocytes were partly antagonistic, and in some respects synergistic. High temperature hyperpolarized RMP, increased $+V_{max}$ and elevated the excitation threshold, while high $[K^+]_o$ depolarized RMP, depressed $+V_{max}$ and reduced the excitation threshold. APD_{50} was considerably shortened and the densities of I_{K1} and I_{Kr} were increased by both high temperature and high $[K^+]_o$. However, the antagonistic effects of high $[K^+]_o$ were so strong that they overrode the effects of high temperature on RMP and $+V_{max}$. Therefore, the effects of high $[K^+]_o$ predominated (Table 4).

Table 4. Effects of high temperature and extracellular K^+ concentration $[K^+]_o$ on AP variables of ventricular myocytes of winter-acclimatized roach. Up and down arrows referred increasing or decreasing in variable values, respectively.

Variable	Temperature (°C)	$[K^+]_o$
RMP	↓	↑
TP	↓	↑
CD	↑	↓
AP amplitude	↑	↓
AP overshoot	↓	↓
APD_{50}	↓	↓
$+V_{max}$	↑	↓
I_{K1}	↑	↑
I_{Kr}	↑	↑

4 DISCUSSION

The body temperature of fish is in close equilibrium with the ambient water temperature, and therefore the heart will encounter all variations of water temperature (Hazel and Prosser, 1974). EE determines the rate and rhythm of the heartbeat and regulates contractility. EE should therefore be sensitive to temperature changes in order to produce temperature-dependent acceleration and deceleration of f_H and coordinate conduction of AP through the heart. At the same time, EE should remain stable in order to avoid severe cardiac arrhythmias (Hassinen et al., 2007; Haverinen and Vornanen, 2009; Vornanen et al., 2014; Vornanen, 2016).

4.1. EXPLANATIONS FOR THERMAL FAILURE OF ROACH HEART

The main findings of the present study, which are particularly relevant when considering the resistance of the roach heart to acute and chronic temperature changes, are mentioned below. Concerning acute heat ramps, cardiac function *in vivo* was much more sensitive to high temperatures than were the APs and ion currents of isolated cardiac myocytes (cellular level). The most heat sensitive functions at the organismal level were T_{ARR} and f_H. At the cellular level, the density of I_{Na} was clearly the most heat-sensitive entity among the ion currents. These findings apply to both winter- and summer-acclimatized roach hearts (Table 3). With regard to *in vivo* f_H and density of I_{Na}, the results from the roach are consistent with the findings from 12°C-acclimated brown trout (Vornanen et al., 2014). The match between the thermal tolerances of I_{Na} and *in vivo* f_H is not quantitatively perfect in the summer roach, suggesting other factors in addition to I_{Na} may be involved in impairing cardiac function at high temperatures. The T_{BP}s of f_H and I_{Na} in winter-acclimatized roach (4°C) were slightly lower (19.8 and 20.3°C, respectively) than in the 12°C-acclimated brown trout (23.5 and 20.9°C, respectively). The respective T_{BP}s of the summer-acclimatized roach (18°C) were clearly higher (28.1 and 23.8°C) than those of the 12°C-acclimated brown trout. Interestingly, there seems to be a positive correlation between acclimation/acclimatization temperature and the thermal tolerance of these variables across the species.

The above findings are consistent with the well-known principle that thermal sensitivity decreases with decreasing complexity of biological organization: the intact organism is the most heat-sensitive and the molecular functions are generally the most heat-resistant (Ushakov, 1964; Prosser and Nelson, 1981; Lagerspetz, 1987). Roach are no exception in this respect: the T_{BP} of f_H is similar or lower than that of the SL ion currents of enzymatically isolated myocytes, with the exception of

the I_{Na} of summer-acclimatized roach. This means that the interaction between cardiac cells and/or the control of organ function by neuronal and humoral systems set the final thermal limits of cardiac function. Indeed, APs can be elicited in enzymatically isolated roach myocytes at temperatures that are higher than the T_{BP} of *in vivo* f_H. Hence, the EE of single myocytes does not fail at high temperatures, if the stimulus used to trigger AP is strong enough.

The most parsimonious explanation for the depression of *in vivo* f_H is temperature-dependent deterioration of the sinoatrial pacemaker mechanism. However, recent results from enzymatically isolated single pacemaker cells of the brown trout heart are contrary to this suggestion: the AP frequency of single pacemaker cells is not limited by temperatures that are depressive to *in vivo* f_H (Haverinen et al., 2017). Moreover, the persistence of the P wave, i.e. atrial depolarization, at high temperatures in the roach ECG suggests that the sinoatrial tissue remains functional at high temperatures, and hence the limitation of f_H must occur at more distal parts of the roach heart. Therefore, I consider the depression of the pacemaker rate as an unlikely explanation for the deterioration of f_H *in vivo*, although it has not yet been directly tested in roach.

It has been speculated that bradycardia at high temperatures is an adaptive (beneficial and regulated) response to hypoxia (Heath and Hughes, 1973). The controlled decrease in f_H could provide protection for the heart under hypoxic conditions by maintaining low $[Ca^{2+}]_i$ levels (Rantin et al., 1998). In this scenario, hypoxia would be the primary cause of thermal deterioration of fish performance, while the depression of f_H would be a mere consequence of that. Regulated depression of f_H in fish occurs via activation of the parasympathetic nervous system. However, currently there is hardly any experimental evidence for the increased cholinergic tone under acute increases of temperature. By contrast, it has been reported that isoprenaline, a β-adrenergic agonist, provides protection against heat-induced depression of *in vitro* f_H in the cold-acclimated rainbow trout (Aho and Vornanen, 2002).

It has recently been shown for the New Zealand wrasse species (*Notolabrus fucicola*, *N.celidotus* and *Thalassam lunare*) that cardiac mitochondrial function is disrupted at temperatures that are below the critical temperature for cardiac failure of these species (Iftikar et al., 2014). Notably, mitochondrial ATP production was disrupted under fully oxygenated conditions. Thus, heat-induced mitochondrial failure is one of the possible reasons why cardiac function fails at high temperatures in fish. However, these experiments were conducted with permeabilized cardiac fibres, and therefore it remains to be shown whether the same limitations are valid for intact animals *in vivo*.

4.2 ROACH HEART AND THE TDEE HYPOTHESIS

One of the major aims of this study was to test the TDEE hypothesis. This hypothesis, formulated by Vornanen, is based on the different temperature dependencies of the outward I_{K1} and inward I_{Na} of the brown trout heart (Vornanen et al., 2014; Vornanen, 2016). I_{K1} and I_{Na} have antagonistic roles in the initiation of atrial and ventricular APs: I_{K1} maintains RMP while I_{Na} provides the necessary depolarization for the initiation of AP (Whalley et al., 1994; Golod et al., 1998). Critical depolarization (CD) is the voltage distance between RMP and TP: the function of I_{Na} is to bring the membrane potential to TP. With moderate increases in temperature, both I_{Na} and I_{K1} increase in a balanced way, and therefore myocytes remain excitable and EE may even improve. With further increases in temperature, the T_{BP} of I_{Na} is reached and it starts to decline. However, thermal tolerance of I_{K1} is much better than that of I_{Na}, and therefore the density of I_{K1} continues to increase; a mismatch between depolarizing I_{Na} and repolarizing I_{K1} develops and I_{Na} is unable to depolarize the membrane to TP (Figs. 9 and 10).

Are the results from roach consistent with the TDEE hypothesis? The results of **Paper IV** seem to support the TDEE hypothesis. At high temperatures, RMP becomes more negative due to higher I_{K1} and, consequently, increases the critical depolarization (CD=TP-RMP). Therefore, stronger depolarization is needed to bring the membrane potential to TP. In addition, the T_{BP}s seem to support the TDEE hypothesis (**Paper II and III**). The T_{BP}s of I_{Na} are much lower (20.3°C and 23.8°C in winter and summer roach, respectively; Table 3) than the T_{BP}s of I_{K1} (40.0°C and 39.4°C for winter and summer roach, respectively; Table 3). Therefore, the mismatch between I_{Na} and I_{K1} might explain the T_{BP}s of f_H in winter (19.8°C) and summer (28.1°C) roach. Even though the T_{BP}s of f_H and I_{Na} are shifted in a similar way by seasonal acclimatization, the quantitative match between the variables is not as good as in the brown trout. This may suggest that other factors are involved.

