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REVIEW

Resilience of cardiac performance in Antarctic notothenioid fishes in a warming climate

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ABSTRACT

Warming in the region of the Western Antarctic Peninsula is occurring at an unprecedented rate, which may threaten the survival of Antarctic notothenioid fishes. Herein, we review studies characterizing thermal tolerance and cardiac performance in notothenioids – a group that includes both red-blooded species and the white-blooded, haemoglobinless icefishes – as well as the relevant biochemistry associated with cardiac failure during an acute temperature ramp. Because icefishes do not feed in captivity, making long-term acclimation studies unfeasible, we focus only on the responses of red-blooded notothenioids to warm acclimation. With acute warming, hearts of the white-blooded icefish *Chaenocephalus aceratus* display persistent arrhythmia at a lower temperature (8°C) compared with those of the red-blooded *Notothenia coriiceps* (14°C). When compared with the icefish, the enhanced cardiac performance of *N. coriiceps* during warming is associated with greater aerobic capacity, higher ATP levels, less oxidative damage and enhanced membrane integrity. Cardiac performance can be improved in *N. coriiceps* with warm acclimation to 5°C for 6–9 weeks, accompanied by an increase in the temperature at which cardiac failure occurs. Also, both cardiac mitochondrial and microsomal membranes are remodelled in response to warm acclimation in *N. coriiceps*, displaying homeoviscous adaptation. Overall, cardiac performance in *N. coriiceps* is malleable and resilient to warming, yet thermal tolerance and plasticity vary among different species of notothenioid fishes; disruptions to the Antarctic ecosystem driven by climate warming and other anthropogenic activities endanger the survival of notothenioids, warranting greater protection afforded by an expansion of marine protected areas.

KEY WORDS: Antarctic fishes, Cardiac function, Temperature, Metabolism, Membranes

Introduction

Ocean warming with its associated decline in oxygen content, coupled with the increase in dissolved CO_2 from the atmosphere, are factors responsible for altering the abundance and distribution of marine organisms worldwide (IPCC, 2019). The relatively rapid changes in the Southern Ocean, surrounding Antarctica, are of particular concern because this marine environment has otherwise remained relatively stable for at least 10 million years. Further, the vast majority of species living there are endemic to the region. Indeed, Antarctic fishes reveal remarkable adaptations to an extreme environment (reviewed in Beers and Jayasundara, 2015; Eastman, 1993), but an exceptional vulnerability to climate warming because of their renowned stenothermy (see Glossary; e.g. Somero and DeVries, 1967). Consequently, how rapidly these remarkable fishes, and other organisms, can acclimate and adapt to a shifting environment is one of the most vital questions being addressed by biologists today.

To contribute to our knowledge of how, and whether, Antarctic fishes can overcome obstacles associated with warming in the Southern Ocean, this Review discusses the resilience of cardiac performance, metabolism and the integrity of biological membranes in Antarctic notothenioid fishes confronting a warming environment. We have chosen to focus on cardiac function, in large part because this physiological system is considered to impart the most significant and well-documented influence on thermal tolerance in teleost fishes (Ekström et al., 2016a; Farrell, 2009; Franklin et al., 2013).

Oceanographic features of the Southern Ocean driving adaptive evolution of notothenioids

The Southern Ocean is delineated by the Antarctic Polar Front (APF), which ranges between 50 and 60°S, depending on the ocean sector (Eastman, 1993). Water temperatures south of the APF have been constantly cold for at least 10–14 million years (Kennett, 1977), with water temperatures over the Antarctic continental shelf near the freezing point of seawater (−1.86°C) with minimal seasonal variation (Eastman, 1993). Because of the inverse relationship between temperature and oxygen solubility in (sea)water, the Southern Ocean is relatively oxygen rich, with approximately 1.6 times more oxygen compared with seawater at 20°C (Clarke, 1983). However, the Southern Ocean is a principal heat sink for increases in atmospheric temperature resulting from both natural and anthropogenic processes. Between 1970 and 2017, the Southern Ocean, for example, accounted for 35–43% of the total heat gained in the upper 2000 m of the world's oceans, despite accounting for only 10% of the world's oceans. Between 2005 and 2017, its total heat gain increased to 45–62%, an amount grossly disproportionate to its size (IPCC, 2019). Moreover, warming is currently localized mostly to the Western Antarctic Peninsula (WAP) region, where sea surface temperatures have increased 1°C during the last 50 years, and subsurface waters are projected to increase by 0.4–0.6°C during the next century and potentially by 1°C by 2200 (Meredith and King, 2005; Yin et al., 2011), under even a modest global warming scenario.

The Antarctic and sub-Antarctic fish fauna comprises 263 identified species, and the most speciose taxa are the Liparididae (snailfishes; 37%), Notothenioidei (35%) and Zoarcidae (eelpouts; 15%) (J. T. Eastman, personal communication; Eastman, 2005). Bursts of diversification occurred approximately 14 million years ago, coinciding with cooling events and the expansion of ice sheets on the

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List of abbreviations

APF	Antarctic polar front
$a–vO_2$	arterio-venous oxygen difference
BPT	breakpoint temperature
Ca_{O_2}	arterial oxygen concentration
COX	cytochrome *c* oxidase
CS	citrate synthase
CT_{max}	critical thermal maximum
Cv_{O_2}	venous oxygen concentration
ETS	electron transport system
f_H	heart rate
Hb	haemoglobin
Hct	haematocrit
HSR	heat shock response
HVA	homeoviscous adaptation
LDH	lactate dehydrogenase
Mb	myoglobin
$\dot{M}_{O_2}$	oxygen consumption rate
MPA	marine protected area
PC	phosphatidylcholine
PE	phosphatidylethanolamine
$\dot{Q}$	cardiac output
ROS	reactive oxygen species
UPR	unfolded protein response
V_S	stroke volume
WAP	Western Antarctic Peninsula

Glossary

Afterload pressure
Pressure against which the heart contracts (ventral aortic pressure in fishes).
Electron transport system
A series of electron transporters in the inner mitochondrial membrane involved in synthesizing ATP by oxidative phosphorylation.
Energy charge
The energy status of the cell, described by the equation: ([ATP]+½[ADP])/([ATP]+[ADP]+[AMP]).
Eurythermal
Capable of withstanding a wide range of temperatures.
Haematocrit
The packed cell volume of red blood cells in the total blood volume.
Heart rate scope
The difference between maximum heart rate and initial resting heart rate.
Hepatosomatic index
The ratio of liver mass to body mass.
Metabolic scope
The difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR), which can be defined as absolute aerobic scope (MMR−SMR) or factorial aerobic scope (MMR/SMR).
Neutral mutation
A change in the DNA sequence that does not impact fitness.
State 3 respiration rate
The rate of oxygen consumption of coupled mitochondria in the presence of saturating levels of ADP, P_i and substrate.
State 4 respiration rate
The rate of mitochondrial oxygen consumption following state 3 when ADP has become depleted; also known as LEAK respiration.
Stenothermy
Capable of withstanding only a narrow range of temperatures.

continent that scoured near-shore habitats, leading to extinctions and opening new areas for colonization (Near et al., 2012). Evolution in this stable, cold, oxygen-rich environment has equipped Antarctic fishes with traits to ensure survival in this extreme environment, such as antifreeze glycoproteins that impede the freezing of body fluids (Chen et al., 1997; DeVries, 1971). But the concurrent loss of other traits may restrict the capacity of Antarctic fishes to thrive in a warmer future. Indeed, many studies have shown that Antarctic fishes display reduced thermal plasticity and thermal tolerance compared with temperate fish species (Bilyk and DeVries, 2011; Bilyk et al., 2018; Somero and DeVries, 1967). Moreover, 88% of Antarctic fishes are endemic to the Southern Ocean and have little or no opportunity for migration further south to cooler waters (Eastman, 2005), unlike temperate fish species.

Some unique features of the Notothenioidei

The Notothenioidei suborder comprises eight families (Eastman and Eakin, 2000), including the Channicthyidae family, or icefishes, which are unique among all adult vertebrates because they lack the oxygen-binding protein haemoglobin (Hb), giving their blood a milky white appearance (Fig. 1A) (Ruud, 1954). The loss of Hb, resulting in a 90% decrement in blood oxygen-carrying capacity (Holeton, 1970), naturally imposes a tremendous constraint on arterial oxygen delivery to peripheral tissues. Icefishes compensate with enlarged hearts that facilitate a greater stroke volume (V_S) (Egginton et al., 2019) and cardiac output ($\dot{Q}$) (Hemmingsen and Douglas, 1972; Holeton, 1970), and accommodate a larger blood volume (Hemmingsen and Douglas, 1970) when compared with red-blooded species at a similar temperature (Table 1). All the same, most icefishes display a relatively inactive lifestyle and have low standard metabolic rates (Hemmingsen and Douglas, 1970). The loss of Hb is considered to be a neutral mutation (see Glossary) under low predation rates and current oceanographic conditions (Sidell and O'Brien, 2006), but may prove to be deleterious in a warmer future. Indeed, icefishes have a lower thermal tolerance compared with red-blooded species (Beers and Sidell, 2011). Nonetheless, the thermal tolerance of icefishes is unaffected by supplemental oxygen, suggesting that traits other than constrained arterial oxygen delivery in the Hb-free condition contribute to the thermal sensitivity of icefishes (Devor et al., 2016).

