Integrated responses of the heart to acute changes in temperature

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Key points

- An acute decrease in temperature impairs cardiac contraction. An increase in temperature has the opposite effect.
- Decreases in temperature are associated with a reduction in the rate of pacemaker firing, an increase in action potential duration, and a decrease in the sensitivity of the myofilaments to Ca^{2+}.
- Increase in temperature are associated with an increase in the rate of pacemaker firing, a decrease in action potential duration, and an increase in the sensitivity of the myofilaments to Ca^{2+}.
- Adrenergic stimulation can ameliorate some of the direct effects of temperature on heart function, but its effects are themselves influenced by temperature.
- As fish approach their upper lethal temperature the counter movement of Na^{+} and K^{+} across the myocyte membrane becomes imbalanced which can lead to arrhythmias and the collapse of heart function.

Glossary

Acclimatization (acclimation) Physiological or morphological changes an organism experiences when exposed to chronic (prolonged) changes in environmental conditions.

Bradycardia A slowing of normal heart rate. In fish, this condition is often associated with hypoxia (low environmental oxygen levels).

Critical thermal maximum (CT_{Max}) The maximum temperature that can be tolerated by an organism. In fish, this variable is most often measured by acutely increasing the temperature (e.g., by 2 °C h^{-1}) until the fish loses equilibrium.

Diastole The period of the cardiac cycle when the ventricle is relaxed or relaxing. It is associated with filling of the ventricle with blood.

Electrocardiogram The electrical recording used to visualize the function of the heart. It is composed of the sequential depolarization and repolarization of the atria and ventricle during the cardiac cycle.

In situ Being in the original position; used to describe experiments where measurements are taken on an organ (tissue) while it is still located within the animal.

Temperature quotient (Q_{10}) A measure of the rate of change of a biological or chemical process as a consequence of temperature increasing or decreasing by 10 °C. The Q_{10} is calculated as: $Q_{10} = R1/R2^{10(T2-T1)}$, where R is the rate at a particular temperature and T the temperature in degrees celsius.

Upper incipient lethal temperature (UILT) Maximum temperature at which 50% of the fish acclimated to a temperature survive after a defined period of exposure to the test temperature.
Thermal inertia The property of a material or substance that determines its ability to resist changes in temperature. It is a measure of how slowly or quickly something heats up or cools down in response to changes in its surroundings.
Thermocline A transition within a body of water, such as a lake, ocean or reservoir, where there is a rapid change in temperature with depth.

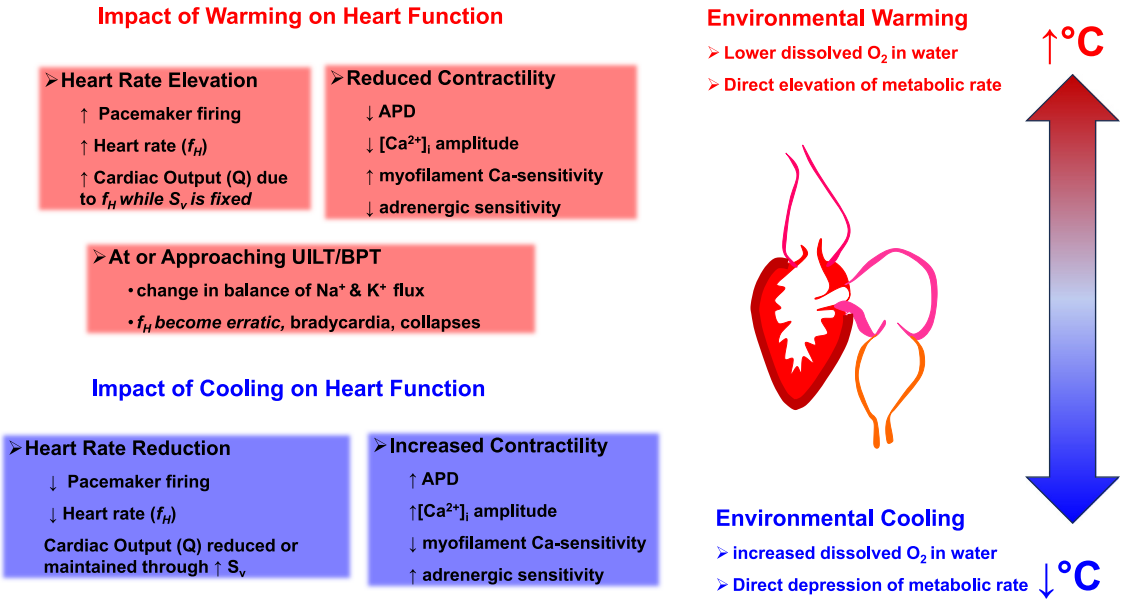
Nomenclature

AP Action Potential
APD Action Potential Duration
AV Atrioventricular
BPT Breakpoint Temperature
cTNC Cardiac Troponin C
Ca^{2+} Calcium
CaO_2 Arterial Oxygen Content
CICR Ca^{2+}-Induced Ca^{2+}-Release
CT_{Max} Critical Thermal Maximum
E-C Coupling Excitation-Contraction Coupling
ECG Electrocardiogram
f_H Heart Rate
f_{PM} Firing Rate of Pacemaker Cells
f_{SA} Firing Rate of Sinoatrial (SA) Node
K^+ Potassium
LTCC L-Type Ca^{2+} Channel
Na^+ Sodium
NCX Na^+-Ca^{2+} Exchanger
Q Cardiac Output
Q_{10} Temperature Quotient
Q_{Max} Maximum Cardiac Output
SA Sinoatrial
SERCA Sarco(Endo)plasmic Reticulum Ca^{2+} - ATPase
SR Sarcoplasmic Reticulum
S_v Stroke Volume
T_aO_2 Arterial Oxygen Transport
TDEE Temperature-Dependent Decline in Electrical Excitability
UILT Upper Incipient Lethal Temperature
V_m Resting Membrane Potential

Abstract

An acute change in physiological temperature can have a significant impact on heart function in fish, and as a result, the delivery of blood to the tissues. A decrease in temperature causes a reduction in the rate of pacemaker firing, an increase in action potential duration, and a decrease in the sensitivity of the myofilaments to Ca^{2+}. Comparatively, an increase in physiological temperature can increase the rate of pacemaker firing, decrease the duration of action potential duration and increase the sensitivity of the myofilaments to Ca^{2+}. These direct effects of temperature on cardiac function can be reduced as a result of adrenergic stimulation. However, too much of an increase in temperature can cause the heart to effectively stop working. As fish approach this temperature (upper lethal temperature) the counter movement of Na^+ and K^+ across the myocyte membrane becomes imbalanced, and this can lead to cardiac arrhythmias and the collapse of heart function.

Teaching slide



Impact of acute temperature change on the fish heart. Details of ion flux at temperatures approaching cold temperature break points are not sufficiently studied to summarise. *f_H heart rate; Q cardiac output, S_v stroke volume, APD, action potential duration, $[Ca^{2+}]_i$ intracellular Ca transient.*

Introduction

Fish have a truly global distribution and live in water with temperatures ranging from −1.8 to ~42 °C. Water temperature can vary over a long-time frame, such as with seasonal changes in temperature. For example, carp species can experience seasonal temperature fluctuations from near 0 °C in winter to 30 °C in summer. Fish also experience temperature changes over short time frames (i.e., from hours to days), or when crossing a thermocline within a body of water. For example, the frillfin goby (*Bathygobius soporator*), which lives in tide pools along the east coast of South America, experiences daily temperature fluctuations from 25 to 40 °C. For fish like lake trout (*Salvelinus namaycush*) living in temperate lakes, the surface of the water may be 15–20 °C warmer than the bottom of the same column of water in the summer, and sometimes thermal inversions can occur in winter. The focus of this article is on how the fish heart responds to short-term (termed **acute**) changes in water temperature. The following article will focus on the mechanisms that facilitate cardiac remodeling associated with long-term (termed **chronic**) changes in temperature.