Are the findings from cellular studies able to explain the heat sensitivity of the fish heart at the organismal level? As noted above, single ventricular myocytes can be excited at high temperatures, when the external stimulus strength is sufficiently strong. However, the stimulus strength is limited in the intact heart. In the cardiac muscle, myocytes are electrically connected forming a functional syncytium. Each cell in the cardiac syncytium functions as a source of current for excitation and as a sink for current flow. The I_{Na} of the upstream myocyte is the source of depolarizing current, and the membrane capacitance and I_{K1} of the downstream cell form the current sink. Membrane capacitance (cell size) does not change with acute temperature changes, so that the propagation of AP is dependent on the size of the I_{Na} of the upstream cell and the size of the I_{K1} of the downstream cell: propagation succeeds if the I_{Na} is high and fast enough to override the I_{K1} of the downstream cell. Therefore, thermal failure of EE in the heart can be seen as a source-sink mismatch,

which prevents the propagation of cardiac AP between cardiac myocytes: either a decrease of I_{Na} or an increase of I_{K1}, or both, could cause the mismatch (Fig. 11).

Theoretically, it is more likely, that propagation of AP between cells fails when the downstream cell has a high density of I_{K1} (large current sink) and/or the upstream cell has a low I_{Na} (I_{Ca} in nodal cells; small current source) (Fig. 11). Ventricular myocytes usually have much higher I_{K1} than atrial myocytes, while I_{Na} is similar in both cell types (Warren et al., 2001; Haverinen and Vornanen, 2006) or higher in atrial than ventricular myocytes (**Paper III**). Therefore, thermal failure is more likely to occur between ventricular myocytes than between atrial myocytes. Impulse transmission between the AV node and the ventricle is expected to be particularly susceptible to failure, since nodal APs are slowly rising and have small amplitude (due to low or absent I_{Na}), i.e. the current source is small. This is especially true because the amplitude of nodal AP is reduced at high temperatures (Haverinen et al., 2017). Impulse transmission between the sinoatrial pacemaker and the atrium might not be as highly endangered, since in many fish species atrial myocytes have a very low I_{K1}. In fact, the types of arrhythmias found in the roach heart support the above assumptions. Absence of QRS complexes, a failure of impulse to propagate from the atrium to the ventricle, is the type of cardiac arrhythmia that appears in roach at relatively low temperatures. The persistence of the P wave indicates that nodal and atrial APs are not suppressed by high temperatures.

Although the heat-sensitivity of I_{Na} seems to be much higher than that of other cardiac ion currents, this does not mean that Na^+ channels will denaturate at 20–25°C. It is a misunderstanding that protein denaturation is involved or required for depression of I_{Na}. The opening and closing of Na^+ channels are multistep changes in the conformation of the channel protein. When the flexibility of the channel protein increases with increasing temperature, it is possible that these changes no longer follow the normal sequence (e.g. some steps could be omitted), which results in failure of the channels to open or to recover from inactivation. Temperature-induced changes in the channel gating or their voltage-dependence probably explain the temperature-related depression of I_{Na}. This is an interesting topic for future research.

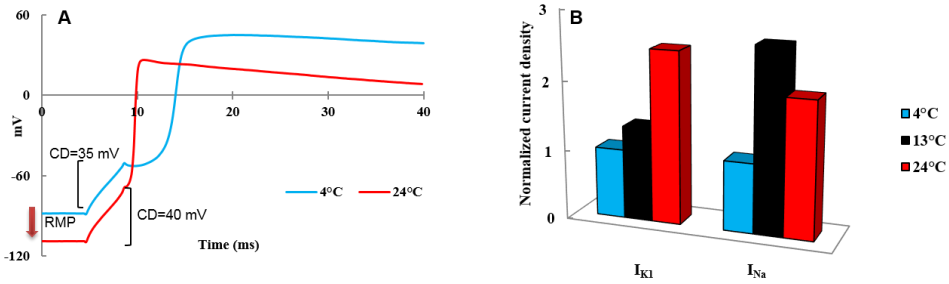

Figure 10. Effect of temperature on EE of roach ventricular myocytes. (A) The first 40 ms of a ventricular AP from winter-acclimatized roach at 4 and 24°C reveals an increase in critical depolarization (CD) and hyperpolarization of RMP at 24°C. (B) Normalized values of the peak of outward I_{K1} and inward I_{Na} at 3 different temperatures show that the density of I_{Na} declines at 24°C, whereas the density of I_{K1} is still increasing (results from winter-acclimatized roach).

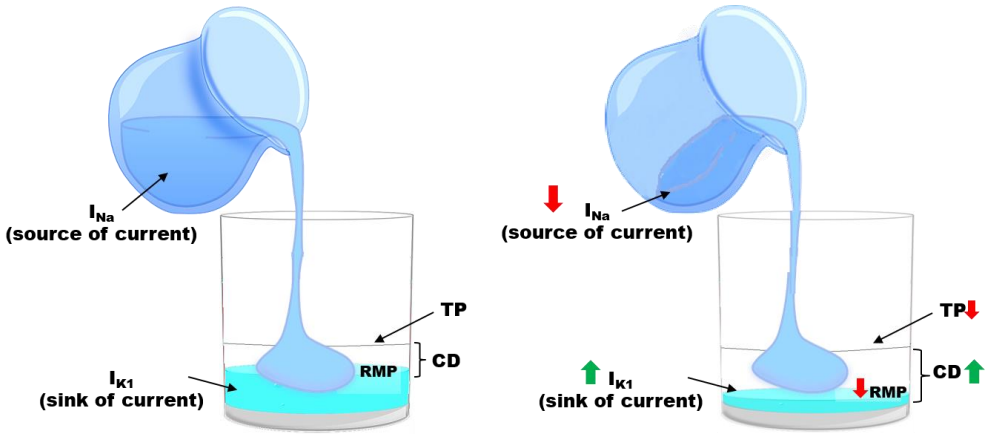

Figure 11. A diagram showing the source-sink relationship in the propagation of AP between cardiac myocytes. In well-coupled cells (left), the source of current (I_{Na}) of the upstream cell is sufficient to shift the membrane potential to the threshold and trigger AP. At high temperatures (right), the myocytes may get uncoupled, because the sink for current flow in the downstream cell is increased (bigger I_{K1}) and the source of current from the upstream cell is reduced (smaller I_{Na}): RMP and TP become more negative (negative direction; red arrows), and CD increased; i.e. a source-sink mismatch develops.

4.3. HEAT-INDUCED CARDIAC ARRHYTHMIA

The missing QRS complexes explain the increased variability of interbeat intervals during heat ramps. Similar heartbeat variability under temperature stress has been reported previously for rainbow trout, cod (*Gadus morhua*) and brown trout (Heath and Hughes, 1973; Gollock et al., 2006; Vornanen et al., 2014). With a further

increase in temperature, short episodes of atrial tachycardia occurred in the roach heart, similar to those found in the brown trout (Vornanen et al., 2014). The episodes of cardiac arrhythmia, in both roach and brown trout, were relatively short-lived and benign, suggesting that they do not have any significant effect on cardiac output. Notably, 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), never occurred in roach. Accordingly, heat-induced bradycardia (T_{BP} of f_H) is the most significant form of arrhythmia with respect to the cardiac pump function and physiology of the fish. Bradycardia was soon followed by complete cessation of the heartbeat. Similar cardiac arrhythmias have been observed in other fish species (Casselman et al., 2012; Anttila et al., 2013; Verhille et al., 2013; Ferreira et al., 2014).

4.4. EFFECTS OF SEASONAL ACCLIMATIZATION ON ELECTRICAL EXCITABILITY

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). There is fairly close match between the T_{BP} of f_H and the upper critical temperature of the fish in both winter-acclimatized/cold-acclimated (19.8°C vs. 22°C) and summer-acclimatized/warm-acclimated (28.1°C vs. 28°C) roach (**Paper I**; Cocking, 1959). These findings are fully consistent with the OCLTT hypothesis (Pörtner, 2001; Eliason et al., 2011), but can it be mechanistically explained?