The loss of the intracellular oxygen-binding protein myoglobin (Mb) in the cardiac muscle of some icefishes (6 of 16 species) may also increase their vulnerability to warming (Fig. 1A) (Moylan and Sidell, 2000; Sidell et al., 1997). The Mb-free condition may impede adequate diffusion of oxygen to heart tissues at elevated temperatures, which may then limit cardiac performance and thermal tolerance if oxygen demand outpaces oxygen supply. The cardiomegaly associated with the Hb-free condition may further exacerbate the mismatch between oxygen supply and demand by increasing the total oxygen demand of the enlarged heart. In support of this, studies of isolated perfused icefish hearts have shown that even under normoxic, ambient conditions, the presence of Mb enhances cardiac performance. For example, when Mb is chemically impaired, $\dot{Q}$ declines at high afterload pressures (see Glossary) in the Mb-positive hearts of *Chionodraco rastrospinosus*, behaving more like the Mb-free hearts of *Chaenocephalus aceratus*, which always perform poorly at high afterloads (Acierno et al., 1997). Perhaps as an evolutionary compensation, cardiac afterload pressure is lower for the Mb-free hearts of *C. aceratus* than in the hearts of the Mb-expressing icefish *C. rastrospinosus*, thereby reducing the heart's workload and oxygen demand (Acierno et al., 1997).

The need for thermal plasticity to cope with a warmer Southern Ocean

Species persistence in a warming climate will require a combination of phenotypic plasticity – the environmentally driven changes in

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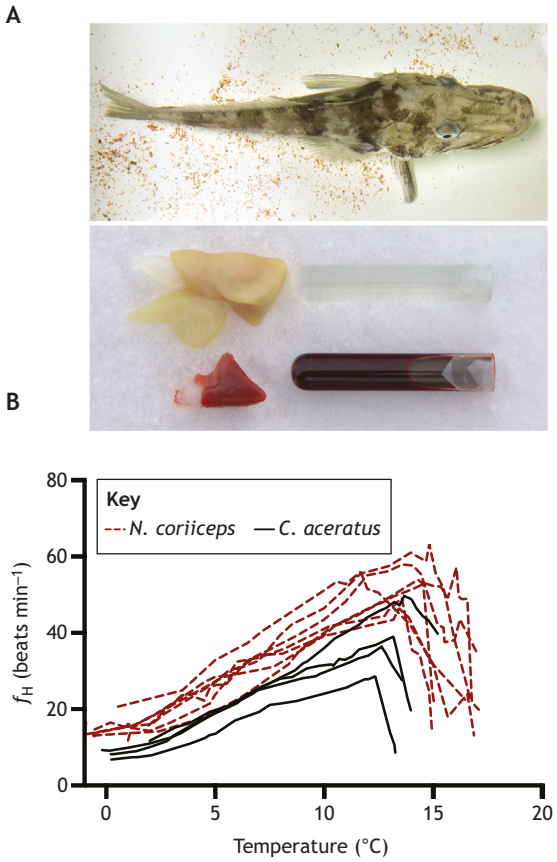

Fig. 1. Cardiac morphometry and performance in the icefish *Chaenocephalus aceratus* and the red-blooded notothenioid *Notothenia coriiceps*. (A) The relative ventricular mass of *C. aceratus* (pictured), which lacks both haemoglobin and myoglobin, is 3-fold larger than that of *N. coriiceps*. Vials of blood from *C. aceratus* (top) and *N. coriiceps* (bottom). (B) *Notothenia coriiceps* displays a higher heart rate (f_H) across temperatures, but a similar breakpoint temperature (BPT) to *C. aceratus* during an acute thermal ramp. Each line represents a different individual. Replotted from Joyce et al. (2018b). Photos by S.E.

phenotype specific to a trait – and evolutionary adaptation (Chevin et al., 2010; Scheiner, 1993; Seebacher et al., 2015). Consequently, the thermal plasticity of several traits in Antarctic notothenioids has been investigated, including gene expression (Bilyk and Cheng, 2014; Bilyk et al., 2018; Huth and Place, 2016), mitochondrial function (O'Brien et al., 2018; Strobel et al., 2013), metabolism (Enzor et al., 2017; Jayasundara et al., 2013; Strobel et al., 2012), membrane integrity (Biederman et al., 2021) and cardiac performance (Franklin et al., 2007; Jayasundara et al., 2013; Joyce et al., 2018a; Seebacher et al., 2005). Collectively, these studies have shown that, at all levels of biological organization, Antarctic notothenioids display less thermal plasticity compared with temperate fish species.

One of the clearest examples of limited thermal plasticity is illustrated by the investigation of gene expression in gills following an acute increase in temperature in three species of notothenioids (Bilyk et al., 2018). Among the three species studied – the cold-temperate *Eleginops maclovinus*, the icefish *C. rastrospinosus* and the high-latitude, cryopelagic *Pagothenia borchgrevinki* – only the cold-temperate *E. maclovinus* induces a heat-shock response (HSR) and endoplasmic reticulum unfolded protein response (UPR). Even more surprising, exposure to heat stress triggers differential expression of only 19 genes in *P. borchgrevinki* (Bilyk et al., 2018), in stark contrast to the 1481 and 1273 genes with altered expression in *E. maclovinus* and *C. rastrospinosus*, respectively, illustrating the diversity in thermal sensitivities among Antarctic notothenioids and reminding us to avoid generalizations based on one or two species (Bilyk et al., 2018). What is unclear from all of these studies is whether the traits probed for thermal plasticity are indeed central to surviving in a warming climate.

Different organismal functions (e.g. growth, reproduction, behaviour, muscle performance) contribute to fitness, and are likely to have different thermal optima, which can vary across life stages and among species (Angilletta, 2009; Kellermann et al., 2019). Nevertheless, ATP is the currency for biological activities and oxygen is essential for generating ATP in a sustained fashion. Ventilation and circulation are the convective mechanisms that power the delivery of oxygen to mitochondria. Therefore, we reasoned that a sound starting place to explore the physiological basis of thermal tolerance would be to focus on cardiac function, as the upper thermal limits of aerobic metabolic scope (see Glossary) in fishes are intrinsically linked to the maximum capacity of cardiac function (Farrell, 2002, 2007, 2009, 2016). Although acute warming will increase kinetic activities such as heart rate (f_H), excessive warming leads to cardiac arrhythmias, as control mechanisms and ionic regulation eventually become compromised. Warm acclimation of temperate fish species can reset intrinsic f_H to a lower value and/or increase the peak f_H, which may restore heart rate scope (see Glossary; Ekström et al., 2016b), but it is less clear whether Antarctic fishes display a similar degree of thermal plasticity. One or more cellular/subcellular processes are likely to underpin cardiac failure at elevated temperatures, including decrements in ATP synthesis (including mitochondrial function) (Iftikar and Hickey, 2013; Iftikar et al., 2014, 2015), loss of membrane integrity (Evans et al., 2021), oxidative damage to macromolecules or macromolecular assemblages (Mueller et al., 2011) and/or disruption to ion channel function (Vornanen et al., 2014). To gain insight into the capacity of Antarctic notothenioids to endure a warming climate, here we describe what is known about the thermal plasticity of cardiac performance and physiological and biochemical processes likely to be associated with loss of cardiac function at upper thermal limits (Fig. 2). Our work has focused on two notothenioid species: the yellowbelly rockcod, *Notothenia coriiceps* (a red-blooded fish expressing cardiac Mb), and the blackfin icefish, *C. aceratus* (a white-blooded fish lacking both Hb and Mb). Comparing these species allows us to evaluate the impact of the loss of respiratory pigments on cardiac performance and thermal tolerance, using earlier work characterizing differences in thermal tolerance between *N. coriiceps* and *C. aceratus* as a foundation (Beers and Sidell, 2011). Equally compelling was the opportunity afforded by our *in vivo* and *in vitro* studies for the first detailed investigation of the basic cardiovascular physiology of icefishes, something that has not been carefully studied since the original work of Hemmingsen and Douglas almost 50 years ago (Hemmingsen and Douglas, 1972; Hemmingsen et al., 1972).