Temperature is considered the "master regulator" as all processes, chemical, physical, and biological are impacted by temperature. The effect of temperature on biological processes is often quantified by calculating a "Q_{10}" value, which describes how many times (fold) a rate changes for a 10 °C change in temperature. Most reactions that underly physiological processes have a Q_{10} value between 2 and 3. For example, a 10 °C increase or decrease in temperature will cause heart rate to increase or decrease between 2- and 3-fold, respectively. Thus, temperature alone can more than double the rate at which a fish heart beats. This article will start by discussing how acute temperature changes impact the function of the heart at the cellular level, and then build toward an integrative perspective on heart function in relation to climate change.

Thermal inertia and acute changes in cardiac temperature

When considering the impacts of acute temperature changes on fish cardiac physiology, it is worth first considering how fast the temperature of the fish heart changes when the fish moves into water of a new temperature. Because fish are ectotherms their body temperature is normally equivalent to that of the water in which they live. But fish, like all organisms, have thermal inertia (an inherent resistance to changes in temperature) so when they move through waters of different temperatures there will be a certain amount of time before the fish's body (and heart) temperature equilibrates with that of the surrounding water. There are a number of factors that impact this rate of temperature change, including the difference between the temperature of the fish and that of the surrounding water, the size of the fish (larger animals have greater thermal inertia than smaller animals), and the physiological characteristics of the fish [changes in blood vessel distribution to retain (e.g., a rete) or eliminate (e.g., vascularized skin) heat]. However, evidence suggests that fish body temperature rapidly equilibrates (within minutes) with that of the water. This is because the respiratory epithelium of the gill is very thin to allow for efficient gas exchange between the water and the blood, and this results

in the rapid equilibration of temperature between these two mediums. Also, blood can return directly to the heart from the gills as venous drainage, or in the coronary artery which perfuses the outside (compact) layer of the heart (~30–40% of fishes have a coronary circulation).

Acute temperature changes on resting heart function

Heart rate and electrical excitability

Heart rate (f_H) is the easiest and most often measured cardiovascular variable with respect to the effects of temperature on fish cardiac function. Resting f_H increases with temperature in a non-linear manner (see Fig. 1A). This non-linear change is reflected in Q_{10} values for this parameter. Many studies indicate that the Q_{10} for f_H is between 1.3 and 3.0 depending on the range of temperatures and species studied. This means that f_H can increase from 30% to 3-fold with an increase of 10 °C. Resting f_H increases with temperature until a few degrees prior to the maximum temperature that a fish can tolerate [called the upper incipient lethal temperature (UILT) or the critical thermal maximum (CT_{Max})]. This breakpoint temperature (BPT) for f_H can be seen from the bell-shaped response of brown trout (*Salmo trutta*) *in vivo* heart rate to acute increases in temperature in Fig. 1B (f_H, diamonds), and is ~15 °C for this species. Beyond this temperature f_H falls (Vornanen et al., 2014). This reduction—or in some cases the collapse of f_H—after the BPT can have dire consequences for the fish's ability to deliver oxygen and metabolites to energy demanding tissues, and can even lead to death. The possible mechanisms underlying collapse of f_H after the BPT are discussed below.

Temperature increases resting f_H partially through its effects on the pacemaker cells of the fish heart. The pacemaker, or sinoatrial (SA) node, is a cluster of specialized cells found at the junction between the sinus venous and the atrium that generates electrical impulses to regulate the rhythm of the heart (Hassinen et al., 2017). These impulses coordinate the contraction of the heart's muscle. Temperature directly impacts the firing rate of the fish's pacemaker cells, such that warming increases f_H and cooling decreases it. This is because temperature affects the probability that ion channels, which regulate the generation and propagation of the electrical impulses (action potentials) of the heart's pacemaker cells, will be open or closed. The firing rate of the pacemaker is also influenced by innervation from the autonomic nervous system [cholinergic (inhibitory) and adrenergic (stimulatory) nerves], and by circulating hormones like adrenaline which increase f_H. Together, these factors combine to set the rate at which the fish heart beats. For example, an acute increase in temperature is normally associated with a decrease in cholinergic tone and an increase in adrenergic tone on the heart, both of which elevate f_H.

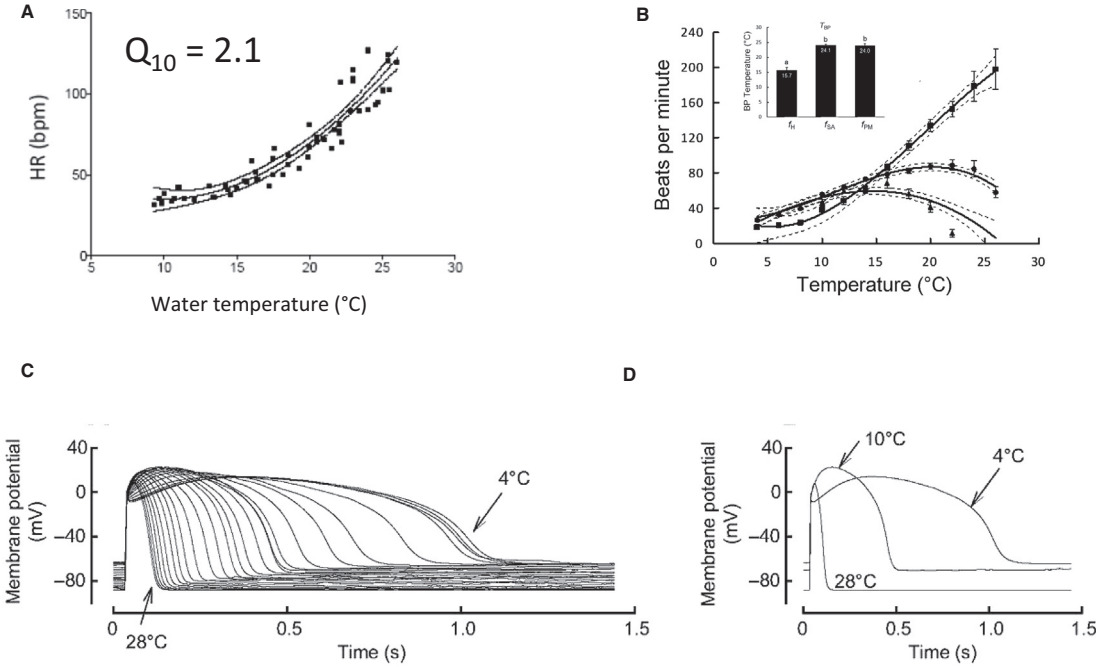


Fig. 1 (A) Acute warming increases *in vivo* heart rate in the brown trout (*Salmo trutta*) (n = 4) fish, non-linear least squares regression fit ±95% confidence intervals, respectively, from heart rate recordings of 256 consecutive cycles; (B) Comparison of the effect of acute warming on *in vivo* heart rate (f_H, diamonds), the beating rate of sinoatrial preparations *in vitro* (f_{SA}, circles), and enzymatically isolated pacemaker cells (f_{PM}, squares). Inset shows mean breakpoint temperate (T_{BP}) under each condition. (C) The effect acute increases in temperature from 4 to 28 °C on the shape of ventricular action potential from isolated myocytes from the brown trout. (D) Three APs at selected temperatures from the myocyte in (C). Notice the progressive shortening of action potential duration and hyperpolarization of the resting membrane potential with warming. (B) Adapted from Vornanen et al. (2014). (D) Adapted from Haverinen et al. (2017).