Seasonal acclimatization affected cardiac function in roach at all levels of biological organization. All variables of *in vivo* ECG were strongly affected by seasonal acclimatization. The T_{BP}s of the summer-acclimatized roach were 6.9−12.4°C higher than those of the winter-acclimatized roach. Most strongly affected were T_{ARR} (12.4°C) and f_H (8.9°C). The effect of acclimatization on the T_{BP} of AP variables was much weaker: the T_{BP}s of the summer-acclimatized roach were (2.2−5°C) higher than those of winter-acclimatized roach. Among the ion currents, the T_{BP} of I_{Na} was significantly increased by summer-acclimatization, while the T_{BP} of I_{K1} remained unchanged. These findings indicate that the effects of seasonal acclimatization appear much more pronouncedly in the intact animal than in single cells. However, thermal tolerance of I_{Na} and f_H were shifted in a similar way in seasonally acclimatized fish, suggesting that source-sink mismatch might explain the seasonal change in thermal tolerance of f_H. The hypothesis that reduction of I_{Na} is involved is supported by strong reductions in the T_{BP}s of QRS duration and PQ interval in the winter roach: the velocity of AP propagation was slowed at relatively low temperatures. Curiously, the T_{BP} of I_{Kr} was depressed in summer roach. I do not have any clear explanation for this, but it may be related to an acute temperature-dependent shift in the voltage-dependence of I_{Kr} in the summer roach myocytes. Thus, at low and moderate temperatures, the shortening of APD is mainly due to

an increase in I_{Kr}, while at extremely high temperatures the relative role of I_{K1} in AP shortening is likely to be more pronounced. Collectively, the results from seasonally acclimatized roach are qualitatively consistent with the OCLTT and TDEE hypotheses, the latter providing a mechanistic explanation at the cellular and molecular levels.

4.5. SIGNIFICANCE OF EXTRACELLULAR K^+ CONCENTRATION

RMP is determined by the concentration gradient of K^+ ions across the cardiac SL. Therefore, small increases in $[K^+]_o$ depolarize RMP and change the function of ion channels. Depolarization of RMP reduces the availability of Na^+ channels and increases the density of I_{Kr} and I_{K1}: the current source is decreased and the current sink increased (**Paper IV**). By this means, the increase of $[K^+]_o$ acts synergistically with high temperature and depresses EE. Moreover, depolarization of RMP reduces the critical depolarization and shifts the TP close to the optimal voltage of I_{Na}. However, the depressive effects of $[K^+]_o$ on EE are much stronger than its permissive effects. In particular, strong depression of $+V_{max}$ can be detrimental for cardiac excitation. The findings from roach ventricular myocytes are consistent with the cable theory of EE and experiments conducted on neuronal membranes and mammalian cardiac preparations (Dominguez and Fozzard, 1970; Kolb, 1990; Lindinger, 1995).

Cardiac myocytes are tightly packed and leaving only a small-restricted space (paracellular) around the cells in the intact heart, where $[K^+]_o$ tends to accumulate at high heart rate. K^+ accumulation is frequency- and temperature-dependent. The effects of high $[K^+]_o$ are likely to be the strongest in exercising fish at high temperatures, when K^+ leakage from the working skeletal muscle fibres elevates plasma $[K^+]_o$. If high $[K^+]_o$ worsens the depressive effects of high temperature on cardiac excitability, this should appear as stronger depression of f_H in exercising fish than in resting fish. Indeed, this has been documented to be the case in the sockeye salmon (*Oncorhynchus nerka*) and coho salmon (*Oncorhynchus kisutch*) (Casselman et al., 2012; Eliason et al., 2013; Eliason and Anttila, 2017).

The effects of high $[K^+]_o$ on cardiac function should be carefully examined in fish which inhabit different thermal habitats and differ in their locomotor abilities. In particular, the role of $[K^+]_o$-induced cardiac depression in popular game fish species should be examined, since it may be associated with the poor recovery of the caught fish after release.

4.6. MOLECULAR BACKGROUND OF CARDIAC ION CURRENTS

Molecular findings are largely consistent with the electrophysiological results and confirm the linkage between gene expression and ion current densities.

As in crucian carp, zebrafish and navaga, Kir2.4 is the main isoform in the roach ventricle (Hassinen et al., 2007; Hassinen et al., 2008b; Hassinen et al., 2014; Hassinen et al., 2015a). In the roach atrium, Kir2.2a is the main isoform with less expression of Kir2.4, as in the zebrafish atrium (Hassinen et al., 2015a). Seasonal acclimatization had no significant effect on Kir2 isoform composition or transcript expression levels of the roach heart, which correlates with the unaltered density of atrial I_{K1}, but differs compared with the increased density of the ventricular I_{K1} in summer roach. The reason for this mismatch remains unknown, but it could involve changes in the concentration of intracellular polyamines, modifiers of the I_{K1} density (Ficker et al., 1994).

The I_{Kr} of the roach heart is generated by Erg2 channels similar to those in the hearts of other fish species (Hassinen et al., 2014; Vornanen and Hassinen, 2016). Consistent with the increased density of I_{Kr} in winter, the Erg2 transcripts were upregulated in the atrium and the ventricle of winter roach heart. Roach is one more fish species that shows this ubiquitous response of fish hearts in exposure to chronic cold.

Seasonal acclimatization had a striking effect on the expression of Na^+ channel transcripts: a prominent increase in winter roach in comparison to summer roach in both atrium and ventricle. The increased expression of Na^+ channel transcripts is consistent with the higher density of I_{Na} in atrial and ventricular myocytes in winter roach. There was also a small shift in isoform composition between summer and winter roach, as the relative number of $Na_v1.4$ was slightly higher in winter than in summer. The I_{Na} generated by the $Na_v1.4$ channels is kinetically faster than the I_{Na} generated by the $Na_v1.5$ channels (Wang et al., 1996). Therefore, the isoform shift towards a kinetically faster isoform could be seen as adaptive at low winter temperatures, but on the other hand, it might make the current more sensitive to deterioration at high temperatures.

4.7. PHYSIOLOGICAL IMPLICATIONS

The present results show that seasonal acclimatization strongly modifies the EE of the roach heart *in vivo*. These changes are probably in many respects beneficial (adaptive). For example, the f_H of the winter-acclimatized roach was higher at low temperatures than that of the summer-acclimatized roach, i.e. there was positive thermal compensation of f_H in winter, while the heat-resistance of f_H was better in summer roach. The physiological plasticity of f_H supports a relatively active life style in both winter and summer for the eurythermal roach. In addition, the T_{BP}s of all ECG variables were shifted in a seasonally appropriate manner: all the variables were more heat-tolerant in summer than in winter roach.

The water temperature of ice-covered lakes in winter is between 0°C and 4°C, while in summer the surface water temperature may be as high as 25−26°C in the lakes of Northern Europe. Concerning the T_{BP}s of f_H, which were 19.8°C and 28.1°C

for winter and summer roach, respectively, the thermal safety margin of cardiac function in the summer-acclimatized roach is only about 2°C. The annual mean temperature in Finland is anticipated to rise 2–5°C and 2–7°C by the 2050s and 2080s, respectively (Lehtonen, 1998). In addition, extreme temperature peaks are likely to be higher and to occur more often. Therefore, even the eurythermal roach may become a "loser" as regards the climate change (Somero, 2010), and they may be forced to seek colder (deeper) habitats in the lakes. It should be remembered, however, that these conclusions concern the adult roach. It is likely that different life stages have different thermal optima and tolerances, which may differ significantly from those of the adult fish (McCauley and Read, 1973; Richardson et al., 1994; Rombough, 1997; Pankhurst and Munday, 2011).

4.8 THEORETICAL IMPLICATIONS

The present findings suggest that EE is a significant factor in the temperature-dependence of heart function in roach. EE of cardiac myocytes probably determines the heat-tolerance (T_{BP}) of f_H. That the T_{BP} of f_H in seasonally acclimatized roach is similar or slightly lower than the upper critical temperature of the fish is consistent with the original OCLTT hypothesis, which states that the temperature limitations of respiratory and cardiovascular systems result in hypoxia and reduction of the aerobic performance of the fish, and ultimately may cause death (Pörtner, 2001). Furthermore, the heat-induced deterioration of EE can be considered as a mechanistic explanation for the OCLTT hypothesis.