Cardiac performance during acute warming of Antarctic notothenioids

In ectothermic animals, acute warming increases metabolic demand and oxygen consumption rate ($\dot{M}_{O_2}$) (Eliason and Anttila, 2017). According to the Fick principle (Farrell et al., 2014; Fick, 1870; Wang et al., 2019), $\dot{M}_{O_2}$ is equivalent to the product of $\dot{Q}$ – a multiple of f_H and stroke volume (V_S) – and arterio-venous oxygen difference (a−vO_2 diff), which is the difference in

Table 1. A comparison of cardiovascular variables between white- and red-blooded Antarctic notothenioids

Species	$\dot{Q}$ (ml min^{-1} kg^{-1})	V_S (ml kg^{-1})	Resting f_H (beats min^{-1})	Vascular resistance (Pa min kg ml^{-1})	Viscosity (cP) (at shear rate 22.5/225 s^{-1})	P_{va} (kPa)	P_{da} (kPa)	Reference
White-blooded species								
Chaenocephalus aceratus	99–153	5.6–8.7	17-18	16.2–10.5		2.4	1.6	Hemmingsen et al., 1972
Chaenocephalus aceratus			17.5		3[A]		1.3	Hemmingsen and Douglas, 1972
Chaenocephalus aceratus	61	4.4	14	26.2		1.9	1.6	Holeton, 1970
Chaenocephalus aceratus	26.6	2.2	9-12	67.7		2.1	1.8	Joyce et al., 2018a
Chaenocephalus aceratus	35.1	4.2	8.5	62.7		2.5	2.2	Joyce et al., 2019
Pseudochaenichthys georgianus	67–94	5.8–7.8	13					Hemmingsen and Douglas, 1977
Chionodraco hamatus					4/3.6			Wells et al., 1990
Cryodraco antarcticus					4.7/3.8			Wells et al., 1990
Red-blooded species								
Notothenia coriiceps	6.5	0.26	25					Joyce et al., 2018b
Notothenia coriiceps	6.5	0.27	24	492.3			3.2	Egginton, 1997
Pagothenia borchgrevinki	29.6	1.4	11.3	94.6		3.6	2.8	Axelsson et al., 1992, 1994
Pagothenia borchgrevinki					6.5/6[B]			Macdonald and Wells, 1991
Trematomus bernacchii	17.6	1.67	10.5			3.1		Axelsson et al., 1992
Trematomus bernacchii					4.9–11.2/ 4.4–6.8[C]			Wells et al., 1990

$\dot{Q}$, cardiac output; V_S, stroke volume; f_H, heart rate; P_{va}, ventral aortic blood pressure; P_{da}, dorsal aortic blood pressure.
[A]Recalculated from centistokes using a density value of 1.025 g ml^{-1}, shear rate of measurements unknown. [B]Data extrapolated from graphs. [C]Range of haematocrit (Hct) for cannulated (7.6%) and acute-sampled fish (15.4%).

oxygen concentration between arterial (Ca_{O_2}) and venous (Cv_{O_2}) blood:

$$\dot{M}_{O_2} = \dot{Q} \times a - vO_2\,\text{diff}, \quad (1)$$

$$\dot{M}_{O_2} = (f_H \times V_S) \times (Ca_{O_2} - Cv_{O_2}). \quad (2)$$

To increase $\dot{M}_{O_2}$, an animal can increase $\dot{Q}$, a–vO_2 diff or a combination of the two. Crucially, temperate fishes invariably increase f_H during acute warming, with very little change in V_S (Eliason and Anttila, 2017; Farrell, 2016). Consequently, the necessary increase in $\dot{Q}$ during warming to meet the increased oxygen demand of the tissues is achieved almost entirely by increasing f_H.

The breakpoint temperature (BPT) for cardiac function (the temperature at which peak f_H occurs) in notothenioids has been revealed by monitoring f_H during an experimental thermal ramp up to the critical thermal maximum (CT_{max}) (Box 1). Although BPT is surprisingly similar for red-blooded (14°C) and white-blooded notothenioids (13°C) when determined in this way (Fig. 1B), other aspects of cardiac performance are associated with species differences in CT_{max} (Joyce et al., 2018b). Most notably, cardiac arrhythmia is a persistent phenomenon at >8°C for white-blooded *C. aceratus*, but not for red-blooded *N. coriiceps*. Hearts of the less thermal-tolerant *C. aceratus* beat rhythmically only up to 8°C, whereas ectopic beats appear temporarily at 1–2°C above ambient temperature in the red-blooded *N. coriiceps*, but rhythmicity returns and is maintained up to the BPT (Joyce et al., 2018b). Thus, the onset of persistent cardiac arrhythmia is a better cardiac indicator of upper thermal tolerance than BPT for peak f_H.

Given their similar cardiac responses up to 8°C, it is not surprising that *N. coriiceps* and *C. aceratus* display similar rates of increase in whole-animal $\dot{M}_{O_2}$ (i.e. Q_{10} of 3.4–3.5) during acute warming from ambient temperature (~0°C) to 8°C (Fig. 3A,D). However, distinctly different mechanisms drive the increases in $\dot{M}_{O_2}$, reflecting the differing capacities of their cardiovascular systems, even though both species increase $\dot{Q}$ primarily by increasing f_H, with little change in V_S. This finding is consistent with the general trend for fishes (Ekström et al., 2016a; Eliason and Anttila, 2017; Joyce and Wang, 2020) (Fig. 3B,E). In *C. aceratus*, the lack of Hb greatly constrains their capacity to decrease Cv_{O_2} during warming, and the already restricted Ca_{O_2} decreases slightly. Consequently, with no change in a–vO_2 diff at 4°C, their $\dot{M}_{O_2}$ increases almost entirely by increasing $\dot{Q}$; a–vO_2 diff actually decreases at 8°C (Fig. 3C,F). Their 2.7-fold increase in $\dot{Q}$ is associated with a large increase in systemic vascular conductance, potentially mediated by adenosine and/or other vasodilators (Egginton et al., 2019; Joyce et al., 2018b). However, in *N. coriiceps*, a–vO_2 diff can, and does, increase during acute warming up to 8°C, and $\dot{Q}$ increases to a lesser extent (2-fold) than in *C. aceratus* (Fig. 3B,C,E,F).

Notothenioid hearts lack a coronary circulation to provide a dedicated arterial oxygen supply, and must rely instead on the residual oxygen in venous blood (Davie and Farrell, 1991). Consequently, any decline in Cv_{O_2} associated with an increase in a–vO_2 diff could compromise oxygen delivery to the heart, especially in icefishes where Cv_{O_2} is already very low given the absence of red blood cells and thus haemoglobin (perhaps explaining their a–vO_2 diff inflexibility during acute warming). Instead, red-blooded notothenioids have another well-documented strategy to increase a–vO_2 diff and cope with increased metabolic demand in general (e.g. that caused by exercise and digestion as well as warming; Axelsson, 2005; Brijs et al., 2020; Franklin et al., 1993): they can considerably boost haematocrit (Hct; see Glossary), thereby increasing Ca_{O_2}. Indeed, Hct can double from 15.3% to 30.3% during thermal ramping to CT_{max} (15.0°C) within ~6 h (Joyce et al., 2018a). Even elite terrestrial athletes, such as

