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REVIEW

Antarctic notothenioid fish: what are the future consequences of 'losses' and 'gains' acquired during long-term evolution at cold and stable temperatures?

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ABSTRACT

Antarctic notothenioids dominate the fish fauna of the Southern Ocean. Evolution for millions of years at cold and stable temperatures has led to the acquisition of numerous biochemical traits that allow these fishes to thrive in sub-zero waters. The gain of antifreeze glycoproteins has afforded notothenioids the ability to avert freezing and survive at temperatures often hovering near the freezing point of seawater. Additionally, possession of cold-adapted proteins and membranes permits them to sustain appropriate metabolic rates at exceptionally low body temperatures. The notothenioid genome is also distinguished by the disappearance of traits in some species, losses that might prove costly in a warmer environment. Perhaps the best-illustrated example is the lack of expression of hemoglobin in white-blooded icefishes from the family Channichthyidae. Loss of key elements of the cellular stress response, notably the heat shock response, has also been observed. Along with their attainment of cold tolerance, notothenioids have developed an extreme stenothermy and many species perish at temperatures only a few degrees above their habitat temperatures. Thus, in light of today's rapidly changing climate, it is critical to evaluate how these extreme stenotherms will respond to rising ocean temperatures. It is conceivable that the remarkable cold specialization of notothenioids may ultimately leave them vulnerable to future thermal increases and threaten their fitness and survival. Within this context, our review provides a current summary of the biochemical losses and gains that are known for notothenioids and examines these cold-adapted traits with a focus on processes underlying thermal tolerance and acclimation capacity.

KEY WORDS: Acclimation capacity, Biochemical adaptation, Climate change, Cold adapted, Hemoglobin, Icefish, Stenothermal, Thermal tolerance

Introduction

Antarctic notothenioids have long fascinated biologists because of their physiological and biochemical adaptations that permit life at sub-zero temperature. This suborder of bony fishes has evolved during the last 10–14 million years to thrive in a vast number of ecological niches and habitats within the frigidly cold and thermally stable waters of the Southern Ocean (Eastman, 1993). Notothenioids have had to overcome one very obvious threat to their well-being – death by freezing. Acquisition of antifreeze glycoproteins has afforded these animals the ability to avert intracellular ice formation and to survive in waters that often hover near −1.9°C, the freezing point of seawater (DeVries and Wohlschlag, 1969; Cheng and Chen, 1999; DeVries

and Cheng, 2005). The notothenioid clade is distinguished not only for gains of essential adaptations for coping with low temperatures but also for the disappearance of traits in some species that would likely prove lethal in warmer and more thermally variable environments. Most noteworthy of these losses is the absence of an inducible heat shock response (HSR) in all Antarctic notothenioids tested to date (Hofmann et al., 2000; Buckley et al., 2004; Place et al., 2004) and the lack of expression of hemoglobin (Hb) in species from the family Channichthyidae (Ruud, 1954; Cocca et al., 1995).

In conjunction with the acquisition of tolerance to extremely cold and stable temperatures, notothenioids have developed a highly stenothermal physiology, with many species subject to death at temperatures only a few degrees above their habitat temperature (Somero and DeVries, 1967; Podrabsky and Somero, 2006; Beers and Sidell, 2011; Bilyk and DeVries, 2011). In light of modern global warming, which is most rapid at high latitudes, it is imperative to examine the question of how notothenioids will respond to rising ocean temperatures in coming years. By no means are we the first to ponder the fate of notothenioids in a warming world. Climate change impact studies on Antarctic fauna have been ongoing for many years and a number of studies and reviews have addressed the topic (for reviews, see Coppes Petricorena and Somero, 2007; Pörtner et al., 2007; Somero, 2010; Patarnello et al., 2011; O'Brien and Crockett, 2013; Peck et al., 2014). Considering the current trends in increasing temperatures, in particular the rapid changes occurring in the Southern Ocean, examining the underlying physiological and biochemical mechanisms of thermal adaptation of notothenioids can help to better assess the vulnerability of these fishes to warming oceans (Somero, 2010). Thus, in an attempt to both summarize and build upon previous work, our primary objectives for this review are threefold: (1) to present an up-to-date summary of the biochemical losses and gains that are known for notothenioids, with emphasis placed on those traits that differentiate the group from other fishes; (2) to examine these losses and gains associated with cold specialization, with a specific focus on processes underlying thermal tolerance and the capacity for thermal acclimation; and (3) to offer ideas and suggestions for future paths of study. To set the stage for our analysis, we first provide a brief history of the development of the Southern Ocean and the evolution of notothenioid fishes.