Although the heat-tolerances of f_H and the upper thermal tolerance of the fish correlate, this does not necessarily mean that cardiac function is the sole, or even the major determinant for heat death in the fish. The TDEE hypothesis, which suggests a heat-induced mismatch between current source and current sink in connected cardiac myocytes as the cause of breaks or complete cessation of f_H, is not limited to the cardiac muscle. Intercellular connections (synapses) are typical of nervous networks and connections between nerves and muscle cells. Nerves and muscles are electrically excitable tissues, and their function follows the same principles as that of the cardiac muscle; a heat-induced mismatch between current source and current sink is equally possible for these tissues as it is for the heart. Thus, it is theoretically possible that the heat death of the roach is due to failure of sensory and/or motor nervous systems, or to integration of body functions by the autonomic nervous system. Several body functions could deteriorate parallel to each other. Indeed, the TDEE hypothesis may not be limited to the cardiac muscle, but could also occur in any electrically excitable tissue. In this respect, the range of application of the TDEE hypothesis will be wider than that of the OCLTT hypothesis: it should apply to all ectotherms that have well-developed nervous and muscular systems, and it should be equally valid for aquatic and for terrestrial animals. These exciting scenarios are interesting topics for future research.

5 BIBLIOGRAPHY

Abramochkin, D.V., Tapilina, S.V. & Vornanen, M. 2014. A new potassium ion current induced by stimulation of M2 cholinoreceptors in fish atrial myocytes. *Journal of Experimental Biology.* 217: 1745-1751.

Abramochkin, D.V. & 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*). *Journal of Comparative Physiology B.* 185: 883-890.

Abramochkin, D.V. & Vornanen, M. 2017. Seasonal changes of cholinergic response in the atrium of Arctic navaga cod (*Eleginus navaga*). *Journal of Comparative Physiology B.* 187: 329-338.

Aguiar, L., Kalinin, A. & Rantin, F. 2002. The effects of temperature on oxygen uptake, gill ventilation and ECG waveforms in serrasalmid fish, *Piaractus mesopotamicus*. *Journal of Thermal Biology.* 27: 299-308.

Aho, E. & Vornanen, M. 2002. Effects of adenosine on the contractility of normoxic rainbow trout heart. *Journal of Comparative Physiology B.* 172: 217-225.

Alday, A., Alonso, H., Gallego, M., Urrutia, J., Letamendia, A., Callol, C. & Casis, O. 2014. Ionic channels underlying the ventricular action potential in zebrafish embryo. *Pharmacological Research.* 84: 26-31.

Anttila, K., Casselman, M.T., Schulte, P.M. & Farrell, A.P. 2013. Optimum temperature in juvenile salmonids: connecting subcellular indicators to tissue function and whole-organism thermal optimum. *Physiological & Biochemical Zoology.* 86: 245-256.

Axelsson, M., Davison, W., Forster, M.E. & Farrell, A.P. 1992. Cardiovascular responses of the red-blooded antarctic fishes *Pagothenia bernacchii* and *P. borchgrevinki*. *Journal of Experimental Biology.* 167: 179-201.

Baker, K., Warren, K.S., Yellen, G. & Fishman, M.C. 1997. Defective "pacemaker" current (I_h) in a zebrafish mutant with a slow heart rate. *Proceedings of the National Academy of Sciences of the United States of America.* 94: 4554-4559.

Ballesta, S., Hanson, L. & Farrell, A. 2012. The effect of adrenaline on the temperature dependency of cardiac action potentials in pink salmon *Oncorhynchus gorbuscha*. *Journal of Fish Biology.* 80: 876-885.

Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M. & Romey, G. 1996. K_vLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature.* 384: 78.

Bean, B.P. 1985. Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity, and pharmacology. *Journal of General Physiology.* 86: 1-30.

Beitinger, T.L. & Bennett, W.A. 2000. Quantification of the role of acclimation temperature in temperature tolerance of fishes. *Environmental Biology of Fishes.* 58: 277-288.

Belardinelli, L. & Isenberg, G. 1983. Actions of adenosine and isoproterenol on isolated mammalian ventricular myocytes. *Circulation Research.* 53: 287-297.

Belyaev, V. 1964. Comparison of changes in the electrical activity of a single node of ranvier after an increase in the potassium ion concentration in the medium and the action of procaine. *Bulletin of Experimental Biology & Medicine.* 58: 1394-1397.

Benitah, J., Alvarez, J.L. & Gómez, A.M. 2010. L-type Ca^{2+} current in ventricular cardiomyocytes. *Journal of Molecular & Cellular Cardiology.* 48: 26-36.

Bennett, W.A. & Beitinger, T.L. 1997. Temperature tolerance of the sheepshead minnow, Cyprinodon variegatus. *Copeia.* 77-87.

Bers, D.M. 2000. Calcium fluxes involved in control of cardiac myocyte contraction. *Circulation Research.* 87: 275-281.

Bers, D.M. 2001. Excitation-contraction coupling and cardiac contractile force. second edn. Springer Science & Business Media.

Bers, D.M. 2002. Cardiac excitation-contraction coupling. *Nature.* 415: 198-205.

Bett, G.C., Morales, M.J., Beahm, D.L., Duffey, M.E. & Rasmusson, R.L. 2006. Ancillary subunits and stimulation frequency determine the potency of chromanol 293B block of the KCNQ1 potassium channel. *Journal of Physiology.* 576: 755-767.

Bezanilla, F. 2005. Voltage-gated ion channels. *IEEE transactions on nanobioscience.* 4: 34-48.

Blank, J.M., Morrissette, J.M., Davie, P.S. & Block, B.A. 2002. Effects of temperature, epinephrine and Ca^{2+} on the hearts of yellowfin tuna (*Thunnus albacares*). *Journal of Experimental Biology.* 205: 1881-1888.

Brett, J.R. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerkd*). *American Zoologist.* 11: 99-113.

Brette, F., Luxan, G., Cros, C., Dixey, H., Wilson, C. & Shiels, H.A. 2008. Characterization of isolated ventricular myocytes from adult zebrafish (*Danio rerio*). *Biochemical & Biophysical Research Communications.* 374: 143-146.

Campbell, H.A., Taylor, E.W. & Egginton, S. 2004. The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin *Myoxocephalus scorpius*. *Journal of Experimental Biology.* 207: 1969-1976.

Campbell, H.A., Leite, C.A., Wang, T., Skals, M., Abe, A.S., Egginton, S., Rantin, F.T., Bishop, C.M. & Taylor, E.W. 2006. Evidence for a respiratory component, similar to mammalian respiratory sinus arrhythmia, in the heart rate variability signal from the rattlesnake, *Crotalus durissus terrificus*. *Journal of Experimental Biology.* 209: 2628-2636.

Carl, L. 1995. Sonic tracking of burbot in Lake Opeongo, Ontario. *Transactions of the American Fisheries Society.* 124: 77-83.

Casselman, M., Anttila, K. & Farrell, A. 2012. Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. *Journal of Fish Biology.* 80: 358-377.

Catterall, W.A. 1992. Cellular and molecular biology of voltage-gated sodium channels. *Physiological Reviews.* 72: S15-S48.

Cech Jr, J.J., Bridges, D.W., Rowell, D.M. & Balzer, P.J. 1976. Cardiovascular responses of winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Canadian Journal of Zoology.* 54: 1383-1388.

Chi, N.C., Shaw, R.M., Jungblut, B., Huisken, J., Ferrer, T., Arnaout, R., Scott, I., Beis, D., Xiao, T. & Baier, H. 2008. Genetic and physiologic dissection of the vertebrate cardiac conduction system. *PLoS Biology.* 6: e109.

Chowdhury, S., Jarecki, B.W. & Chanda, B. 2014. A molecular framework for temperature-dependent gating of ion channels. *Cell.* 158: 1148-1158.

Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G. & Farrell, A.P. 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 295: R1631-R1639.

Cocking, A.W. 1959. The effects of high temperatures on roach (*Rutilus rutilus*). *Journal of Experimental Biology.* 36: 217-226.

Coraboeuf, E. 1978. Ionic basis of electrical activity in cardiac tissues. *American Journal of Physiology.* 234: H101-H116.

Danylchuk, A.J., Suski, C.D., Mandelman, J.W., Murchie, K.J., Haak, C.R., Brooks, A.M. & Cooke, S.J. 2014. Hooking injury, physiological status and short-term mortality of juvenile lemon sharks (*Negaprion bevirostris*) following catch-and-release recreational angling. *Conservation Physiology.* 2: cot036.

Dickson, K.A. & Graham, J.B. 2004. Evolution and consequences of endothermy in fishes. *Physiological & Biochemical Zoology: PBZ.* 77: 998-1018.