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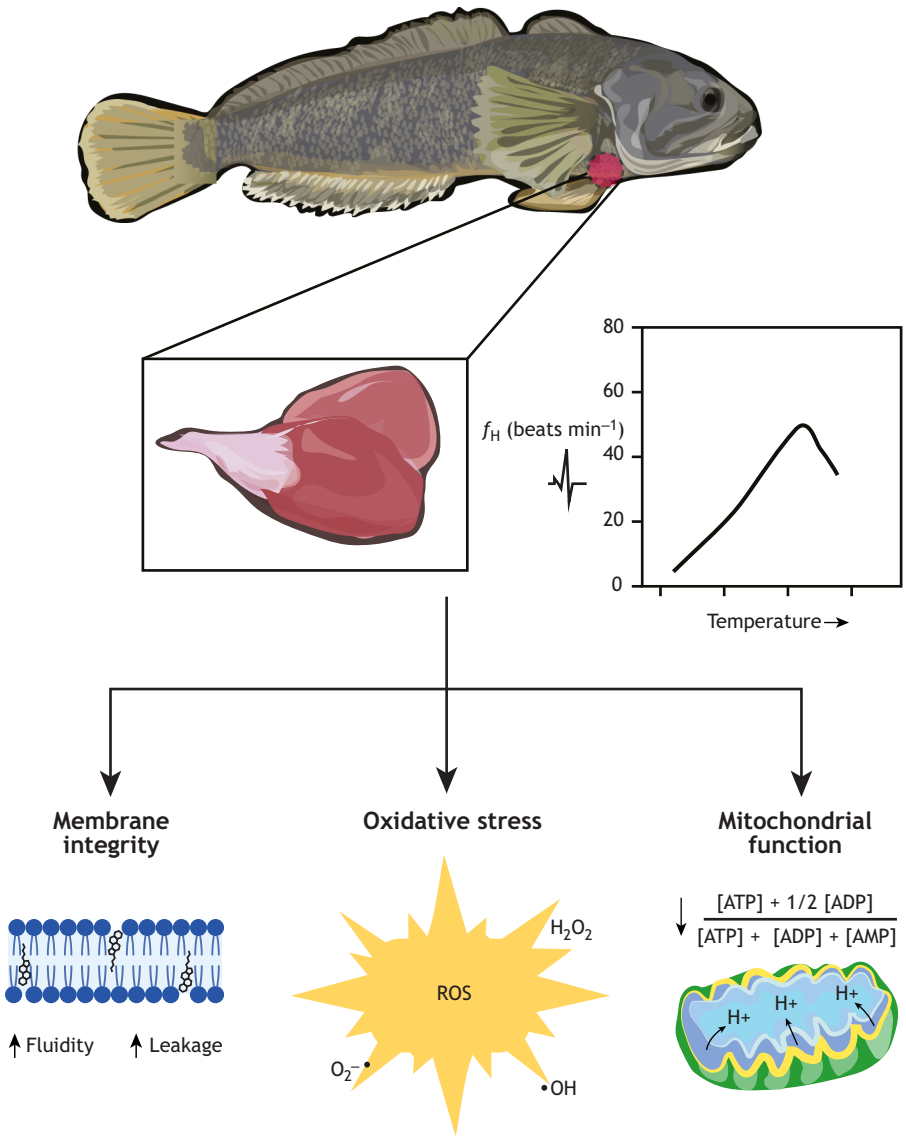

Fig. 2. Physiological and biochemical underpinnings of thermal tolerance. Schematic diagram of *N. coriiceps* and its heart (top left). The BPT of f_H (top right) may be defined by loss of membrane integrity, leading to an increase in fluidity and leakage (bottom left), an increase in the production of reactive oxygen species (ROS) that damage biological macromolecules (bottom centre) and/or a decrement in mitochondrial function leading to a decline in energy charge (bottom right). Figure by Megan Perra.

greyhound dogs and thoroughbred racehorses, cannot achieve such an acute change (Neuhaus et al., 1992; Ohmura et al., 2013). Of course, by reducing their routine Hct to such a low level (routine Hct more typically is around 30% in temperate fish species; Brijs et al., 2020; Gallaugher and Farrell, 1998; Gallaugher et al., 1995), red-blooded notothenioids benefit by reducing blood viscosity, providing a sustained reduction in vascular resistance and perhaps cardiac workload, until oxygen demand increases (Table 1).

It is likely that red-blooded notothenioids finely regulate Hct using the spleen, as do most vertebrates. At rest, the spleen can sequester a large population of red blood cells. In *P. borchgrevinki*, surgical removal of the spleen (splenectomy) greatly reduces the capacity to increase $\dot{M}_{O_2}$ following enforced exercise (Brijs et al., 2020). Going forward, it would be informative to determine whether splenectomy lowers the CT_{max} in red-blooded notothenioids. However, in contrast to mammals, in which splenic contraction is under adrenergic control (Hurford et al., 1996), in notothenioids the spleen depends primarily on cholinergic innervation (Nilsson et al., 1996). *In vitro*, the spleen of the Hb-free *C. aceratus* also constricts in response to cholinergic agonists, likely representing a vestigial trait (Joyce and Axelsson, 2021). Haemoconcentration, owing to increased plasma efflux, may also contribute to the increased Hct (Hedrick et al., 2020). The extent of this is yet to be experimentally determined in notothenioids, but it is small in rainbow trout (Gallaugher et al., 1992).

Taken together, these data indicate that cardiovascular performance contributes to acute thermal tolerance of Antarctic notothenioid fishes. In fact, the large factorial scope for $\dot{Q}$ during acute warming in *C. aceratus* was an unexpected response, given a much higher ($\geq$100 ml min^{-1} kg^{-1}) previous report of *in vivo* routine $\dot{Q}$ (Hemmingsen et al., 1972). Our *in vivo* routine $\dot{Q}$ measurements are severalfold lower (Fig. 3B, Table 1) than those of Hemmingsen et al. (1972), which are closer to maximum $\dot{Q}$ as determined by our *in vitro* measurements in *C. aceratus* (see below). This discrepancy is likely due to lower levels of animal stress during the measurements and methodological differences (Joyce et al., 2018b). Furthermore, our measurement of cardiac work in *C. aceratus* is in line with that of most red-blooded notothenioids (Joyce et al., 2018b), indicating that the loss of Hb does not confer a selective advantage by reducing cardiac work.

Although important differences have emerged between the thermal performance of Hb-free and red-blooded notothenioids – some intuitive and some less so – the challenge remains to identify

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molecular mechanisms that limit cardiac performance at elevated temperature, and the thermal plasticity of those processes. Below, we review what is known of these mechanisms.

Box 1. A comparative approach and a reference point for understanding the limits of thermal tolerance

One of the most widely used metrics for assessing thermal tolerance in fishes is the critical thermal maximum (CT_{max}), determined as the temperature at which an animal loses its motor function (most often measured in fishes as the loss of equilibrium) when heated at a rate sufficient to enable warming of the body core without initiating processes associated with acclimation (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997). Although the CT_{max} for a given species, by definition, is above the temperature at which those individuals could survive for more than a few minutes, thereby limiting its ecological value, it may reflect tolerance to short-term temperature exposures that are likely to become more frequent as global temperatures rise. CT_{max} is correlated with tolerance of slower rates of warming (Asheim et al., 2020), and so it is used to predict impacts of climate change (e.g. shifts in species range) (Sunday et al., 2012). Although other measures of thermal tolerance, such as temperature preference tests, may provide more ecologically relevant information, the logistical constraints of fieldwork in extreme, remote environments largely preclude their use. Thus, CT_{max} serves as a suitable reference point against which to probe the thermal tolerance of other physiological and biochemical measurements, as well as a comparator with other fish species. When heated at a rate of 3.6°C h^{-1}, the CT_{max} for *N. coriiceps* is 17.1°C and that for *C. aceratus* is 13.9°C (Beers and Sidell, 2011).

Impact of acute warming on cardiac metabolism of Antarctic notothenioids

The robust increase in $\dot{Q}$ that supports the rise in metabolic rate during warming will increase cardiac ATP demand (Egginton et al., 2019; Franklin et al., 2007; Jayasundara et al., 2013; Joyce et al., 2018a). Higher rates of ATP production can be achieved by enhanced activity of aerobic and/or anaerobic pathways of energy metabolism, although Antarctic notothenioids, in general, have lower anaerobic capacities compared with temperate teleosts (Crockett and Sidell, 1990; Sidell et al., 1995).

As temperature increases, the activity of metabolic enzymes, and thus ATP synthesis, naturally increases because of the thermodynamic impact on the catalytic rate of enzymes (the Q_{10} effect) (Hochachka and Somero, 2002). The higher f_H of *N. coriiceps* compared with that of *C. aceratus* at ambient temperature and during a temperature ramp (Fig. 1B) is supported by the activity of citrate synthase (CS, a key enzyme in the Krebs cycle), which is 1.5-fold higher in hearts of *N. coriiceps*. *Notothenia coriiceps*, but not *C. aceratus*, appears to be capable of boosting cardiac ATP supply further by increasing levels of mitochondrial enzymes. Following exposure to CT_{max}, cardiac levels of CS, malate dehydrogenase and isocitrate dehydrogenase increase 1.5- to 1.8-fold in *N. coriiceps*, whereas there is no change in any mitochondrial protein expression in *C. aceratus* (O'Brien et al., 2018).