The Southern Ocean

Contemporary Antarctica consists of an ice-covered, dry land mass that is surrounded by the Southern Ocean. It is known as one of the coldest and windiest regions on Earth. Over the course of the last ~55 million years, several key geologic events isolated the continent from other land masses, permitting circular water flow around the entire perimeter of the continent for the first time (Eastman, 1993; Scher and Martin, 2006). These geological and oceanographic changes established the circumpolar current system that we see

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List of symbols and abbreviations

ACC	Antarctic Circumpolar Current
AFGP(s)	antifreeze glycoprotein(s)
APF	Antarctic Polar Front
CCT	chaperonin-containing t-complex polypeptide-1
CS	citrate synthase
CT_{max}	critical thermal maximum
Hb	hemoglobin
HSPs	heat shock proteins
HSR	heat shock response
k_{cat}	substrate turnover number
K_m	Michaelis–Menten constant
LDH	lactate dehydrogenase
Mb	myoglobin
MCA	metabolic cold adaptation
ND6	NADH dehydrogenase subunit 6 gene
SOT	systemic oxygen transport
WAP	Western Antarctic Peninsula

today (Fig. 1). Development of the Antarctic Circumpolar Current (ACC) in particular has led to the extremely cold and stable temperatures characteristic of today's Southern Ocean and has been a significant driver in the evolution of Antarctic marine animals
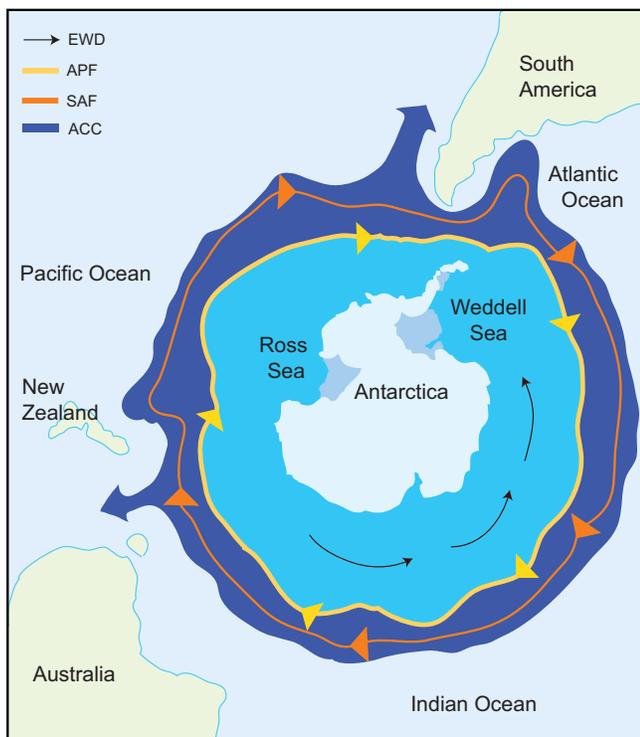
Fig. 1. The Southern Ocean and its oceanographic current system surrounding Antarctica. Following isolation of the continent from other landforms ~55 million years ago when tectonic rifting separated South America and Antarctica, two noteworthy events pursued: (i) the opening of the Drake Passage, the body of water flowing between South America and the Antarctic Peninsula, ~40 million years ago, and (ii) the opening of the Tasmanian Gateway between Antarctica and Australia, ~25 million years ago (Eastman, 1993; Scher and Martin, 2006). Combined, these geologic events allowed water to flow around the entirety of the continent for the first time and led to present-day oceanographic features [e.g. the Antarctic Circumpolar Current (ACC) and the Antarctic Polar Front (APF)] of the Southern Ocean. The ACC is located between 50°S and 60°S and is the largest current on Earth in terms of both length and transport capacity (Gordon, 1999). SAF, Sub-Antarctic Front; EWD, East Wind Drift.