Dobrzynski, H., Marples, D.D., Musa, H., Yamanushi, T.T., Hendersonxyl, Z., Takagishi, Y., Honjo, H., Kodama, I. & Boyett, M.R. 2001. Distribution of the muscarinic K^+ channel proteins Kir3.1 and Kir3.4 in the ventricle, atrium, and sinoatrial node of heart. *Journal of Histochemistry & Cytochemistry.* 49: 1221-1234.

Dominguez, G. & Fozzard, H.A. 1970. Influence of extracellular K^+ concentration on cable properties and excitability of sheep cardiac Purkinje fibers. *Circulation Research.* 26: 565-574.

Edsall, T.A., Kennedy, G.W. & Horns, W.H. 1993. Distribution, abundance, and resting microhabitat of burbot on Julian's Reef, southwestern Lake Michigan. *Transactions of the American Fisheries Society.* 122: 560-574.

Ehrlich, J.R. 2008. Inward rectifier potassium currents as a target for atrial fibrillation therapy. *Journal of Cardiovascular Pharmacology.* 52: 129-135.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G. & Farrell, A.P. 2011. Differences in thermal tolerance among sockeye salmon populations. *Science (New York, N.Y.).* 332: 109-112.

Eliason, E.J., Clark, T.D., Hinch, S.G. & Farrell, A.P. 2013. Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conservation Physiology*. 1, cot008.

Eliason, E.J. & Anttila, K. 2017. Temperature and the cardiovascular system. In: Fish Physiology, A. Kurt Gamperl, Todd E. Gillis, Anthony P. Farrell, Colin J. Brauner (Eds). Academic Press, San Diego. 36B: 235-297.

Elliott, J. & Elliott, J. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. *Journal of Fish Biology*. 77: 1793-1817.

Endo, M., Foscarini, R. & Kuroki, A. 1988. Electrocardiogram of a marine fish, *Pagrus major*, exposed to red tide plankton, *Chattonella marina*. *Marine Biology*. 97: 477-481.

Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *American Journal of Physiology*. 245: C1-C14.

Farrell, A.P. 2009. Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology*. 212: 3771-3780.

Farrell, A.P. & Smith, F. 2017. Cardiac form, function and physiology. In: Fish Physiology, A. Kurt Gamperl, Todd E. Gillis, Anthony P. Farrell, Colin J. Brauner (Eds). Academic Press, San Diego. 36A: 155-264.

Ferreira, E.O., Anttila, K. & Farrell, A.P. 2014. Thermal optima and tolerance in the eurythermic goldfish (*Carassius auratus*): relationships between whole-animal aerobic capacity and maximum heart rate. *Physiological & Biochemical Zoology*. 87: 599-611.

Ficker, E., Taglialatela, M., Wible, B.A., Henley, C.M. & Brown, A.M. 1994. Spermine and spermidine as gating molecules for inward rectifier K^+ channels. *Science*. 266: 1068-1073.

Fozzard, H.A. 1977. Cardiac muscle: Excitability and passive electrical properties. *Progress in Cardiovascular Diseases*. 19: 343-359.

Fozzard, H.A. & Hanck, D.A. 1996. Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. *Physiological Reviews*. 76: 887-926.

Franklin, C.E., Davison, W. & Seebacher, F. 2007. Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *Journal of Experimental Biology*. 210: 3068-3074.

Fry, F.E.J. 1947. Effects of the Environment on Animal Activity. University of Toronto Press.

Gale, M.K., Hinch, S.G. & Donaldson, M.R. 2013. The role of temperature in the capture and release of fish. *Fish & Fisheries*. 14: 1-33.

Galli, G.L., Lipnick, M.S. & Block, B.A. 2009. Effect of thermal acclimation on action potentials and sarcolemmal K^+ channels from Pacific bluefin tuna

cardiomyocytes. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 297: R502-R509.

Galli, G.L., Lipnick, M.S., Shiels, H.A. & Block, B.A. 2011. Temperature effects on Ca^{2+} cycling in scombrid cardiomyocytes: a phylogenetic comparison. *Journal of Experimental Biology.* 214: 1068-1076.

Gamperl, A.K. & Farrell, A.P. 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. *Journal of Experimental Biology.* 207: 2539-2550.

Genge, C., Hove-Madsen, L. & Tibbits, G.F. 2012. Functional and structural differences in atria versus ventricles in teleost hearts. In: New Advances and Contributions to Fish Biology, Hakan Türker (Eds). InTech. pp. 221-245.

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

Golod, D.A., Kumar, R. & Joyner, R.W. 1998. Determinants of action potential initiation in isolated rabbit atrial and ventricular myocytes. *American Journal of Physiology.* 274: H1902-H1913.

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

Hassinen, M., Paajanen, V., Haverinen, J., Eronen, H. & Vornanen, M. 2007. Cloning and expression of cardiac Kir2.1 and Kir2.2 channels in thermally acclimated rainbow trout. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 292: R2328-R2339.

Hassinen, M., Haverinen, J. & Vornanen, M. 2008a. Electrophysiological properties and expression of the delayed rectifier potassium (ERG) channels in the heart of thermally acclimated rainbow trout. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 295: R297-R308.

Hassinen, M., Paajanen, V. & Vornanen, M. 2008b. A novel inwardly rectifying K^+ channel, Kir2.5, is upregulated under chronic cold stress in fish cardiac myocytes. *Journal of Experimental Biology.* 211: 2162-2171.

Hassinen, M. 2010. Thermal responses of inward and delayed rectifier potassium channels in fish cardiac myocytes. University of Eastern Finland. p. 18

Hassinen, M., Laulaja, S., Paajanen, V., Haverinen, J. & Vornanen, M. 2011. Thermal adaptation of the crucian carp (*Carassius carassius*) cardiac delayed rectifier current, I_{Ks}, by homomeric assembly of $K_V7.1$ subunits without MinK. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 301: R255-R265.

Hassinen, M., Abramochkin, D.V. & Vornanen, M. 2014. Seasonal acclimatization of the cardiac action potential in the Arctic navaga cod (*Eleginus navaga*, Gadidae). *Journal of Comparative Physiology B.* 184: 319-327.

Hassinen, M., Haverinen, J., Hardy, M.E., Shiels, H.A. & Vornanen, M. 2015a. Inward rectifier potassium current (I_{K1}) and Kir2 composition of the zebrafish (*Danio rerio*) heart. *Pflügers Archiv-European Journal of Physiology.* 467: 2437-2446.

Hassinen, M., Haverinen, J. & Vornanen, M. 2015b. Molecular basis and drug sensitivity of the delayed rectifier (I_{Kr}) in the fish heart. *Comparative Biochemistry & Physiology Part C: Toxicology & Pharmacology.* 176: 44-51.

Haverinen, J. & Vornanen, M. 2004. Temperature acclimation modifies Na^+ current in fish cardiac myocytes. *Journal of Experimental Biology.* 207: 2823-2833.

Haverinen, J. & Vornanen, M. 2006. Significance of Na^+ current in the excitability of atrial and ventricular myocardium of the fish heart. *Journal of Experimental Biology.* 209: 549-557.

Haverinen, J. & Vornanen, M. 2007. Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 292: R1023-R1032.

Haverinen, J. & Vornanen, M. 2009. Responses of action potential and K^+ currents to temperature acclimation in fish hearts: phylogeny or thermal preferences? *Physiological & Biochemical Zoology.* 82: 468-482.

Haverinen, J., Egginton, S. & Vornanen, M. 2014. Electrical excitation of the heart in a basal vertebrate, the European river lamprey (*Lampetra fluviatilis*). *Physiological & Biochemical Zoology.* 87: 817-828.

Haverinen, J., Abramochkin, D.V., Kamkin, A. & Vornanen, M. 2017. Maximum heart rate in brown trout (*Salmo trutta fario*) is not limited by firing rate of pacemaker cells. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 312: R165-R171.

Haworth, T.E., Haverinen, J., Shiels, H.A. & Vornanen, M. 2014. Electrical excitability of the heart in a Chondrostei fish, the Siberian sturgeon (*Acipenser baerii*). *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 307: R1157-R1166.

Hazel, J.R. & Prosser, C.L. 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiological Reviews.* 54: 620-677.

Heath, A.G. & Hughes, G.M. 1973. Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology.* 59: 323-338.

Hibino, H., Inanobe, A., Furutani, K., Murakami, S., Findlay, I. & Kurachi, Y. 2010. Inwardly rectifying potassium channels: their structure, function, and physiological roles. *Physiological Reviews.* 90: 291-366.