Importantly, the BPT for pacemaker function is higher than that for f_H. This is illustrated in Fig. 1B. The firing rate of an isolated SA node preparation from brown trout has a BPT of ~24 °C (f_{PM}, square symbols), as compared to ~15 °C for f_H, (see f_{SA}, round symbols) (Vornanen et al., 2014). These data tell us that it is not failure of the pacemaker cells, or propagation of the electrical impulse from the SA node that fails at the BPT when the fish heart warms (Vornanen et al., 2014). Rather, there is failure at the level of the working heart (myocardial) cells. This observation may be explained by the Temperature-Dependent Decline in Electrical Excitability (TDEE) hypothesis. It suggests that heart failure occurs at BPT due to reduced electrical excitability of the ventricular cardiomyocytes, and that this is due to a temperature-driven mismatch between the ion currents responsible for initiating and propagating action potentials in this heart chamber. Specifically, the TDEE hypothesis suggests that when temperatures rise, the increase in the outward movement of K^+ (leakage) leads to a heightened requirement for inward Na^+ currents to initiate a ventricular action potential. This K^+ leakage is shown as the decrease in resting membrane potential (V_m) in panels C and D of Fig. 1. However, acute warming also decreases the open duration of Na^+ channels, and this results in a decrease in inward Na^+ current, just when more Na^+ is needed. This difference in the temperature sensitivity of repolarizing K^+ currents and depolarizing Na^+ currents in the ventricular cells compromises the generation of ventricular action potentials. This can be observed as atrioventricular (AV) block, where action potentials generated by the atrium, and that pass through the AV node, are unable to depolarize cardiomyocytes of the ventricle.

Interesting, this interplay between Na^+ and K^+ currents, and temperature, is a universal phenomenon in excitable tissues, suggesting that the TDEE plays a key role in determining the upper temperature limits of various tissues, such as neurons, skeletal muscle cells, smooth muscle cells, and some glandular cells (Vornanen et al., 2014; Haverinen and Vornanen, 2020). It has also been suggested that loss of mitochondrial function at high temperatures contributes to the upper temperature limit of the heart (Iftikar and Hickey, 2013).

Excitation-contraction coupling

At a given contraction frequency (f_H), cooling tends to increase myocardial contractility (i.e., force generation and rate of relaxation), whereas warming tends to have the opposite effect (Shiels et al., 2002b) (Fig. 2A). Changes in contraction of the whole heart are determined by changes at the cellular level; i.e., in the cardiomyocytes that make up the heart. Excitation-contraction coupling (E-C coupling) links depolarization of the cardiomyocyte with the inward movement of calcium (Ca^{2+}) ions through L-type Ca^{2+} channels (LTCC) in the sarcolemma (cell membrane). The subsequent release of Ca^{2+} from the sarcoplasmic reticulum (SR) of the heart cells results in contraction, and relaxation is dependent upon the removal of Ca^{2+} from the heart's cells via pumps on the sarcoplasmic reticulum membrane and Na^+/Ca^{2+} exchangers on the sarcolemma. The more Ca^{2+} that enters the cytosol during a heartbeat, the greater the strength of contraction. The faster the Ca^{2+} rises and falls in the cytosol of the cell with each beat, the faster the rates of contraction and relaxation. Temperature impacts all of these processes to some degree (Vornanen et al., 2002; Vornanen, 2016).

Effects of acute temperature change on the action potential

Acute warming increases the rate at which Na^+ channels open and close, as well as impacting open probability. Faster opening and closing means that the action potential is faster (i.e., of shorter duration), at warm compared with cooler temperatures. This can be seen in Fig. 1C and D where the action potential duration (APD) at 50% repolarization is ~800 ms at 7 °C and <100 ms at 28 °C (Haverinen et al., 2017). Temperature-dependent changes in the shape of the action potential impact how long Ca^{2+} flows from the extracellular space to the inside of the cell, thus impacting contraction of the myofilaments.

Effects of temperature on the Ca^{2+} transient

The rate and amplitude of the rising phase of the Ca^{2+} transient is directly affected by temperature. This is illustrated for a rainbow trout (*Oncorhynchus mykiss*) atrial myocyte in Fig. 2. Panel (B) shows the effect of a rapid (within seconds) temperature change on the Ca^{2+} transient, and panel (C) shows the effect on Ca^{2+} influx through LTCCs. Warm temperatures tend to speed up the rise of the Ca^{2+} transient. This is partially because of the effect of temperature on Ca^{2+} influx via the LTCCs, which is faster and greater in amplitude at warm temperatures (shown by the larger downward deflection in Fig. 2C). Cool temperatures tend to slow down the rate of the rise in Ca^{2+} levels, but also increase the amplitude of the Ca^{2+} transient. This is because the slower kinetics allow more time for Ca^{2+} influx across the sarcolemma through the LTCC during the AP.

Temperature also impacts the decay phase of the Ca^{2+} transient. This is because the activity of the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) pumps, which move Ca^{2+} from the cytosol back into the sarcoplasmic reticulum (allowing the myocyte to relax), is also temperature-dependent in fish. This can be seen as the slower decay rate of the tuna ventricular Ca^{2+} transient in Fig. 2C. It is important to note that tuna are athletic fish which actively utilize the SR during E-C coupling. SR-dependent acute temperature effects will vary with the degree of SR utilization during E-C coupling between species (Shiels and Galli, 2014; Vornanen, 2021).

Interestingly the activity of the Na^+- Ca^{2+} exchanger, which is both a Ca^{2+} influx pathway across the sarcolemmal membrane during the rising phase, and an efflux pathway during the falling phase, is relatively insensitive to temperature change (Xue et al., 2001). This means that this pathway operates continuously despite changes in the thermal environment. This could certainly be beneficial for heart function in fish living in fluctuating thermal environments.