Because of the general need to enhance ATP production during warming, mitochondrial function has been implicated as a weak link that limits thermal tolerance in fishes, and studies have shown that, in some tissues, mitochondria become less efficient at using oxygen

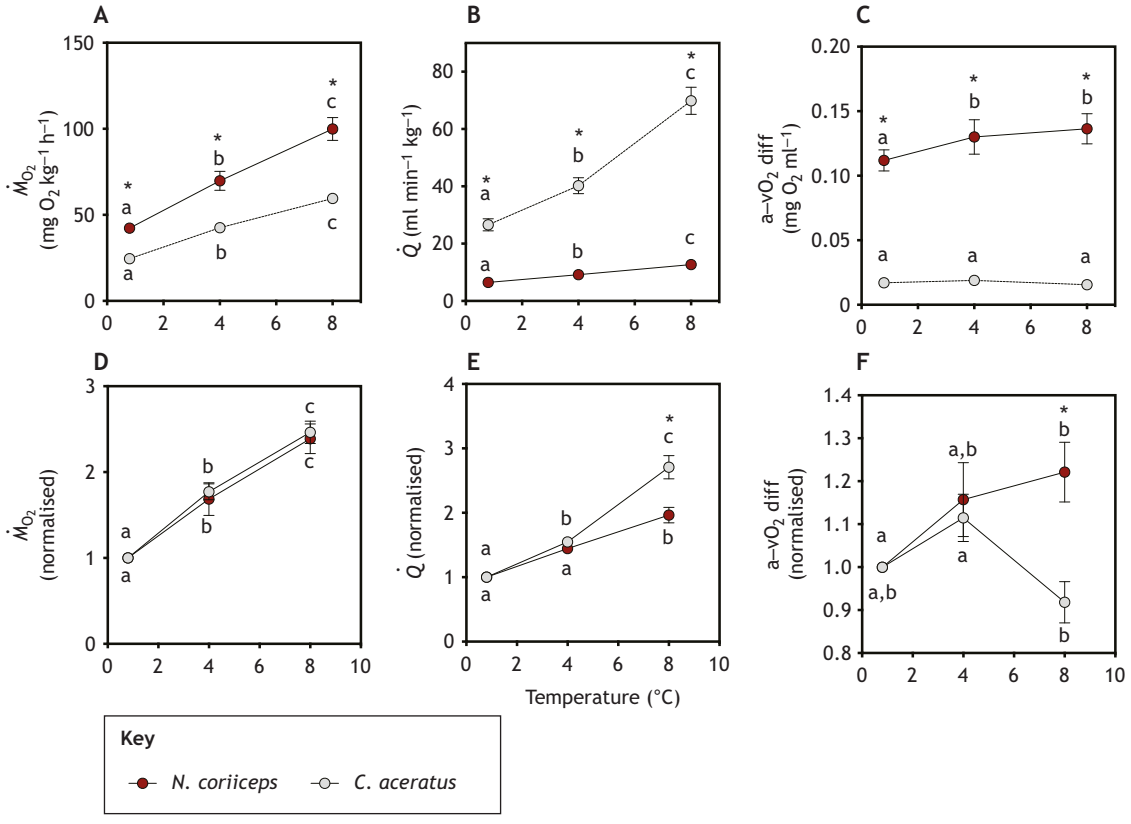

Fig. 3. The absolute and relative changes in oxygen consumption rate, cardiac output and arterio-venous oxygen difference in notothenioids at rest. Data are replotted from Joyce et al. (2018a,b), and arterio-venous oxygen difference (a–vO_2 diff) was calculated from oxygen consumption ($\dot{M}_{O_2}$) and cardiac output ($\dot{Q}$) using the Fick principle. Asterisks indicate significant differences between the species; dissimilar letters indicate significant differences between temperatures within a species (P<0.05; two-way ANOVA with repeated measures for temperature). Values are means±s.e.m.

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to generate ATP at high temperature (Iftikar and Hickey, 2013; Iftikar et al., 2015). This would place an increased load on the oxygen delivery system. However, few studies have specifically examined cardiac mitochondrial function. State 3 respiration rates (see Glossary) of mitochondria isolated from heart ventricles are similar between *C. aceratus* and *N. coriiceps* and increase in response to CT_{max} (Fig. 4A), but the two species have several notable differences in mitochondrial function that may contribute to greater cardiac performance of *N coriiceps* at elevated temperature (O'Brien et al., 2018). First, the activity of cytochrome *c* oxidase (COX) per milligram of mitochondrial protein is 1.8- to 1.9-fold higher in *N. coriiceps* than in *C. aceratus* (O'Brien et al., 2018). Excess COX activity relative to state 3 rate permits greater flux through the electron transport system (ETS; see Glossary) when needed, minimizes the production of reactive oxygen species (ROS; Payne and Chinnery, 2015), and may contribute to greater hypoxia tolerance in fishes (Hilton et al., 2010); the last of these is something that might accompany cardiac warming (Breitburg et al., 2018). The two species have a second important difference in mitochondrial function: state 4 respiration rate (see Glossary), which increases in *C. aceratus* but not in *N. coriiceps* following exposure to CT_{max} (Fig. 4B). The thermal sensitivity of state 4 respiration is higher in *C. aceratus*, indicating that the efficiency of ATP production likely declines more precipitously in the hearts of *C. aceratus* than in those of *N. coriiceps* during warming (O'Brien et al., 2018). Thus, hearts of *C. aceratus* must consume more oxygen for a given level of ATP production and cardiac work than hearts of *N. coriiceps*, assuming that mechanical efficiency of the heart remains unchanged. Indeed, ATP levels and energy charge (see Glossary) fall to lower levels in hearts of *C. aceratus* than in those of *N. coriiceps* following exposure to CT_{max} (O'Brien et al., 2018), again emphasizing the importance of Cv_{O_2} as noted above.

The activity of lactate dehydrogenase (LDH) is higher in *C. aceratus* heart than in *N. coriiceps* heart, suggesting a greater capacity for, and reliance on, anaerobic metabolism that may compensate for their more limited aerobic capacity (O'Brien et al., 2018). Consistent with this, when exposed to their CT_{max}, cardiac lactate levels increase to a greater extent in *C. aceratus* than in *N. coriiceps* (Devor et al., 2016; O'Brien et al., 2018), which is consistent with a potentially limited cardiac oxygen supply with no Hb or Mb. However, expression of genes encoding enzymes of anaerobic metabolism, such as lactate dehydrogenase-A and aldolase-A, does not increase following exposure to CT_{max} in either species, despite an increase in hypoxia inducible factor-1, the transcription factor that regulates expression of these genes (O'Brien et al., 2020). This suggests a limited capacity to increase anaerobic metabolism during warming. The differences in metabolic capacity reflect differing strategies for meeting cardiac energetic demands, which may be equally effective under ambient conditions, but clearly the more limited aerobic metabolic capacity of *C. aceratus* hearts may constrain function at elevated temperature.

An undesirable potential consequence of increased aerobic metabolism with elevated temperature is elevated oxidative stress. As temperature increases, flux through the ETS increases, enhancing rates of ROS production (Abele et al., 2002; Mueller et al., 2011). Also, an increase in ROS production can occur if electron transfer becomes disrupted or proton motive force increases (Cadenas and Boveris, 1980). The high degree of coupling between electron transfer and proton pumping in icefish mitochondria, reflected in their high mitochondrial membrane potential, is associated with higher rates of ROS production when the ETS is inhibited, and likely contributes to the increase in oxidative damage incurred by hearts of *C. aceratus* following exposure to CT_{max} (Mueller et al., 2012, 2011). Additionally, hearts of *C. aceratus* lack the two mitochondrial isoforms of creatine kinase, localized to the intermembrane space. These isoforms maintain a high rate of flux through the ETS by keeping ATP levels low and ADP levels high, which also helps to minimize ROS production (Schlattner et al., 2009; Tepp et al., 2011). Thus, it is conceivable that oxidatively damaged proteins and lipids in hearts of *C. aceratus* may contribute to their persistent arrhythmia above 8°C. In support of this, an imbalance between ROS production and antioxidant defence is associated with heart failure in humans as a result of oxidative modifications to proteins involved in excitation–contraction coupling, including the ryanodine receptor and calcium ATPase, which regulate calcium dynamics in the heart (Tsutsui et al., 2011).

Do biophysical properties of biological membranes underpin thermal tolerance?