(Eastman, 1993). Of equal significance is the Antarctic Polar Front (APF), the northern boundary of the ACC that is distinguished by a prominent change in seawater surface temperature of about 3–4°C (Knox, 1970). The APF forms a thermal and physical barrier that inhibits species from migrating between sub-Antarctic and Antarctic waters (McDonald et al., 1987). However, this barrier may not be as impermeable as once thought (Page and Linse, 2002; Clarke et al., 2005; Thornhill et al., 2008), and trans-APF movement of marine animals is likely to occur more frequently as the ocean warms.

Water temperatures of the Southern Ocean have been well below 5°C for the last 10–14 million years and currently range between +1.5 and −1.9°C at the most northerly and southerly latitudes, respectively (Littlepage, 1965). There is little variation in temperature across seasons or as a function of depth because of the well-mixed nature of the ACC and APF (DeWitt, 1970; Hunt et al., 2003), and the waters are also noted for their exceptionally high oxygen content. Another important historical consideration is that cooling over the last ~5 million years has not been completely monotonic because of cyclical freezing and warming events, which have resulted in corresponding advances and retreats of the continental ice sheet, thereby introducing new habitats and niches for faunal radiation (Eastman, 1993). Thus, a suite of abiotic environmental conditions has been highly influential in shaping both the biological specialization and biogeographic distribution of marine species in the Southern Ocean.

Evolution of notothenioid fishes
The origins of the modern Antarctic fish fauna are poorly understood as a result of a minimal fossil record (Eastman, 1993). It is generally thought that today's fauna originated from a sluggish, benthic stock that colonized and diversified in Southern Ocean waters sometime during the fragmentation period of Gondwana 40–60 million years ago, when cooling of the circumpolar current first began (Eastman, 1993). Additionally, it is hypothesized that the contemporary fish fauna has been very successful in its radiation of this marine system largely because of the persistence of an ancestral stock that survived a dramatic collapse in fish species diversity ~30 million years ago (Eastman, 1993). Governed primarily by a few groups, today's Antarctic fish fauna consists of 227 species that belong to 18 highly endemic families of the region. The most speciose taxa are from two perciform assemblages, the suborder Notothenioidei (notothenioids) and the Zoarcidae (eelpouts), and one scorpaeniform family, Liparidae (snailfish) (Eastman, 1993; Eastman and McCune, 2005). The monophyletic Notothenioidei in particular has flourished in the Southern Ocean and dominates the fish fauna (Fig. 2) (Gon and Heemstra, 1990; Eastman, 1993, 2005; Eastman and Eakin, 2000). Evolution in geographic isolation in this environment has led to adaptations to tolerate freezing conditions and to optimize overall fitness, including reproductive, sensory and locomotory functions at exceptionally cold body temperature (di Prisco et al., 1991; Eastman, 1993; Wilson et al., 2002; Near et al., 2012).

Adaptations to living in chronically frigid waters
The evolutionary histories of notothenioids have been marked by both gains and losses of biological traits. The acquisition of antifreeze proteins (DeVries and Wohlschlag, 1969; Cheng and Chen, 1999; DeVries and Cheng, 2005) and enzymes and mitochondria that maintain function at low temperatures (Fields and Somero, 1998; Johnston et al., 1998; Weinstein and Somero, 1998; Hardewig et al., 1999; O'Brien and Sidell, 2000; Kawall
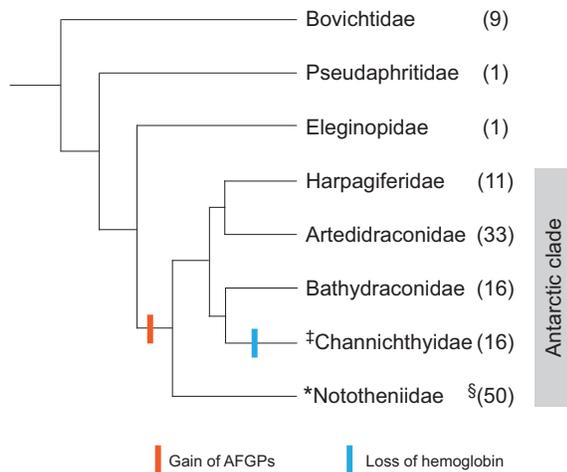
Fig. 2. Phylogenetic distribution of the perciform suborder Notothenioidei. Dominating the fish fauna of the Southern Ocean, notothenioids encompass eight families, 44 genera and 137 species to date (compiled by J. T. Eastman and R. R. Eakin; updated August 2014 at www.oucom.ohiou.edu/dbms-eastman), and account for approximately 46% of fish species and 90% of fish biomass south of the APF (Ekau, 1990; Eastman, 1993, 2005). Phylogenetic relationships among notothenioid families are as described by Bargelloni et al. (2000), Near et al. (2004) and Near and Cheng (2008). The total species count for each family is noted in parentheses. The red bar symbolizes the genetic gain of antifreeze glycoproteins (AFGPs) while the blue bar signifies the loss of hemoglobin (Hb) in Channichthyid icefishes. [‡]Some species also lack the oxygen-binding protein myoglobin (Mb). [*]Secondary loss of AFGPs in certain Notothenid species. [§]Some species within the family are non-Antarctic.