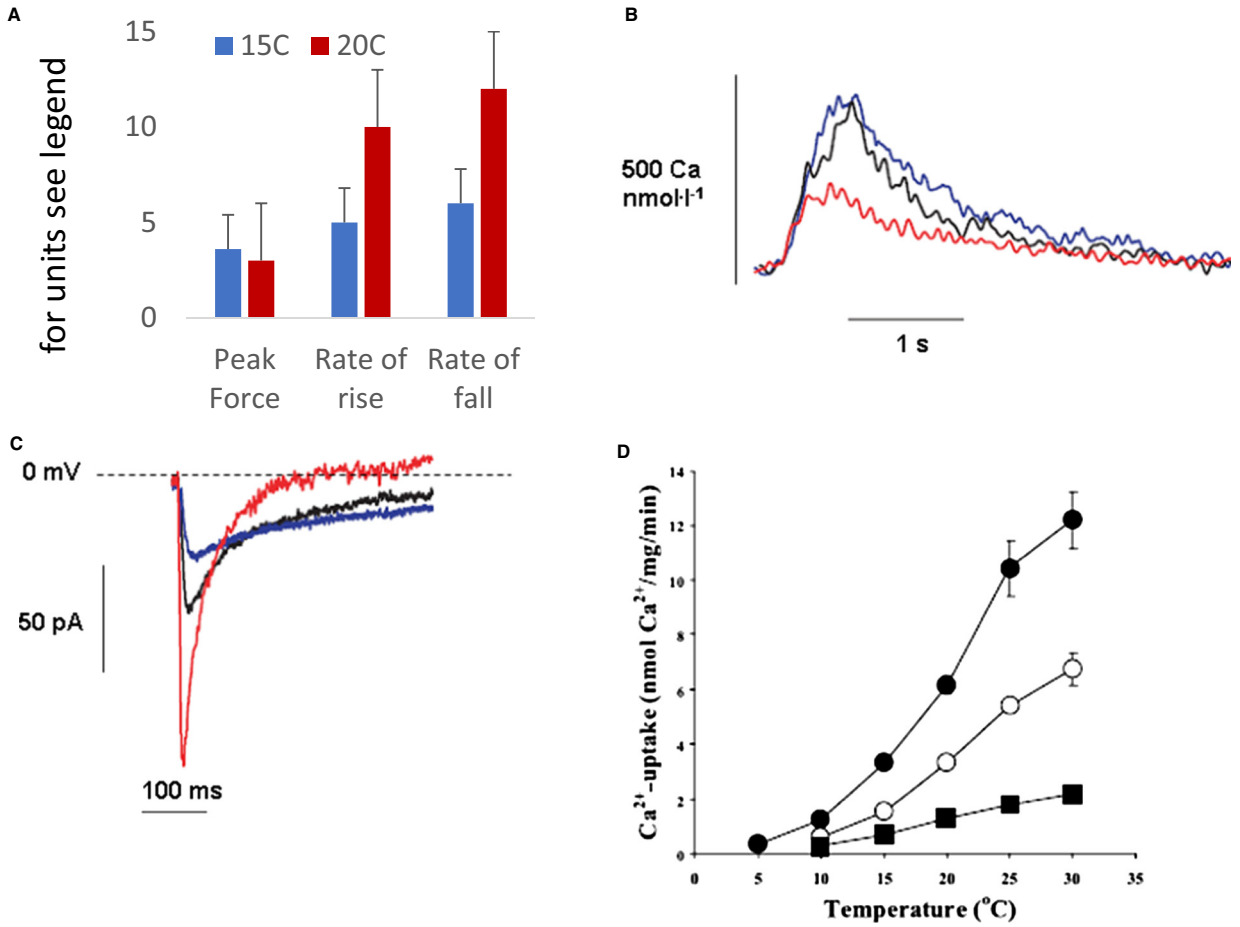


Fig. 2 The effect of acute temperature change on calcium flux in fish myocardium. (A) Effect of acute temperature change on ventricular contractile parameters from Pacific mackerel (*Scomber japonicus)* ventricle. Peak force is in mm mN^{-2} and rates are in mm mN^{-2} sec^{-1}. (B) The effect of acute temperature change on the cellular Ca^{2+} transient and (C) the LTCC currents recorded simultaneously from an atrial myocyte from a 14 °C acclimated rainbow trout (*Oncorhynchus mykiss*) tested acutely at either 7 (blue), 14 (black) and 21 (red) °C. In this example the myocytes were stimulated at 0.2 Hz with a 500 ms square pulse from −80 to +10 mV to highlight the effect of acute temperature on the Ca^{2+} transient and current. (A) Shows the effect of temperature on the Ca^{2+} transient in this species. Warm temperatures result in smaller Ca^{2+} transients than cold temperatures. (B) Shows Ca^{2+} influx as an ionic current (measured in pico Amps, pA) across the SL membrane through the LTCCs. Warm temperature gives a faster current with a larger peak amplitude than colder temperatures. However, at colder temperatures the ion channels stay open longer so that more Ca^{2+} crosses the SL and the Ca^{2+} transient is bigger. (D) The temperature dependence of SR Ca^{2+} uptake in isolated SR membrane preparations from different tuna species. ● Pacific bluefin (*Thunnus orientalis)*; ○, albacore (*Thunnus alalunga)*; ■ yellowfin (*Thunnus albacares)*. The figure clearly shows how SERCA function depends on species and also how cold temperature reduces SERCA activity. Values are means ± SE of 3–4 experiments. (A) Adapted from Shiels and Farrell (2000). (B) Data adapted from Shiels et al. (2002a). (D) Adapted with permission from **Fig. 2**A in Landeira-Fernandez et al. (2004).

Effect of adrenergic stimulation on the temperature sensitivity of EC coupling

Fish cannot avoid the effects of temperature on their heart cells as they swim through water of different temperatures. However, certain hormones can compensate for the effects of temperature on cellular Ca^{2+} flux. For example, adrenaline is released when a fish moves rapidly into water of a new temperature, and it increases Ca^{2+} flux through the LTCC. The direct effect of an acute temperature change on Ca^{2+} influx into rainbow trout myocytes (i.e., through LTCC in the sarcolemmal membrane) can be seen in **Fig. 3**A. The amplitude of Ca^{2+} inflow increases with warming, and the rate of change is faster (Shiels et al., 2003). Overall, this means that less Ca^{2+} flows into the cell when acutely warmed as compared to when acutely cooled. Further, although adrenaline increases the flow of Ca^{2+} into the cell at all temperatures, adrenaline is more effective at acutely cold than acutely warm temperatures (Shiels et al., 2003). The reason for this response is still unclear. In ventricular myocytes from the bluefin tuna (Shiels et al., 2015) the amplitude of the intracellular Ca^{2+} transient is also impacted by acute temperature change (**Fig. 3**B); however, adrenergic stimulation increased the amplitude of the transient at all temperatures. This may be because tuna routinely cross thermoclines in the ocean when foraging for food, whereas this is less common for trout living in lakes.