Membrane fluidity is influenced by extrinsic and intrinsic factors, notably temperature, pressure, osmolarity, and the packing density and composition of polar and neutral lipid molecules (Los and Murata, 2004; Subczynski et al., 2017). Influences of fluidity on membrane function are wide ranging, including the responses of ion

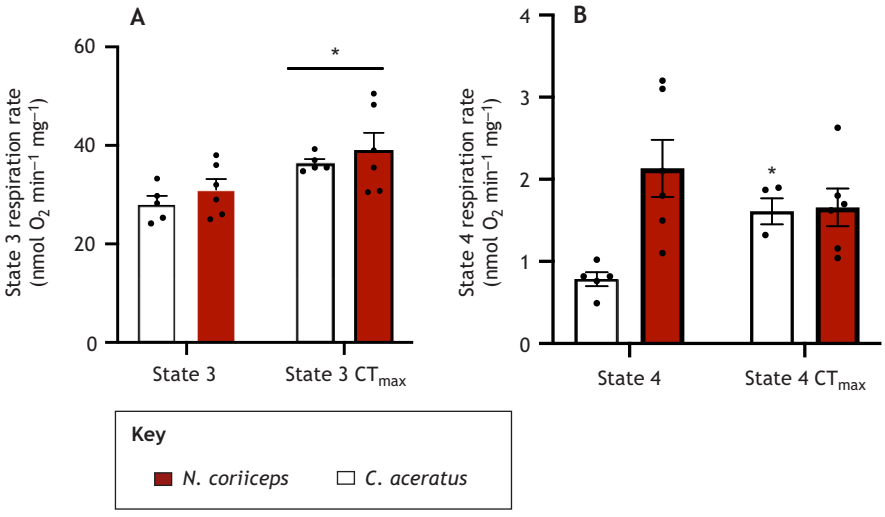

Fig. 4. The effect of temperature on cardiac mitochondrial respiration rates of notothenioids. (A) State 3 respiration rates in mitochondria isolated from heart ventricles of *N. coriiceps* and *C. aceratus* are similar between species when animals are held at ambient temperature but increase following exposure to CT_{max} (measured at 2°C). (B) State 4 rates, indicative of proton leak, increase in *C. aceratus* following exposure to CT_{max}. Asterisks indicate significant differences between experimental conditions (P<0.05). Values are means±s.e.m. Figure is modified from O'Brien et al. (2018).

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channels and a large number of membrane-associated catalysts, many of which have key roles in processes such as membrane transport and energy metabolism (Cornelius et al., 2015; Gu and de Groot, 2020; Lee, 2004). Structural integrity of the membrane is fundamental to selective passive permeability of small molecules, including ions, and a breach in membrane integrity can lead to critical disruption of function, particularly in excitable cells, including cardiac myocytes. As in most animals, cardiac contractility depends on the appropriate balance of ions, which is largely coordinated by the opening and closing of channels associated with the sarcolemmal membrane, and the active movement of ions by transmembrane pumps, which are all temperature sensitive (Vornanen, 2016). Thus, thermal limits to regular cardiac rhythm can arise from disturbance of channel function, and the capacity to repolarize the sarcolemma (Aho and Vornanen, 1999; Haverinen and Vornanen, 2020; Vornanen, 1998; Vornanen et al., 2002). Further, membrane fluidity may directly influence Na^+ channel function, and thereby cardiomyocyte excitability, in fishes during warming (Haverinen and Vornanen, 2020).

To date, few studies have directly examined thermal profiles of membrane fluidity and/or structural integrity from the hearts of Antarctic notothenioids. Yet, recent comparisons reveal several striking contrasts, which may indeed influence cardiac function, and ultimately limit thermal tolerance (Biederman et al., 2019a; Evans et al., 2021).

Fluidity, measured as the inverse of fluorescence polarization, of both mitochondrial and microsomal (mostly sarcolemmal) membrane preparations from the ventricle varies significantly among notothenioid species (Biederman et al., 2019a; Evans et al., 2021). When compared at any single temperature, ventricular mitochondrial membranes are more fluid in the icefish *C. aceratus* than in the red-blooded *N. coriiceps* (Fig. 5A) (Biederman et al., 2019a). Similarly, ventricular sarcolemmal membranes of four icefishes – *C. aceratus*, *Champsocephalus gunnari*, *C. rastrospinosus* and *Pseudochaenichthys georgianus* – as well as the red-blooded *Gobionotothen gibberifrons*, are all more fluid than those prepared from the red-blooded *N. coriiceps* (Evans et al., 2021). Although phosphatidylcholine (PC) and phosphatidylethanolamine (PE) dominate the phospholipid classes that compose ventricular

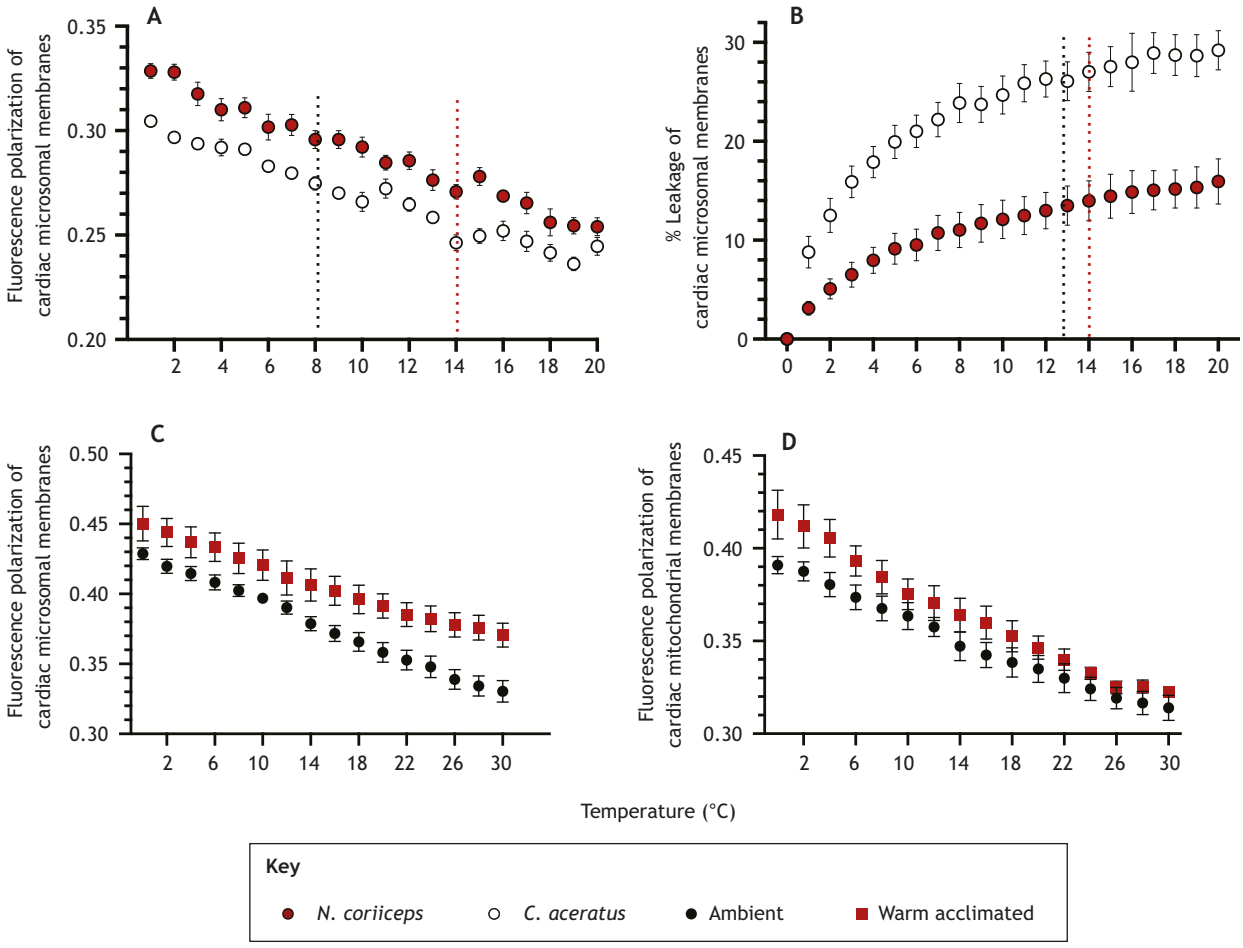

Fig. 5. The effect of temperature on fluidity and membrane leakage in cardiac membranes of notothenioids. Membrane fluidity (A) and leakage (B) are higher in microsomal (i.e. sarcolemmal) membranes from hearts of the icefish *C. aceratus* than in membranes from the red-blooded *N. coriiceps*. Membrane fluidity is similar at the temperature at which each species' heart becomes persistently arrhythmic (A), and the fold-change in percentage leakage from ambient to BPT temperatures is similar (3-fold) between the two species (B), as indicated by the black dotted line for *C. aceratus* and red dotted line for *N. coriiceps*. In response to warm acclimation to 5°C for 6 weeks, microsomal membranes from hearts of *N. coriiceps* (C) and cardiac mitochondrial membranes (D) become less fluid compared with those of animals held at ambient temperature, indicative of homeoviscous adaptation. Figures are re-plotted from Evans et al. (2021) and Biederman et al. (2021).

membranes in notothenioids, lower PE relative to PC in cardiac mitochondria of *C. aceratus* compared with those of *N. coriiceps* accounts for much of the difference in membrane fluidity between these two species (Biederman et al., 2019a; Evans et al., 2021). In contrast, a higher content of unsaturated acyl tails in phospholipids from icefishes than in those from red-blooded notothenioids best explains differences in sarcolemmal membrane fluidity across the six notothenioid species (Evans et al., 2021).