Hodgkin, A.L. & Huxley, A.F. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. *Journal of Physiology.* 116: 449-472.

Holopainen, I.J., Tonn, W.M. & Paszkowski, C.A. 1997. Tales of two fish: the dichotomous biology of crucian carp (*Carassius carassius* (L.)) in northern Europe. *Annales Zoologici Fennici.* pp. 1-22.

Hove-Madsen, L. & Gesser, H. 1989. Force frequency relation in the myocardium of rainbow trout. *Journal of Comparative Physiology B: Biochemical, Systemic & Environmental Physiology.* 159: 61-69.

Hove-Madsen, L. & Tort, L. 1998. L-type Ca^{2+} current and excitation-contraction coupling in single atrial myocytes from rainbow trout. *American Journal of Physiology.* 275: R2061-R2069.

Icardo, J.M. 2006. Conus arteriosus of the teleost heart: dismissed, but not missed. *Anatomical Record.* 288: 900-908.

Icardo, J.M. & Colvee, E. 2011. The atrioventricular region of the teleost heart. A distinct heart segment. *Anatomical Record.* 294: 236-242.

Iftikar, F.I. & Hickey, A.J. 2013. Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in *Notolabrus celidotus*. *PloS One.* 8: e64120.

Iftikar, F.I., MacDonald, J.R., Baker, D.W., Renshaw, G.M. & Hickey, A.J. 2014. Could thermal sensitivity of mitochondria determine species distribution in a changing climate? *Journal of Experimental Biology.* 217: 2348-2357.

Irisawa, H., Brown, H. & Giles, W. 1993. Cardiac pacemaking in the sinoatrial node. *Physiological Reviews.* 73: 197-227.

Isenberg, G. & Klöckner, U. 1980. Glycocalyx is not required for slow inward calcium current in isolated rat heart myocytes. *Nature.* 284: 358-360.

Jain, K.E. & Farrell, A.P. 2003. Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology.* 206: 3569-3579.

Jaleel, N., Nakayama, H., Chen, X., Kubo, H., MacDonnell, S., Zhang, H., Berretta, R., Robbins, J., Cribbs, L., Molkentin, J.D. & Houser, S.R. 2008. Ca^{2+} influx through T- and L-type Ca^{2+} channels have different effects on myocyte contractility and induce unique cardiac phenotypes. *Circulation Research.* 103: 1109-1119.

Johnson, A.C., Turko, A.J., Klaiman, J.M., Johnston, E.F. & Gillis, T.E. 2014. Cold acclimation alters the connective tissue content of the zebrafish (*Danio rerio*) heart. *Journal of Experimental Biology.* 217: 1868-1875.

Johnston, I.I., Guderley, H., Franklin, C., Crockford, T. & Kamunde, C. 1994. Are Mitochondria Subject to Evolutionary Temperature Adaptation? *Journal of Experimental Biology.* 195: 293-306.

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D.J., Lefevre, S., Nilsson, G.E., Metcalfe, N.B., Hickey, A.J.R., Brijs, J., Speers-Roesch, B., Roche, D.G., Gamperl, A.K., Raby, G.D., Morgan, R., Esbaugh, A.J., Grans, A., Axelsson, M., Ekstrom, A., Sandblom, E., Binning, S.A., Hicks, J.W., Seebacher, F., Jorgensen, C., Killen, S.S., Schulte, P.M. & Clark, T.D. 2018. Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *Journal of Experimental Biology.* 221: 10.1242/jeb.169615.

Keen, J., Vianzon, D., Farrell, A. & Tibbits, G. 1994. Effect of temperature and temperature acclimation on the ryanodine sensitivity of the trout myocardium. *Journal of Comparative Physiology B.* 164: 438-443.

Keen, A.N., Klaiman, J.M., Shiels, H.A. & Gillis, T.E. 2017. Temperature-induced cardiac remodelling in fish. *Journal of Experimental Biology.* 220: 147-160.

Kim, C.S., Coyne, M.D. & Gwathmey, J.K. 2000. Voltage-dependent calcium channels in ventricular cells of rainbow trout: effect of temperature changes in vitro. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 278: R1524-R1534.

Klaiman, J.M., Fenna, A.J., Shiels, H.A., Macri, J. & Gillis, T.E. 2011. Cardiac remodeling in fish: strategies to maintain heart function during temperature change. *PloS One.* 6: e24464.

Klaiman, J.M., Pyle, W.G. & Gillis, T.E. 2014. Cold acclimation increases cardiac myofilament function and ventricular pressure generation in trout. *Journal of Experimental Biology.* 217: 4132-4140.

Kleber, A.G. & Rudy, Y. 2004. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiological Reviews.* 84: 431-488.

Kolb, H. 1990. Potassium channels in excitable and non-excitable cells. In: Special Issue on Ionic Channels II. Springer. pp. 51-91.

Kubly, K.L. & Stecyk, J.A. 2015. Temperature-dependence of L-type Ca^{2+} current in ventricular cardiomyocytes of the Alaska blackfish (*Dallia pectoralis*). *Journal of Comparative Physiology B.* 185: 845-858.

Lagerspetz, K.Y. 1987. Temperature effects on different organization levels in animals. *Symposia of the Society for Experimental Biology.* 41: 429-449.

Langheinrich, U., Vacun, G. & Wagner, T. 2003. Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe arrhythmia. *Toxicology & Applied Pharmacology.* 193: 370-382.

Lannig, G., Bock, C., Sartoris, F.J. & Pörtner, H.O. 2004. Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L., studied by magnetic resonance imaging and on-line venous oxygen monitoring. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 287: R902-R910.

Lee, J., Gomora, J.C., Cribbs, L.L. & Perez-Reyes, E. 1999. Nickel block of three cloned T-type calcium channels: low concentrations selectively block α1H. *Biophysical Journal.* 77: 3034-3042.

Lefevre, S. 2016. Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction. *Conservation Physiology.* 4: cow009.

Lehtonen, H. 1998. Does global warming threat the existence of Arctic charr, *Salvelinus alpinus* (Salmonidae), in northern Finland? *Italian Journal of Zoology.* 65: 471-474.

Lillywhite, H.B., Zippel, K.C. & Farrell, A.P. 1999. Resting and maximal heart rates in ectothermic vertebrates. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology.* 124: 369-382.

Lin, E., Ribeiro, A., Ding, W., Hove-Madsen, L., Sarunic, M.V., Beg, M.F. & Tibbits, G.F. 2014. Optical mapping of the electrical activity of isolated adult zebrafish hearts: acute effects of temperature. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 306: R823-R836.

Lindinger, M.I. 1995. Potassium regulation during exercise and recovery in humans: implications for skeletal and cardiac muscle. *Journal of Molecular & Cellular Cardiology.* 27: 1011-1022.

Maltsev, V. & Undrovinas, A. 1998. Relationship between steady-state activation and availability of cardiac sodium channel: evidence of uncoupling. *Cellular & Molecular Life Sciences.* 54: 148-151.

Maltsev, V.A., Vinogradova, T.M. & Lakatta, E.G. 2006. The emergence of a general theory of the initiation and strength of the heartbeat. *Journal of Pharmacological Sciences.* 100: 338-369.

Mangoni, M.E., Couette, B., Marger, L., Bourinet, E., Striessnig, J. & Nargeot, J. 2006. Voltage-dependent calcium channels and cardiac pacemaker activity: from ionic currents to genes. *Progress in Biophysics & Molecular Biology.* 90: 38-63.

Maricondi-Massari, M., Kalinin, A., Glass, M. & Rantin, F. 1998. The effects of temperature on oxygen uptake, gill ventilation and ECG waveforms in the Nile tilapia, *Oreochromis niloticus*. *Journal of Thermal Biology.* 23: 283-290.

Martem'yanov, V. 2001. Ranges of regulation of sodium, potassium, calcium, magnesium concentrations in plasma, erythrocytes, and muscle tissue of Rutilus rutilus under natural conditions. *Journal of Evolutionary Biochemistry & Physiology.* 37: 141-147.

Matikainen, N. & Vornanen, M. 1992. Effect of season and temperature acclimation on the function of crucian carp (*Carassius carassius*) heart. *Journal of Experimental Biology.* 167: 203-220.

Maylie, J. & Morad, M. 1995. Evaluation of T- and L-type Ca^{2+} currents in shark ventricular myocytes. *American Journal of Physiology.* 269: H1695-H1703.