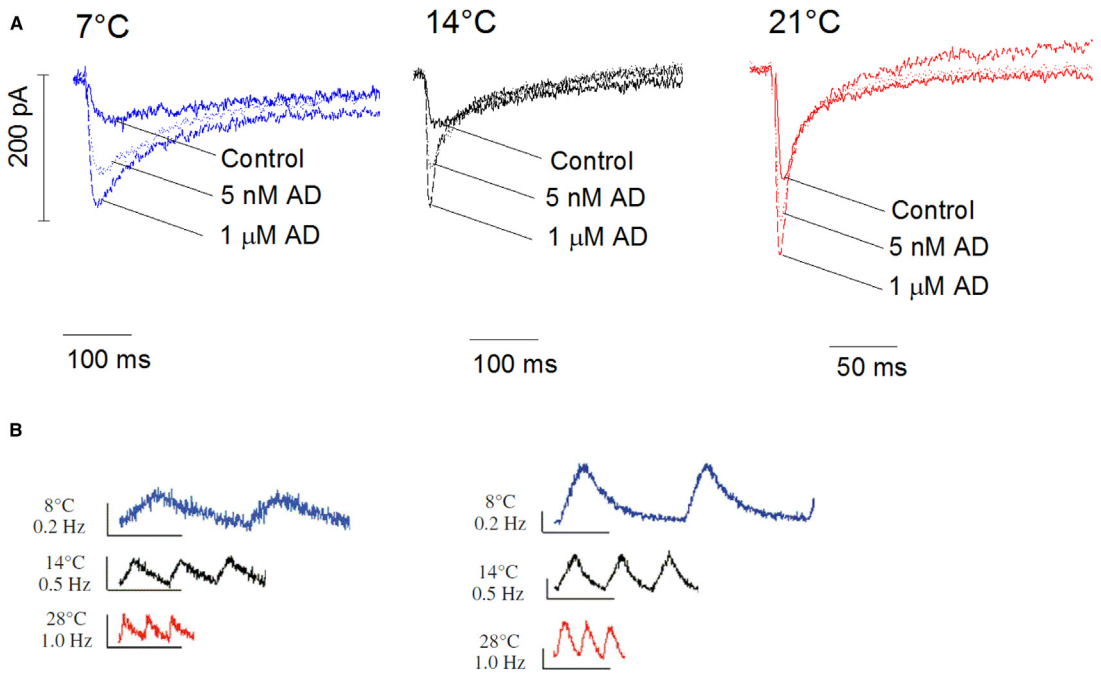


Fig. 3 Effect of adrenaline on cellular Ca^{2+} flux during acute temperature change. (A) Acute temperature change on calcium influx (downward deflection) through the LTCC in atrial myocytes from the rainbow trout under control conditions, and in the presence of low and high adrenergic stimulation. Notice the direct effect of temperature on calcium influx (I_{Ca}, the Ca^{2+}-current) and its impact on the response to adrenaline (Shiels et al., 2003). (B) The effect of acute temperature change on the intracellular Ca^{2+} transient in the bluefin tuna ventricular myocyte control conditions. (C) The impact of acute temperature on the intracellular Ca^{2+} transient of the bluefin tuna ventricular myocyte when the cell was exposed to 500 nM adrenergic stimulation. The temperature and the frequency of contraction (Hz) are written on the left of each panel. The frequencies of contraction reflect the *in vivo* heart rate of the tuna at each of these acute temperatures. The horizontal bars show the time course of the transient and represent 3 s in each image. The vertical bars show the amplitude of contraction in arbitrary units and is 1 in all images. Notice how adrenaline increases the amplitude of the Ca^{2+} transient at temperatures in the tuna.

Effects of temperature on myofilament function

An acute change in heart temperature also alters the Ca^{2+} sensitivity of the cardiac myofilaments. More specifically, an increase in temperature increases the Ca^{2+} sensitivity of the myofilament while a reduction in temperature has the opposite effect. The end result is a change in the force generating capacity of the muscle (Churcott et al., 1994; Harrison and Bers, 1987; Stephenson and Williams, 1985). The decrease in Ca^{2+} sensitivity of cardiac muscle with an acute reduction in temperature has been reported in multiple species, including trout, frogs, mice, rabbits, ferrets and ground squirrels (Churcott et al., 1994; Harrison and Bers, 1987; Liu et al., 1990, 1993). One reason for this effect is that the Ca^{2+} affinity of cardiac troponin C (*c*TnC), which is the Ca^{2+}-activated trigger for muscle contraction, decreases with temperature (Gillis et al., 2000, 2005; Gillis and Tibbits, 2002). A comparison of force generation by trout cardiac myofilaments to that from a number of mammalian species reveals that the Ca^{2+} sensitivity of trout preparations is approximately 10 times that of mammals (Harrison and Bers, 1989) when measured at the same temperature. It is thought that the higher Ca^{2+} sensitivity of the trout myofilaments helps to compensate for the comparatively lower temperature that fish species live at (Gillis et al., 2000). This higher Ca^{2+} sensitivity of the trout myofilament is due, in part, to *c*TnC in the trout heart having an ~2 fold greater Ca^{2+} affinity than in the mammalian heart (Gillis et al., 2000) (**Fig. 4**).

Integrative effect of acute temperature change on fish heart function

Fish must be able to adjust the delivery of oxygen and energy substrates to meet temperature-dependent changes in tissue demand. In addition, they must try to accommodate these temperature-related demands while providing for other life processes that are critical to the fish's survival and fitness (e.g., growth, digestion, swimming, migration, and reproduction).

In fishes, the oxygen delivered from the gills to the tissues (T_aO_2, arterial oxygen transport) is determined by cardiac output (Q, the amount of blood pumped by the heart per unit time) and the amount of oxygen carried in the arterial blood (C_aO_2, arterial oxygen content) according to the following equation: $T_aO_2 = Q \times C_aO_2$. Since Q can vary by up to 3−5-fold in fishes, changes in Q can have an enormous influence on how much oxygen is delivered to the tissues. In fact, any environmental change that alters the fish's oxygen consumption usually involves a change in Q. Furthermore, arterial blood is normally nearly fully saturated with

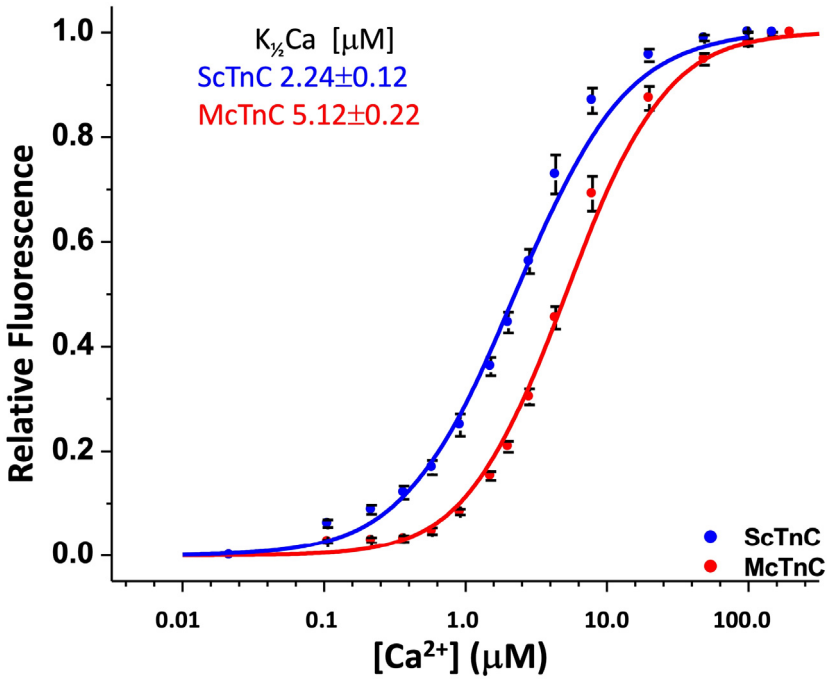


Fig. 4 Difference in Ca^{2+} sensitivity between bovine cardiac troponin C (BcTnC) and salmonid cardiac troponin C (ScTnC). The curves generated by fitting the data with the Hill equation have been added for comparison against the data points. Titration of fluorescence of BcTnC (n = 10) and ScTnC (n = 9) was completed at 21.0 °C, pH 7.0. Data are normalized with respect to the maximal fluorescence of each Ca^{2+} titration and presented as means ± SE. The $K_{1/2}$ of the curves are significantly different from each other ($P < 0.05$). Adapted from Gillis et al. (2000).

oxygen, and it remains this way when temperature changes. Therefore, changes in C_aO_2 are not appreciable as compared with Q when the fish experiences changes in temperature.

When exposed to acute changes in temperature there are two ways in which Q can be increased to meet this change in energy demand. Cardiac output is the product of f_H and stroke volume (S_V; the amount of blood pumped per heart beat), and thus, a change in either of these parameters will affect Q. Interestingly, when fish at rest are acutely exposed to warmer temperatures they only increase f_H (Fig. 4A), and heart rate continues to increase until approximately 1–2 °C before the fish reaches its critical thermal maximum (CT_{Max}). At this point, contraction of the ventricle becomes irregular (arrhythmic), and can even settle at a new (lower) contraction rate as the fish is further warmed; i.e., there is a "ventricular bradycardia" (Figs. 4 and 5). The onset of arrhythmias and the sustained bradycardia that follows are not related to reduced excitability or dysfunction of the pacemaker cells as detailed above, rather they are associated with ventricular failure as explained by the TDEE hypothesis. Additional support for this hypothesis is that electrical excitation [as assessed by electrocardiogram (ECG) recordings] between different parts of the heart was coordinated when