Membrane integrity of the cardiac sarcolemma of notothenioid fishes was recently examined using leakage as measured by the release of 5(6)-carboxyfluorescein from liposomes prepared with isolated ventricular membranes (expressed as a percentage of total leak – this represents the difference between initial fluorescence and maximum fluorescence when liposomes are fully ruptured; Evans et al., 2021). Although leakage increases with temperature in liposomes prepared from all species, as expected, thermal sensitivity (i.e. the slope of the leakage versus temperature curve) is particularly pronounced between 0 and 5°C but differs among species (Fig. 5B; Evans et al., 2021). Specifically, leakage for a given temperature is 2-fold greater, and thermal sensitivity of leakage is also significantly greater, in membranes of the icefish *C. aceratus* than in those of the red-blooded *N. coriiceps* (Fig. 5B; Evans et al., 2021). These data indicate both a greater extent of disruption to membrane integrity in the icefish on acute warming, and disruption at a significantly lower temperature than in the red-blooded species. Again, the higher degree of unsaturation in fatty acid tails is the biochemical correlate for greater sarcolemmal membrane leakage in the icefishes compared with the red-blooded species (Evans et al., 2021).

How might these observations of biological membranes from Antarctic notothenioids be viewed within the context of cardiac function and/or thermal tolerance? When examining measures of cardiac performance for *C. aceratus* and *N. coriiceps* (the species with the lowest and highest overall CT_{max}, respectively, compared with three other notothenioid species; Beers and Sidell, 2011), one can see that several differences between these two species warrant closer attention. Membranes from *N. coriiceps* would have to be warmed by 6°C to match the fluidity of those from *C. aceratus* (Fig. 5A); this is the same temperature difference for the two species noted above for the onset of persistent arrhythmia. There is also notable similarity in the change in percentage leakage from ambient temperature (e.g. 1°C) to the temperatures associated with the BPT of cardiac function (Fig. 5B). For example, at the species' BPT, the change in percentage leakage is 3-fold for membranes from both *C. aceratus* and *N. coriiceps*. Again, these results indicate that disruption of cardiac rhythmicity and, ultimately, failure of the heart could be underpinned by a significant compromise to membrane fluidity and integrity, respectively (Evans et al., 2021).

An intriguing question is why icefishes have evolved more fluid cardiac membranes than red-blooded fishes. The answer may lie in the large dimensional changes the heart must endure with each heartbeat, because their cardiac stroke volume is routinely 8 times larger and maximum $\dot{Q}$ is up to 2.4 times larger than that of red-blooded species (Egginton et al., 2019). However, higher membrane fluidity in icefishes is also observed in synaptic membranes from the brain (Biederman et al., 2019b). Thus, although enhanced fluidity may represent a liability in the face of a warming climate, it may also confer physiological benefits (i.e. higher oxygen flux) at cold temperatures (Box 2).

Box 2. Does enhanced membrane fluidity of icefishes represent a physiological advantage?

Molecular oxygen crosses several biological membranes by diffusion before being utilized, and its diffusion can be summarized by the Meyer–Overton rule (Missner and Pohl, 2009) as derived from the Fick equation:

$$P_M = K_P D_M / d.$$

The membrane permeability coefficient (P_M) is a function of K_P (the oil/water partition coefficient), D_M (the diffusion coefficient of the solute within the membrane) and d (the distance over which the solute must diffuse – in this case, the thickness of the membrane). Although this analysis assumes a relatively homogeneous membrane (Missner and Pohl, 2009), it can predict how membrane properties influence oxygen permeability, and thus oxygen flux. Recent models can better account for the heterogeneous nature of biological membranes (e.g. Shinoda, 2016; Zocher et al., 2013); however, we lack some of the critical data for notothenioids.

The most profound influence on P_M is temperature (Widomska et al., 2007). Warming leads to greater fluidity and a reduction in packing density, thereby enabling oxygen to diffuse more readily across the bilayer (Subczynski et al., 1989; Widomska et al., 2007). Elevated fluidity of the biological membranes of icefishes compared with counterpart membranes in the red-blooded species *N. coriiceps* should permit enhanced oxygen flux (Evans et al., 2021). Combined with anatomical (enhanced non-cardiac vascularization) and physiological (greater $\dot{Q}$) features that ensure sufficient oxygen delivery in the Hb-free icefishes (Egginton and Rankin, 1998; Sidell and O'Brien, 2006; Wujcik et al., 2007), the more-fluid membranes of the icefish likely contribute to robust oxygen delivery to the electron transport chain. The need for a lower resistance to oxygen diffusion from the blood of icefishes will apply to all aerobic tissues, not just heart and brain. Loss of myoglobin (Mb) creates further resistance to diffusion outside of the blood (Wittenberg and Wittenberg, 1987). Although greater membrane fluidity should improve oxygen flux across membranes and benefit icefishes in a frigid Southern Ocean, this benefit becomes a liability for other essential membrane functions under warming conditions. With less dissolved oxygen in seawater at elevated temperatures (Schmidtko et al., 2017), greater membrane fluidity may aid oxygen extraction, although increased ventilation will be required, which will be costly.

In summary, studies to date demonstrate that cardiac metabolic capacity and membrane characteristics contribute to cardiac performance and thermal tolerance of Antarctic notothenioids. The increase in membrane fluidity, mitochondrial proton leak and oxidative damage to mitochondrial proteins in hearts of *C. aceratus* likely contribute to reduced cardiac performance and thermal tolerance compared with *N. coriiceps*. Probing the thermal plasticity of these processes in response to warm acclimation provides further insight regarding the capacity of notothenioids to survive in a warming environment, and this is considered in the next section.

Impact of warm acclimation on Antarctic notothenioids

The effects of warm acclimation have only been studied in red-blooded notothenioids, as icefishes do not feed in captivity, making long-term acclimation studies unfeasible. Below, we consider what is known about how acclimation to higher temperatures affects the physiology of red-blooded notothenioids.

Effects on oxygen uptake and cardiac performance

The thermal plasticity of both cardiac performance and whole-animal $\dot{M}_{O_2}$ varies among Antarctic notothenioid fishes, although methodologies for measuring each also vary among studies. When red-blooded *P. borchgrevinki* that have been warm acclimated to 4°C for 4–5 weeks are compared with fish held at an ambient temperature of −1°C, $\dot{Q}$, $\dot{M}_{O_2}$and ventilation rate at their respective

acclimation temperatures are equivalent (Franklin et al., 2007; Robinson and Davison, 2008). Although this indicates perfect thermal compensation, factorial scope to increase both $\dot{Q}$ and f_H (measured as the maximum value during exercise/resting value) increases after warm acclimation (Franklin et al., 2007). In contrast, when red-blooded *Trematomus bernacchii* acclimated to −1.0, 2.0 or 4.5°C for 14 days are compared, absolute f_H scope during acute warming declines with temperature (Jayasundara et al., 2013), indicating that cardiac function of *T. bernacchii* has little thermal plasticity, at least over a shorter acclimation period. This may be an important difference between species at such frigid water temperatures.

The red-blooded *N. coriiceps* responds differently to warm acclimation compared with *P. borchgrevinki* or *T. bernacchii*. $\dot{Q}$ is 2.7-fold higher in *N. coriiceps* acclimated to 5°C than in those held at 0°C (Joyce et al., 2018a), a difference driven primarily by a larger V_S rather than an increase in f_H (contrasting with the effects of acute warming). The doubling of V_S is associated with a doubling of central venous pressure, potentially indicating an increased blood volume (Joyce et al., 2018a). The increase in $\dot{Q}$ in *N. coriiceps* more than compensates for the 1.4-fold higher $\dot{M}_{O_2}$, which is not thermally compensated over the 6–9 week acclimation period (Egginton and Campbell, 2016; Joyce et al., 2018a), thereby reducing the need to increase routine arterio-venous extraction and preserving the scope to increase f_H, both of which may improve thermal tolerance and contribute to the increased BPT of cardiac function from 15.0°C to 17.7°C (Joyce et al., 2018a). Similar to that in *N. coriiceps*, $\dot{M}_{O_2}$ remains elevated in the congeneric species *Notothenia rossii* after 4 weeks of acclimation to 7°C (Strobel et al., 2012).