McCauley, R. & Read, L. 1973. Temperature selection by juvenile and adult yellow perch (*Perca flavescens*) acclimated to 24 C. *Journal of the Fisheries Board of Canada.* 30: 1253-1255.

McDonald, T.F., Pelzer, S., Trautwein, W. & Pelzer, D.J. 1994. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiological Reviews.* 74: 365-507.

Mendonça, P.C. & Gamperl, A.K. 2010. The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (*Pseudopleuronectes americanus*). *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology.* 155: 245-252.

Mitra, R. & Morad, M. 1986. Two types of calcium channels in guinea pig ventricular myocytes. *Proceedings of the National Academy of Sciences of the United States of America.* 83: 5340-5344.

Molina, C.E., Gesser, H., Llach, A., Tort, L. & Hove-Madsen, L. 2007. Modulation of membrane potential by an acetylcholine-activated potassium current in trout atrial myocytes. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 292: R388-R395.

Morita, A. & Tsukuda, H. 1994. The effect of thermal acclimation on the electrocardiogram of goldfish. *Journal of Thermal Biology.* 19: 343-348.

Nemtsas, P., Wettwer, E., Christ, T., Weidinger, G. & Ravens, U. 2010. Adult zebrafish heart as a model for human heart? An electrophysiological study. *Journal of Molecular & Cellular Cardiology.* 48: 161-171.

Nerbonne, J.M. & Kass, R.S. 2005. Molecular physiology of cardiac repolarization. *Physiological Reviews.* 85: 1205-1253.

Nielsen, J.S. & Gesser, H. 2001. Effects of high extracellular [K^+] and adrenaline on force development, relaxation and membrane potential in cardiac muscle from freshwater turtle and rainbow trout. *Journal of Experimental Biology.* 204: 261-268.

Nilius, B., Hess, P., Lansman, J.B. & Tsien, R.W. 1986. A novel type of cardiac calcium channel in ventricular cells. *Biomedica biochimica acta.* 45: S167-S170.

Nilsson, G.E., Crawley, N., Lunde, I.G. & Munday, P.L. 2009. Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology.* 15: 1405-1412.

Noble, D. 1984. The surprising heart: a review of recent progress in cardiac electrophysiology. *Journal of Physiology.* 353: 1-50.

Olson, K.R. & Farrell, A.P. 2006. The cardiovascular system. In: The Physiology of Fishes, D. H. Evans, J. B. Claiborne (Eds)., third edn. CRC Press. Boca Raton. pp. 119-152.

Opie, L. 1998. The heart-Physiology from cell to circulation. Lippincott Williams & Wilkins, Philadelphia.

Paajanen, V. & Vornanen, M. 2004. Regulation of action potential duration under acute heat stress by I_{KATP} and I_{K1} in fish cardiac myocytes. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 286: R405-R415.

Pääkkönen, J.J. & Marjomäki, T.J. 2000. Feeding of burbot, *Lota lota*, at different temperatures. *Environmental Biology of Fishes.* 58: 109-112.

Pahapill, P. & Schlichter, L. 1990. Modulation of potassium channels in human T lymphocytes: effects of temperature. *Journal of Physiology.* 422: 103-126.

Pankhurst, N.W. & Munday, P.L. 2011. Effects of climate change on fish reproduction and early life history stages. *Marine & Freshwater Research.* 62: 1015-1026.

Patel, S.P. & Campbell, D.L. 2005. Transient outward potassium current, 'I_{to}', phenotypes in the mammalian left ventricle: underlying molecular, cellular and biophysical mechanisms. *Journal of Physiology.* 569: 7-39.

Poon, K.L. & Brand, T. 2013. The zebrafish model system in cardiovascular research: A tiny fish with mighty prospects. *Global Cardiology Science & Practice.* 1: 4.

Pörtner, H.O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften.* 88: 137-146.

Pörtner, H.O. & Farrell, A.P. 2008. Physiology and climate change. *Science.* 690-692.

Pörtner, H.O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology.* 213: 881-893.

Pörtner, H.O., Bock, C. & Mark, F.C. 2018. Connecting to ecology: a challenge for comparative physiologists? Response to 'Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology'. *Journal of Experimental Biology.* 221: 10.1242/jeb.174185.

Powers, D.A. 1989. Fish as model systems. *Science (New York, N.Y.).* 246: 352-358.

Prosser, C.L. & Nelson, D. 1981. The role of nervous systems in temperature adaptation of poikilotherms. *Annual Review of Physiology.* 43: 281-300.

Randall, D. 1968. Functional morphology of the heart in fishes. *American Zoologist.* 8: 179-189.

Randall, D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. *Journal of Experimental Biology.* 100: 275-288.

Rantin, F., Gesser, H., Kalinin, A., Guerra, C., De Freitas, J. & Driedzic, W. 1998. Heart performance, Ca^{2+} regulation and energy metabolism at high temperatures in *Bathygobius soporator*, a tropical marine teleost. *Journal of Thermal Biology.* 23: 31-39.

Richardson, J., Boubée, J.A. & West, D.W. 1994. Thermal tolerance and preference of some native New Zealand freshwater fish. *New Zealand Journal of Marine & Freshwater Research.* 28: 399-407.

Riemann, B., Sondergaard, M., Persson, L. & Johansson, L. 1986. Carbon metabolism and community regulation in eutrophic, temperate lakes. In*:* Carbon Dynamics in Eutrophic, Temperate Lakes. Elsevier Science Publishing Co., New York. pp. 267-283.

Roden, D.M., Balser, J.R., George Jr, A.L. & Anderson, M.E. 2002. Cardiac ion channels. *Annual Review of Physiology.* 64: 431-475.

Roden, D.M. & Yang, T. 2005. Protecting the heart against arrhythmias: potassium current physiology and repolarization reserve. *Circulation.* 112: 1376-1378.

Rombough, P.J. 1997, The effects of temperature on embryonic and larval development. In: Seminar Series-Society For Experimental Biology. Cambridge University Press. 61: 177-224.

Rosati, B. & McKinnon, D. 2004. Regulation of ion channel expression. *Circulation Research.* 94: 874-883.

Rottbauer, W., Baker, K., Wo, Z.G., Mohideen, M.P., Cantiello, H.F. & Fishman, M.C. 2001. Growth and function of the embryonic heart depend upon the cardiac-specific L-type calcium channel α1 subunit. *Developmental Cell.* 1: 265-275.

Saito, T. & Tenma, K. 1976. Effects of left and right vagal stimulation on excitation and conduction of the carp heart (*Cyprinus carpio*). *Journal of Comparative Physiology B: Biochemical, Systemic & Environmental Physiology.* 111: 39-53.

Sandblom, E. & Axelsson, M. 2007. Venous hemodynamic responses to acute temperature increase in the rainbow trout (*Oncorhynchus mykiss*). *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 292: R2292-R2298.

Sanguinetti, M., Curran, M., Zou, A., Shen, J., Specter, P., Atkinson, D. & Keating, M. 1996. Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature.* 384: 80.

Santer, R.M. 1985. Morphology and innervation of the fish heart. *Advances in Anatomy, Embryology, & Cell Biology.* 89: 1-102.

Satchell, G.H. 1991. Physiology and form of fish circulation. Cambridge University Press.

Schmitt, N., Grunnet, M. & Olesen, S.P. 2014. Cardiac potassium channel subtypes: new roles in repolarization and arrhythmia. *Physiological Reviews.* 94: 609-653.

Schram, G., Pourrier, M., Melnyk, P. & Nattel, S. 2002. Differential distribution of cardiac ion channel expression as a basis for regional specialization in electrical function. *Circulation Research.* 90: 939-950.

Schram, G., Pourrier, M., Wang, Z., White, M. & Nattel, S. 2003. Barium block of Kir2 and human cardiac inward rectifier currents: evidence for subunit-heteromeric contribution to native currents. *Cardiovascular Research.* 59: 328-338.

Sedmera, D., Reckova, M., deAlmeida, A., Sedmerova, M., Biermann, M., Volejnik, J., Sarre, A., Raddatz, E., McCarthy, R.A. & Gourdie, R.G. 2003. Functional and morphological evidence for a ventricular conduction system in zebrafish and Xenopus hearts. *American Journal of Physiology-Heart & Circulatory Physiology.* 284: H1152-H1160.