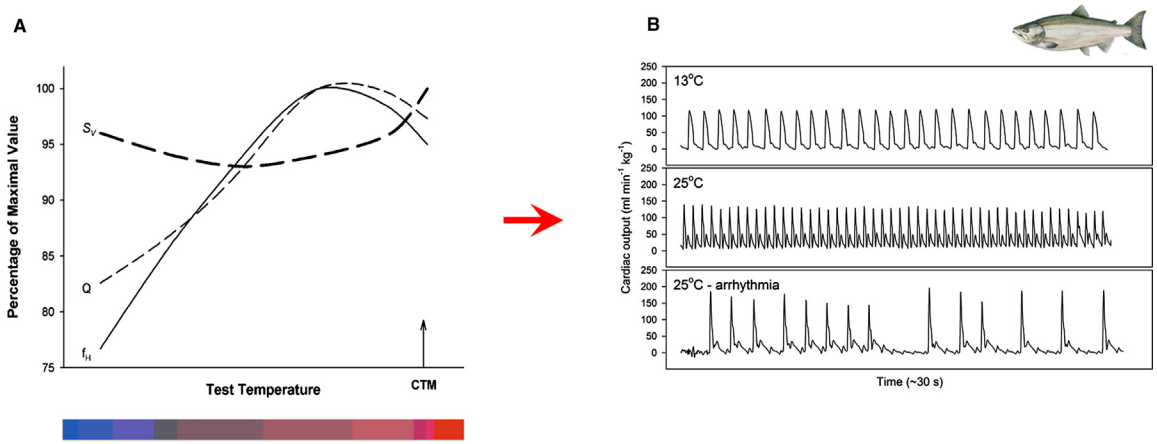


Fig. 5 (A) Schematic representation of how an acute increase in temperature affects resting cardiac parameters: heart rate (f_H), stroke volume (Sv) and cardiac output (Q). CT_{Max} is the fish's critical thermal maximum. (B) Traces of blood flow (cardiac output) in chinook salmon (*Oncorhynchus tshawytscha*) at their acclimation temperature, and when temperature was acutely increased to 25 °C. The two traces at 25 °C show Q in the salmon just before, and after, the heart became arrhythmic. (A) Modified from Gamperl et al. (2011). (B) Modified from Clark et al. (2008).

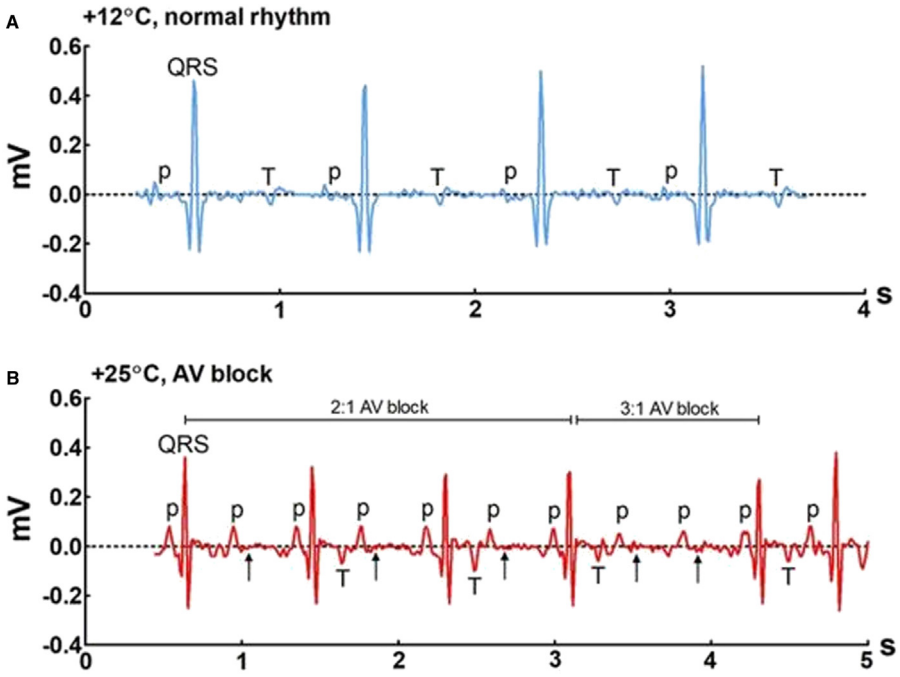


Fig. 6 Effects of acute warming on the electrocardiogram (ECG recording) from a rainbow trout (*Oncorhynchus mykiss*) heart. In these recordings, the "QRS" complex indicates ventricular depolarization (contraction), whereas the "P" wave indicates atrial depolarization. (A) ECG recording at 12 °C, note how the QRS and P waves are 1:1 and occur at the same timepoint in every heartbeat. (B) This recording was taken at 25 °C, and shows that ventricular depolarization is not able to keep up with that of the atrium. The arrows indicate where we would normally be expecting a QRS complex. The ratio changes from 1:1 to 2:1, and there is even a point at which the ratio is 3:1. This is considered atrioventricular (AV) block. S = seconds.

rainbow trout preparations were warmed from 12 to 25 °C. Yet, with further warming, while the atrial contraction rate continued to increase and peaked at value of ~188 beats min^{-1} at 27 °C, the ventricular contraction rate dropped from 124 to ~110 beats min^{-1} (**Fig. 6**, Haverinen and Vornanen, 2020). Further, research has shown that the delay in electrical conduction across the tissue which separates the atrium and ventricle (the AV junction/canal) is insensitive to the effects of warm temperatures (Kuzmin et al., 2022). Thus, it appears that the ventricular "bradycardia" or other forms of ventricular arrhythmia that occur at high temperatures in fish are due to the inability of the action potential that passes through the AV canal to appropriately stimulate (depolarize) the ventricular cells in this region. The molecular mechanism for this was described above in the discussion of the TDEE hypothesis.

Having the atrium and ventricle contract in an uncoordinated fashion is obviously detrimental to Q, and this parameter falls when temperatures approach the fish's Upper Incipient Lethal Temperature (UILT). Some authors have reported that an increase in cholinergic tone is observed at high temperatures and speculated that the resultant slowing of action potential generation in the cardiac pacemaker may serve to synchronize the pacemaker's rate with the functional depolarization/contraction rate of the ventricle, thus avoiding (or reducing the severity of) AV block at higher temperatures (Gilbert et al., 2019). However, more recent studies could not replicate this effect. For example, Ekström et al. (2021) were unable to show that cholinergic blockade (using the drug atropine) affected the temperature at which f_H peaked or the CT_{Max} of yellow perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*).

While cholinergic blockade of the heart does not appear to influence the sensitivity of the heart to high temperatures, there is evidence that sympathetic stimulation (i.e., via circulating catecholamines or sympathetic innervation) plays an important role in supporting *in vivo* heart function when fish are exposed acutely to high temperatures. Blocking the fish's β-adrenergic receptors resulted in a decrease in the temperature at which f_H peaked and the fish's CT_{Max}, and a higher prevalence of arrhythmias at high temperatures (Ekström et al., 2021). This is not surprising as β-adrenergic stimulation is known to protect cardiac contractility during adverse conditions associated with acute warming [e.g., hypoxia, acidosis and/or hyperkalemia; Hanson et al. (2006)]. In addition, Gilbert et al. (2019), suggested that adrenergic stimulation of the heart may reduce/partially rectify the imbalance in Na^+ and K^+ flux rates in ventricular myocytes that are responsible for the failure of myocardial action potential conduction and cardiac excitability (i.e., the TDEE hypothesis), and a declining f_H at high temperatures.