Effects on metabolism

The higher routine $\dot{M}_{O_2}$ of warm-acclimated *N. coriiceps* is likely to require a sustained increase in foraging success. In *N. rossii*, the hepatosomatic index (see Glossary) decreases by 50% in warm-acclimated animals compared with those at ambient temperature (Strobel et al., 2012). Despite thermal compensation of routine $\dot{M}_{O_2}$ in *T. bernacchii* acclimated to 4°C, growth rate and condition index decline with *ad libitum* feeding, probably as a result of lower assimilation rates (Enzor et al., 2017; Sandersfeld et al., 2015). Together, these data suggest that ocean warming will likely alter the energy budget of Antarctic notothenioids, diverting resources from reproduction and growth to maintain routine metabolic rate.

Overall, metabolic thermal plasticity varies widely among Antarctic fish species, tissue types and life stages, and does not always correlate with changes in cardiac performance or $\dot{M}_{O_2}$ (Davis et al., 2018; Enzor et al., 2017; Jayasundara et al., 2013; Lannig et al., 2005; Seebacher et al., 2005; Windisch et al., 2011). For example, although f_H in *T. bernacchii* increases with warm acclimation, CS, LDH and 3-hydroxyacyl CoA dehydrogenase (a biomarker for fatty acid oxidation) cardiac activity all decline. This could be indicative of either thermal compensation or a decrement in cardiac function (Jayasundara et al., 2013). Thermal plasticity in mitochondrial function of Antarctic notothenioids appears to be more limited than that of temperate teleosts (Strobel et al., 2013). For example, state 3 respiration rates and proton leak in liver mitochondria are not altered in *N. rossii* acclimated to 7°C (Strobel et al., 2013).

Warm acclimation may lower oxidative stress and potentially improve cardiac performance (Enzor and Place, 2014), thereby offsetting the increase seen with acute warming in *C. aceratus* (Mueller et al., 2012). Antarctic notothenioids have higher levels of molecular chaperones and ubiquitinated proteins, as well as genes encoding antioxidants and proteins of the ubiquitin–proteasomal pathway compared with temperate fishes, suggesting that cold temperature denatures proteins and that oxidative stress may be exacerbated by higher oxygen solubility in cold waters (Bilyk and Cheng, 2013; Kim et al., 2019; Place et al., 2004; Todgham et al., 2007). Warm acclimation seems to alleviate both of these stressors, because decreases are seen in: (1) transcript levels of the antioxidant catalase in hearts of *N. coriiceps* (Mueller et al., 2014); (2) levels of oxidized proteins in gill and liver of *Trematomus bernacchii* and *P. borchgrevinki* (Enzor and Place, 2014); and (3) expression of molecular chaperones and ubiquitin in livers of *P. borchgrevinki* (Bilyk and Cheng, 2014). Together, these data suggest that oxidative stress declines with a modest increase in acclimation temperature, providing some potential relief to life in a warmer environment (Todgham et al., 2007), and potentially improving cardiac performance.

Membrane remodelling

Biological membranes require an appropriate mix of polar and neutral lipids to support optimal function. As such, lipid remodelling to achieve homeoviscous adaptation (HVA) is a common response to temperature acclimation in many eurythermal organisms (see Glossary; Hazel, 1995). A good example of HVA over an evolutionary time scale is derived from studies of synaptic membranes from cold- and warm-bodied vertebrates. When compared at their physiological temperatures, Antarctic notothenioids have similar values for membrane anisotropy (a measure of membrane viscosity, the inverse of fluidity) to those for temperate ectotherms, and even endothermic organisms (Behan-Martin et al., 1993; Logue et al., 2000). Whether extreme stenotherms, like Antarctic notothenioids, have lost the capacity for HVA during warm acclimation has been examined by measuring membrane lipid composition. The results are mixed. For some species, tissues can alter the fatty acid composition of membrane phospholipids with warm acclimation (e.g. Truzzi et al., 2018), but in other cases, neither fatty acid unsaturation (Gonzalez-Cabrera et al., 1995; Strobel et al., 2013) nor cholesterol content (Malekar et al., 2018) changes with increases in temperature. Variable outcomes may result from different acclimation regimes, as warm-induced adjustments to fatty acid composition may require exposure above a threshold temperature (Malekar et al., 2018).

A clearer picture has emerged from direct measurements of membrane fluidity. Mitochondrial and microsomal membranes from the cardiac ventricle – and plasma membranes from gills – of *N. coriiceps* demonstrate perfect HVA efficacy following a minimum 6 week acclimation to 5°C (Fig. 5C,D) (Biederman et al., 2021). However, synaptic, mitochondrial and myelin membranes from the brain do not. A major feature of the HVA response in microsomal membranes (heart) and plasma membranes (gill) is an increased cholesterol content; plasma membranes from the gill and mitochondrial membranes from the heart also display longer chains among the fatty acids associated with phospholipids. All membrane types display modest adjustments in the composition of phospholipids with an increase in temperature. Taken together, these results indicate greater thermal plasticity of membranes in the heart and gill than in the brain. Considering the fact that brain synaptic membranes exhibit HVA over an evolutionary time course (Behan-Martin et al., 1993; Logue et al., 2000), brain membranes possibly require longer periods of warming than other tissues for structural remodelling. Thus, to achieve HVA efficacy, the time necessary for restructuring synaptic membranes and the rate of

continued warming in the Southern Ocean will matter. If the rate of climate warming is indeed too rapid, impaired brain function may underpin the limits to thermal tolerance in notothenioid fishes.

Conclusions

To predict with any accuracy the impacts of climatic warming on the fate of Antarctic notothenioids, their adaptive capacity for key traits limiting thermal tolerance must be thoroughly assessed. Research to date indicates that thermal plasticity varies considerably among Antarctic notothenioids both by trait and by species. Yet, in general, cardiac function and the molecular processes central to cardiac performance are malleable, although more so in some species than others. The sizable increase in $\dot{Q}$ with acute warming, even in a species with relatively low thermal tolerance (*C. aceratus*), together with the remodelling of cardiac membranes after weeks of warm acclimation in another notothenioid (*N. coriiceps*), provides the expectation that Antarctic notothenioids can make some of the physiological adjustments necessary to withstand some temperature elevation in the Southern Ocean. However, the extent to which notothenioids can survive a warming environment could ultimately be constrained by limited changes to both mitochondrial respiration in the heart and enzymatic activities associated with energy metabolism, combined with a lack of remodelling of membranes associated with the nervous system.

A factor not considered here is the impact of multiple stressors encountered in the natural environment; the combined effects of ocean acidification and warming are more deleterious and result in less metabolic remodelling than the effects of either stressor alone (reviewed by Todgham and Mandic, 2020). Of additional concern is the paucity of data regarding genetic diversity of Antarctic notothenioids. One of the few studies examining genetic diversity found that *Dissostichus mawsoni* (Antarctic toothfish) individuals sampled from three circumpolar sites display little genetic diversity in mitochondrial or nuclear DNA sequences (Smith and Gaffney, 2005). Nothing is known about the adaptive capacity of traits associated with thermal tolerance in notothenioids (i.e. gene expression, metabolism, membrane structure/function, cardiac performance). A recent study of changes in gene expression in rainbowfish (*Melanotaenia* sp.) showed that plasticity of 23 genes is under divergent selection for tolerance to heat stress, and notably among these genes were those encoding heat shock proteins (Sandoval-Castillo et al., 2020) – a family of genes that Antarctic notothenioids lack the capacity to modulate (Bilyk et al., 2018; Bogan and Place, 2019).

Lastly, notothenioids are central components of a rapidly changing Antarctic ecosystem, linking lower trophic levels (e.g. krill) with higher ones (e.g. seals, whales). Their survival hinges not only on their ability to withstand abiotic stressors but also on the impacts of disease (Harvell et al., 1999), invasive species (Hughes et al., 2020), changes in prey availability (Flores et al., 2012) and pressures from commercial fishing (Watters et al., 2020). The last of these is exacerbated by the fact that the majority of the catch is illegal, underreported or unregulated (O'Brien and Crockett, 2013). Thus, increasing the number of marine protected areas (MPAs) within the Southern Ocean is essential for ensuring the survival of Antarctic notothenioids. The establishment of a MPA within the WAP region is of critical importance given the acute impacts of climate warming in this region (Clarke et al., 2007). Unfortunately, the proposal for this MPA failed during the 2020 meeting of the Commission for the Conservation of Marine Living Resources (CCAMLR) but will be revisited in 2021 and hopefully enacted thereafter.

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Competing interests

The authors declare no competing or financial interests.

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