Shiels, H. & Farrell, A. 1997. The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca^{2+} to force development in isolated ventricular trabeculae from rainbow trout. *Journal of Experimental Biology.* 200: 1607-1621.

Shiels, H.A., Vornanen, M. & Farrell, A.P. 2000. Temperature-dependence of L-type Ca^{2+} channel current in atrial myocytes from rainbow trout. *Journal of Experimental Biology.* 203: 2771-2780.

Shiels, H.A., Vornanen, M. & Farrell, A.P. 2002. The force–frequency relationship in fish hearts—a review. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology.* 132: 811-826.

Shiels, H.A., Blank, J.M., Farrell, A.P. & Block, B.A. 2004. Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from bluefin tuna and Pacific mackerel. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 286: R659-R668.

Shiels, H.A., Paajanen, V. & Vornanen, M. 2006. Sarcolemmal ion currents and sarcoplasmic reticulum Ca^{2+} content in ventricular myocytes from the cold stenothermic fish, the burbot (*Lota lota*). *Journal of Experimental Biology.* 209: 3091-3100.

Shiels, H.A. & Galli, G.L. 2014. The sarcoplasmic reticulum and the evolution of the vertebrate heart. *Physiology (Bethesda, Md.).* 29: 456-469.

Shiels, H.A., Galli, G.L. & Block, B.A. 2015. Cardiac function in an endothermic fish: cellular mechanisms for overcoming acute thermal challenges during diving. *Proceedings of Biological Sciences / The Royal Society.* 282: 20141989.

Sidhu, R., Anttila, K. & Farrell, A. 2014. Upper thermal tolerance of closely related Danio species. *Journal of Fish Biology.* 84: 982-995.

Somero, G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology.* 213: 912-920.

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

Tessadori, F., van Weerd, J.H., Burkhard, S.B., Verkerk, A.O., de Pater, E., Boukens, B.J., Vink, A., Christoffels, V.M. & Bakkers, J. 2012. Identification and functional characterization of cardiac pacemaker cells in zebrafish. *PloS One.* 7: e47644.

Tibbits, G.F., Hove-Madsen, L. & Bers, D.M. 1991. Calcium transport and the regulation of cardiac contractility in teleosts: a comparison with higher vertebrates. *Canadian Journal of Zoology.* 69: 2014-2019.

Tibbits, G.F., Moyes, C.D. & Hove-Madsen, L. 1992. Excitation–Contraction Coupling in the Teleost Heart. In: Fish Physiology. Academic Press. 12: 267-304.

Tikkanen, E., Haverinen, J., Egginton, S., Hassinen, M. & Vornanen, M. 2017. Effects of prolonged anoxia on electrical activity of the heart in crucian carp (*Carassius carassius*). *Journal of Experimental Biology.* 220: 445-454.

Tort, L., Watson, J.J. & Priede, I.G. 1987. Changes in *in vitro* heart performance in rainbow trout, *Salmo gairdneri* Richardson, infected with *Apatemon gracilis* (Digenea). *Journal of Fish Biology.* 30: 341-347.

Trautwein, W., McDonald, T. & Tripathi, O. 1975. Calcium conductance and tension in mammalian ventricular muscle. *Pflügers Archiv European Journal of Physiology.* 354: 55-74.

Ushakov, B. 1964. Thermostability of Cells and Proteins of Poikilotherms and its Significance in Speciation. *Physiological Reviews.* 44: 518-560.

Vassort, G., Talavera, K. & Alvarez, J.L. 2006. Role of T-type Ca^{2+} channels in the heart. *Cell Calcium.* 40: 205-220.

Verde, C., Parisi, E. & di Prisco, G. 2006. The evolution of thermal adaptation in polar fish. *Gene.* 385: 137-145.

Verhille, C., Anttila, K. & Farrell, A.P. 2013. A heart to heart on temperature: impaired temperature tolerance of triploid rainbow trout (*Oncorhynchus mykiss*) due to early onset of cardiac arrhythmia. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology.* 164: 653-657.

Vornanen, M. 1989. Regulation of contractility of the fish (*Carassius carassius* L.) heart ventricle. *Comparative Biochemistry & Physiology Part C: Comparative Pharmacology.* 94: 477-483.

Vornanen, M. 1997. Sarcolemmal Ca^{2+} influx through L-type Ca^{2+} channels in ventricular myocytes of a teleost fish. *American Journal of Physiology.* 272: R1432-R1440.

Vornanen, M. 1998. L-type Ca^{2+} current in fish cardiac myocytes: effects of thermal acclimation and beta-adrenergic stimulation. *Journal of Experimental Biology.* 201: 533-547.

Vornanen, M., Ryökkynen, A. & Nurmi, A. 2002a. Temperature-dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 282: R1191-R1199.

Vornanen, M., Shiels, H.A. & Farrell, A.P. 2002b. Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology.* 132: 827-846.

Vornanen, M. & Paajanen, V. 2004. Seasonality of dihydropyridine receptor binding in the heart of an anoxia-tolerant vertebrate, the crucian carp (*Carassius carassius* L.). *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 287: R1263-R1269.

Vornanen, M., Stecyk, J.A. & Nilsson, G.E. 2009. The anoxia-tolerant crucian carp (*Carassius carassius* L.). In: Fish physiology. Elsevier. pp. 397-441.

Vornanen, M., Hälinen, M. & Haverinen, J. 2010. Sinoatrial tissue of crucian carp heart has only negative contractile responses to autonomic agonists. *BMC Physiology.* 10: 10.

Vornanen, M., Hassinen, M. & Haverinen, J. 2011. Tetrodotoxin sensitivity of the vertebrate cardiac Na^+ current. *Marine Drugs.* 9: 2409-2422.

Vornanen, M., Haverinen, J. & Egginton, S. 2014. Acute heat tolerance of cardiac excitation in the brown trout (*Salmo trutta fario*). *Journal of Experimental Biology.* 217: 299-309.

Vornanen, M. 2016. The temperature dependence of electrical excitability in fish hearts. *Journal of Experimental Biology.* 219: 1941-1952.

Vornanen, M. & Hassinen, M. 2016. Zebrafish heart as a model for human cardiac electrophysiology. *Channels.* 10: 101-110.

Vornanen, M. 2017. Electrical excitability of the fish heart and its autonomic regulation. In: Fish Physiology, A. Kurt Gamperl, Todd E. Gillis, Anthony P. Farrell, Colin J. Brauner (Eds). Academic Press, San Diego. 36A: 99-153.

Wang, D.W., George, A. & Bennett, P.B. 1996. Comparison of heterologously expressed human cardiac and skeletal muscle sodium channels. *Biophysical Journal*. 70: 238-245.

Wang, Z., Yue, L., White, M., Pelletier, G. & Nattel, S. 1998. Differential distribution of inward rectifier potassium channel transcripts in human atrium versus ventricle. *Circulation.* 98: 2422-2428.

Warmke, J.W. & Ganetzky, B. 1994. A family of potassium channel genes related to eag in Drosophila and mammals. *Proceedings of the National Academy of Sciences of the United States of America.* 91: 3438-3442.

Warren, K.S., Baker, K. & Fishman, M.C. 2001. The *slow mo* mutation reduces pacemaker current and heart rate in adult zebrafish. *American Journal of Physiology-Heart & Circulatory Physiology.* 281: H1711-H1719.

Wells, R., McIntyre, R., Morgan, A. & Davie, P. 1986. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. *Comparative Biochemistry & Physiology Part A: Physiology.* 84: 565-571.

Whalley, D.W., Wendt, D.J., Starmer, C.F., Rudy, Y. & Grant, A.O. 1994. Voltage-independent effects of extracellular K^+ on the Na^+ current and phase 0 of the action potential in isolated cardiac myocytes. *Circulation Research.* 75: 491-502.

Widmark, J., Sundström, G., Ocampo Daza, D. & Larhammar, D. 2010. Differential evolution of voltage-gated sodium channels in tetrapods and teleost fishes. *Molecular Biology & Evolution.* 28: 859-871.

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

Zhang, S. 2006. Isolation and characterization of I_{Kr} in cardiac myocytes by Cs^+ permeation. *American Journal of Physiology-Heart & Circulatory Physiology.* 290: H1038-H1049.

Zhang, P.C., Llach, A., Sheng, X.Y., Hove-Madsen, L. & Tibbits, G.F. 2011. Calcium handling in zebrafish ventricular myocytes. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology.* 300: R56-R66.