Interestingly, Ekström et al. (2021) showed that blocking cholinergic tone the heart of European perch and roach allowed these species to achieve much higher f_H values than in control fish when warmed to their CT_{Max} (~160−180 vs. 120−130 beats min^{-1}). That cholinergic tone is not completely removed at high temperatures in fish makes sense physiologically, as the maximum contraction frequency of the ventricle is much lower than that of the atrium, and allowing the pacemaker cells to fire at a faster rate (i.e., resulting in an increase in the rate of atrial contraction), would only exacerbate the discrepancy in the rate of contraction of the two chambers (i.e., ventricle vs. atrium), and potentially have a negative impact on Q.

Role of stroke volume in modulating cardiac output with acute changes in temperature

Why resting fish don't increase S_V alone, or both f_H and S_V, when warmed is still a mystery for several reasons. First, fish can often increase S_V by 2−3-fold when swimming maximally. Second, if increases in f_H with temperature are prevented using the drug zatebradine, temperature-dependent increases in trout Q, and maximum Q, are not different as compared to control fish due to a compensatory augmentation of S_V (Gamperl et al., 2011; Keen and Gamperl, 2012, **Fig. 7**). Thus, if f_H does not increase with temperature, an increase in S_V would provide the extra pumping capacity required at high temperatures. This finding suggests that increases in f_H are unavoidable because of the direct effects of temperature on the frequency of pacemaker discharge, and possibly, that increasing cholinergic tone on the heart to slow f_H may not be an option. Acetylcholine has negative inotropic effects on atrial muscle (Holmgren, 1977), and thus, spillover of this neurotransmitter from the sino-atrial node to the atrium could have significant negative consequences for ventricular filling. In addition, it is possible that increasing cholinergic tone at high temperatures may result in arrhythmias, and thus, diminish cardiac function due to effects on pacemaker function. Third, increasing S_V, as opposed to f_H, would benefit the heart by: (1) avoiding negative force−frequency effects on myocardial contractility (Shiels et al., 2002b); (2) maintaining/improving oxygen delivery to the myocardium, and (3) enhancing the efficiency of ventricular muscle contraction (see Farrell, 2007). Finally, although it had been previously suggested that f_H must increase with temperature to match the increase in ventilation rate (i.e., to ensure that there is cardiorespiratory synchrony), Keen and Gamperl (2012) did not find any effects of slowing f_H with zatebradine on the oxygenation of trout arterial blood.

Given the increases in f_H observed in resting fish with temperature, there are a number of explanations as to why S_v does not increase as the fish is warmed. It may be difficult to fill the heart with blood. Central venous pressure (which is a key determinant of cardiac filling) does not change with increased temperature until ventricular bradycardia occurs (likely due to pooling of blood in the venous vessels at the point of cardiac failure) (Sandblom and Axelsson, 2007; Clark et al., 2008), and the time available to fill the heart decreases as f_H increases. Also, the negative force-frequency affect (Shiels et al., 2002b) means that it becomes increasingly difficult for the ventricle to eject blood into the circulation.

With regard to how acute increases in temperature affect the contribution of f_H and *Sv* when fish need to swim maximally, the results mirror those reported for resting fish. For example, Steinhausen et al. (2008) showed that sockeye salmon (*Oncorhynchus nerka*) acclimated to 15 °C increased their Q by about ∼2.5-fold as compared to resting values, due to an ∼1.75-fold increase in S_v but only an ∼1.25-fold increase in f_H (**Fig. 8**). However, when they are swum after acute exposure to increasingly higher temperatures (up to 24 °C), there was no capacity to increase S_v, and the ∼20% increase in Q measured when exercised maximally at higher temperatures was achieved solely by increases in f_H (**Fig. 8**). This indicates that while S_v is responsive to exercise, maximum S_v has no capacity to increase as temperatures warm acutely, and the capacity of fishes to perform at higher temperatures is determined by their maximum f_H or scope for f_H.

Integrated function of the fish heart during acute cooling

There are very few studies that have looked at the effects of acute decreases in temperature on cardiac function in fishes, and the available data on this environmental challenge are quite varied. This is likely due to different responses between species and the experimental protocols/preparations used. For example, *in situ* maximum cardiac output (Q_{Max}) was ∼40−45% lower when the hearts from summer-acclimated (12−14 °C) sea raven (*Hemitripterus americanas*) were tested at 3.3 °C as compared to at 13.3 °C (Graham and Farrell, 1985). Lurman et al. (2012) showed that Q_{Max} was only ∼25% lower when *in situ* hearts from cod (*Gadus morhua*) were tested at 10 °C vs. acutely exposed to 0 °C, and that this was solely due to a decrease in intrinsic f_H (from ∼41 to 24 beat min^{-1}) because maximum S_v was not affected. Finally, Porter and Gamperl (2023) showed that resting Atlantic salmon (*Salmo salar*) acutely exposed to 1 °C had a Q value that was the same as 8 °C-acclimated fish (measured at 8 °C). This result was because S_v compensated for the temperature dependent decrease in f_H (from ∼65 to 27 beats min^{-1}), but that this meant that the capacity to increase S_v when exercised was constrained and this resulted in a much lower Q_{Max} at 1 °C. This latter result was particularly interesting, as the capacity of salmon to increase f_H when they exercise at cold temperatures was also very limited, and this meant that fish acutely exposed to a decrease in temperature had to rely almost entirely on an increase in the amount of blood extracted by the tissues (not Q) to meet the metabolic demands of maximal exercise. With regards to the control of cardiac function at these temperatures, the data are extremely limited. However, Porter et al. (2022) recently showed that salmon acutely exposed to a temperature decrease from 8 to 1 °C had an unexpectedly high adrenergic (sympathetic) tone on the heart. These data suggest that this stimulation of the heart is critical to supporting cardiac function at such low temperatures. Clearly, a lot more research needs to be conducted before we understand how acute decreases in temperature to near a fish's lower thermal limit affect cardiac function at rest and during exercise.

Summary

As global temperatures rise due to climate change, fish face several key challenges in maintaining cardiac function. Warm water holds less dissolved oxygen than cooler water, and this leads to a reduction in oxygen availability in their environment. This is coupled with the increased demand for oxygen that is associated with elevated metabolic rate at warm temperatures. With decreased oxygen availability and increased metabolic demand, fish must pump blood more rapidly to compensate, and we have discussed