et al., 2002) are prominent examples of biochemical essentials underlying cold adaptation of these fishes. Similarly, the gain of cold-stable proteins, such as tubulins (Detrich et al., 1989, 2000; Redeker, et al., 2004), the evolution of membranes that maintain their fluidity at cold temperatures, i.e. homeoviscous adaptation (Hazel, 1984; Logue et al., 2000), and the reorganization of metabolic pathways (Crockett and Sidell, 1990; Magnoni et al., 2013) play a significant role in optimizing function at sub-zero temperature. In contrast, the loss of critical functional traits such as the inducible HSR (Hofmann et al., 2000; Buckley et al., 2004; Place et al., 2004) and the oxygen-binding proteins Hb and myoglobin (Mb), which are tolerated because of low and stable temperatures, is thought to play a key role in determining the extreme stenothermy of notothenioids (Ruud, 1954; Cocca et al., 1995; Sidell et al., 1997). In the following sections, we discuss how the gain of novel traits and the loss of other physiological capacities have simultaneously led to the success of notothenioids in their cold and stable habitat and poised them for challenges in the face of climate change.

Gain of functions needed for life at stable cold temperatures
Antifreeze proteins
First discovered in the blood serum of Antarctic notothenioids in the late 1960s, antifreeze glycoproteins (AFGPs) are one of the most significant adaptations to supporting life in extremely cold environments (DeVries and Wohlschlag, 1969; Cheng and Chen, 1999; DeVries and Cheng, 2005). Notothenioid AFGPs are differentiated by molecular weight into eight distinct types and the primary chemical structure is highly conserved in all notothenioids studied to date (Komatsu et al., 1970; DeVries, 1971; Ahlgren and DeVries, 1984). AFGPs exert their 'antifreeze' properties by attaching to existing ice crystals to prevent further

growth, a mechanism known as adsorption-inhibition (Raymond and DeVries, 1977; DeVries, 1984). Through this growth-inhibiting effect, small ice crystals that may arise *de novo* or are acquired while drinking seawater (part of the osmoregulatory strategy of all marine bony fish) are effectively inhibited from growing to sizes large enough to damage tissues and cells.

Antifreeze proteins are not unique to Antarctic fish, but have evolved in a multitude of different organisms that live at sub-zero temperatures (Barrett, 2001; Duman, 2001). A variety of protein-encoding genes have been exploited to generate antifreezes in high-latitude fishes (Fletcher et al., 2001), but the AFGP gene in notothenioids has specifically evolved from a pancreatic trypsinogen precursor (Chen et al., 1997a,b). Notothenioid AFGPs are produced in pancreatic tissue and the anterior portion of the stomach, where they enter the gut cavity to exert their cryoprotective effects upon ingested ice. However, the pathway and mechanism of entry into the gut remain unknown (Cheng et al., 2006).

Interestingly, a recent study revealed that AFGPs also inhibit the melting of ice crystals (Cziko et al., 2014). Cziko and collaborators found that AFGP-stabilized ice crystals resist melting at temperatures above the expected equilibrium freezing/melting point. They observed what they termed 'superheated ice' in notothenioid serum samples and in solutions of purified AFGPs, and confirmed similar findings in natural populations. Cziko and colleagues conjecture that the evolutionary benefit of possessing life-saving AFGPs might be tempered by the accumulation of damaging internal ice over the course of a lifetime (Cziko et al., 2014), a paradoxical cost that accompanies the benefit of freezing prevention.