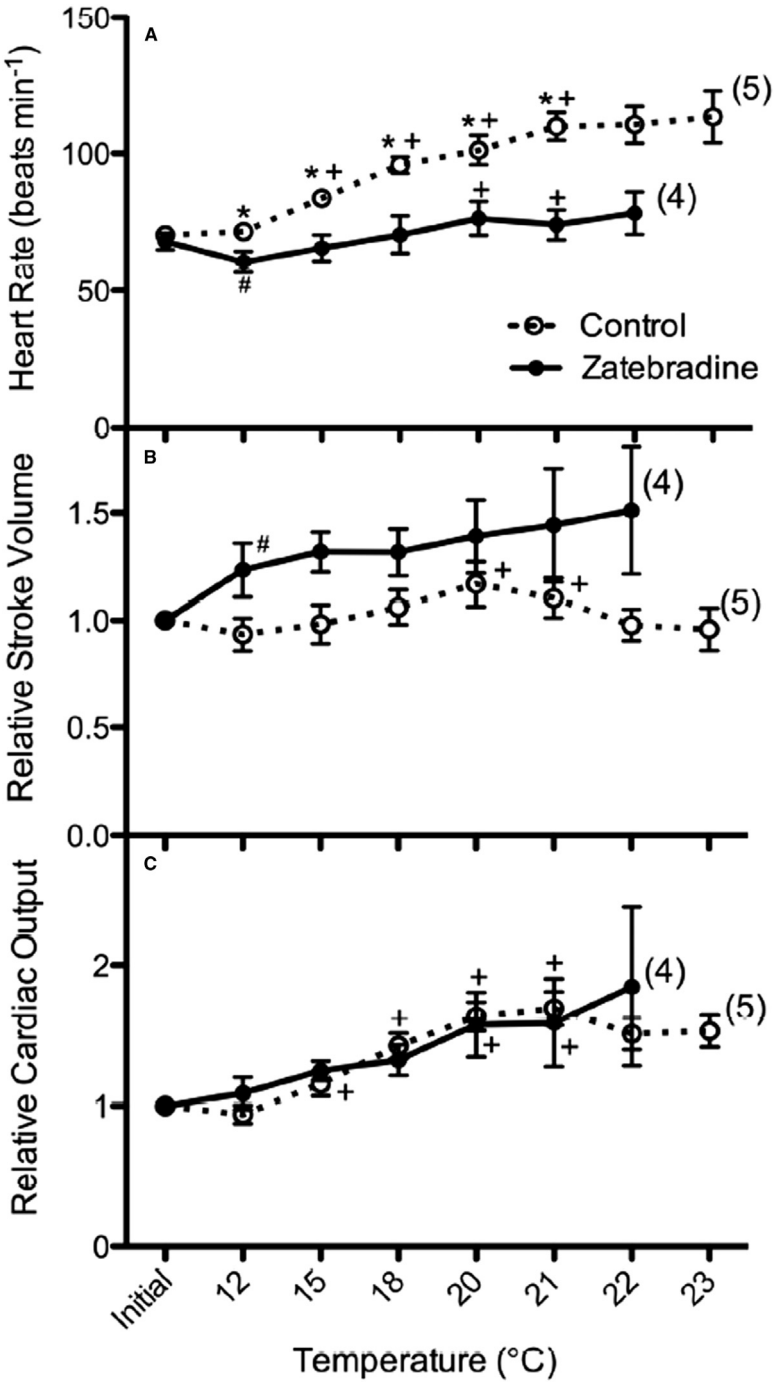


Fig. 7 Effect of zatebradine hydrochloride (1 mg kg^{-1}) on trout (*Oncorhynchus mykiss*) *in vivo* cardiovascular variables during an incremental temperature increase from 12 °C to the fish's CT_{Max}. $^{+}$Indicates a significant difference from values at 12 °C within each group. $^{\#}$Indicates a significant difference between pre-injection (saline or zatebradine; "initial") values and those 30 min post-injection (12 °C). *Indicates a significant difference between groups at a particular temperature. Values are means ± S.E. (n = 7–8). Numbers beside the symbols indicate data points with a reduced number of animals. Modified from Keen and Gamperl (2012).

here how fish increase Q during warming primarily by increasing f_H. However, temperature also impacts the flow of ions that coordinate the electrical and contractile activity of the heart, altering the shape of the cardiac action potential and the ability of the contractile elements to deliver force. These direct effects of temperature on cellular ion flux are most extreme as temperatures are increased or decreased beyond the fish's optimal thermal range, and this suggests that temperature-dependent cardiac performance may be a major determinant of the thermal tolerance of fishes. Each fish species has a specific range of temperatures within which they can function optimally, although this can be adjusted seasonally or with thermal acclimation. When temperatures approach

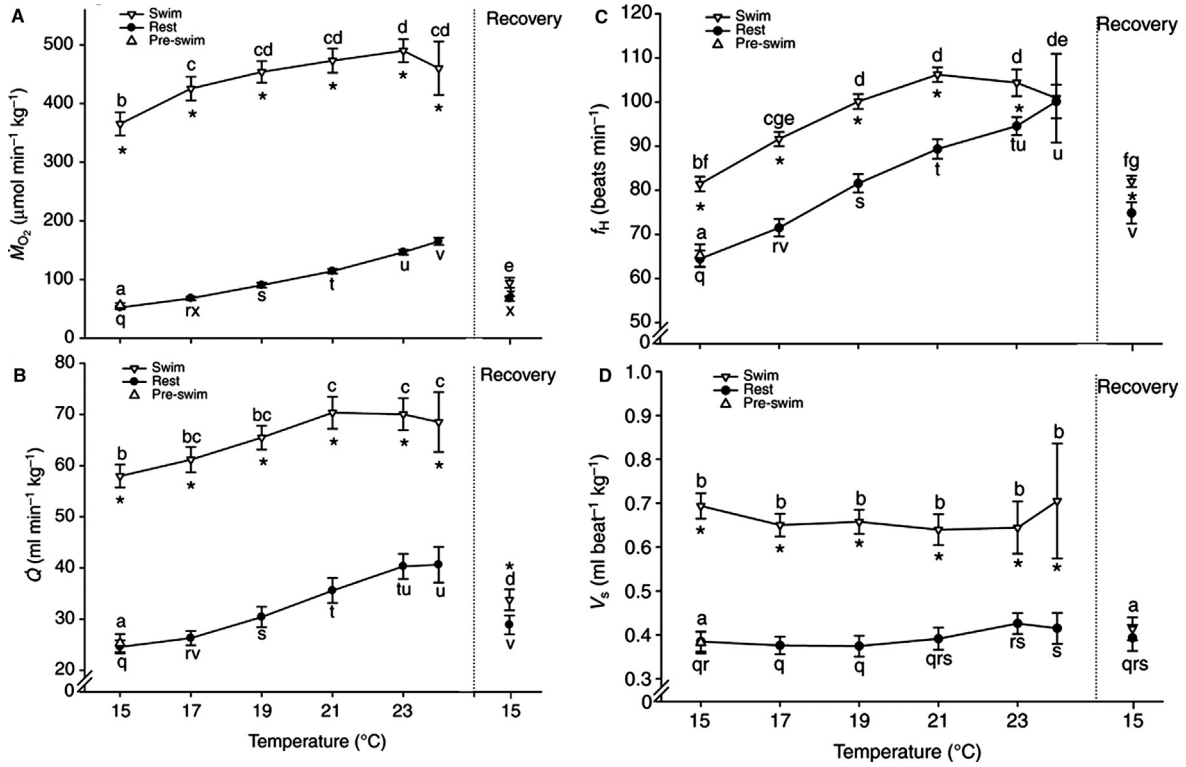


Fig. 8 Effect of acute increases in temperature on the resting and swimming oxygen consumption (MO_2), heart rate (f_H), cardiac output (Q) and stroke volume (Sv) of 15 °C acclimated sockeye salmon (*Oncorhynchus nerka*). The fish were forced to swim constantly at 75% of their maximum aerobic swimming speed after being acutely exposed to a temperature increase from 15 °C to their test temperature. Different letters indicate significant differences between temperatures within the same group (rest or swim). An asterisk (*) indicates a significant difference between resting and swimming fish at a given temperature. Modified from Steinhausen et al. (2008).

the fish's upper thermal tolerance (i.e., CT_{Max} or UILT), imbalances in ion fluxes occur (e.g., the TDEE hypothesis) and heart function can become severely compromised. Because warming often occurs in conjunction with other environmental stressors, such as pollution or hypoxia, these additional pressures can exacerbate the thermal challenges faced by fish hearts, which is why there is currently a major research effort to understand these processes.

See Also: Anatomy in fishes and the associated coronary circulation; Cardiac morphology; Cardiac thermal acclimation and adaption of the heart to extreme temperatures; Effects of temperature: An introduction; Electrical excitation, action potential and impulse conduction; Energy metabolism of cardiac pumping; Excitation-contraction coupling in fish cardiomyocytes; Integrated cardiovascular responses of fish to swimming; Integrated response of the cardiovascular system to hypoxia; Measures of thermal tolerance; Physiology of cardiac pumping; Temperature and fish biology: Insights from metabolism.

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