Cold adaptation of metabolic processes
Organismal capacity to modify metabolic processes through acclimatory and adaptive mechanisms to optimize ATP production at a given temperature is crucial to overall fitness. Therefore, cold adaptation of metabolic processes is a key aspect of specialization to low temperatures. Whether notothenioids have compensated for the effects of low temperature, such that their oxygen consumption rates are similar to those of warm-adapted teleosts at a higher temperature (a concept termed metabolic cold adaptation or MCA), has generated an intriguing and somewhat contentious discussion in the literature. Metabolic rate comparisons between cold- and temperate-adapted species are inherently difficult considering that numerous factors, including levels of locomotory activity, physiological state and body size, are associated with establishing the metabolic requirements of a given organism (White et al., 2012). Interspecific comparisons involving brain tissues might be considered most pertinent because metabolic activity in the brain is largely independent of locomotory demands and the reproductive and nutritional state of an organism. In fact, oxygen consumption of brain tissues of the notothenioid *Trematomus bernacchii* exhibits a substantial, yet incomplete, compensation for the effects of low temperature (Somero et al., 1968). The mechanisms underlying this compensation for the effects of cold have been partially elucidated through studies of enzyme activities and mitochondrial function, as discussed below.

Metabolic enzyme activity
Metabolic enzyme activity data from several notothenioid species suggest a partial thermal compensation for the effects of low temperature and demonstrate a capacity for modifying activity levels with long-term exposure to higher temperatures. Based on maximal enzyme activities of citrate synthase (CS) and lactate dehydrogenase (LDH) in brain homogenates of Antarctic and tropical fishes, Kawall and colleagues showed a partial temperature compensation

of ATP-generating capacity in notothenioids (Kawall et al., 2002), consistent with earlier observations of partial compensation in brain oxygen consumption rate (Somero et al., 1968). MCA is further supported by the observation that CS enzyme activities, adjusted for assay temperature, of several notothenioid species are higher than those of their temperate counterparts (Sidell et al., 1987; Crockett and Sidell, 1990; Johnston et al., 1994; Jayasundara et al., 2013). More recently, White and colleagues illustrated metabolic compensation on multiple fronts, including the whole animal, and at the level of enzymatic and mitochondrial function (White et al., 2012). Similar to past studies, White and colleagues observed only a partial metabolic compensation and concluded that evolutionary adaptation and thermal plasticity in fishes appear to be insufficient to fully overcome the acute thermodynamic effects of temperature (Johnston et al., 1994; White et al., 2012).

In contrast to research corroborating MCA, there have been a number of studies that might appear to call into doubt the occurrence of MCA. For example, in the study by Kawall and colleagues that demonstrated MCA in activities of brain enzymes, no compensation was suggested in the case of white skeletal muscle (Kawall et al., 2002). However, this 'failure' to observe MCA is likely a reflection of the sluggish lifestyle of the notothenioids studied and the large role played by labriform locomotion involving the pectoral fin assemblage. Thus, whereas white skeletal muscle associated with trunk muscle-driven locomotion did not exhibit high levels of ATP-generating enzymes, the red muscle that powers labriform swimming had extremely high activities of CS. This observation again emphasizes the difficulties of making metabolic rate comparisons among different species that employ different locomotory strategies.

Comparison of metabolic enzyme levels across differently thermally adapted species must also take into account the organization of metabolism; specifically, the differential reliance on diverse substrates as sources of energy for ATP production. Magnoni and colleagues recently reported that enzymes important in gluconeogenesis did not display activity values consistent with MCA, and found this to be true in comparisons with non-Antarctic notothenioids and at multiple experimental temperatures (Magnoni et al., 2013). They concluded that their results might simply indicate that the use of glycerol as a precursor for glucose synthesis is of low physiological importance to these fish, and offered additional evidence to corroborate their final analyses (Magnoni et al., 2013). At the least, these results provide additional backing for the importance of metabolic reorganization that has been shown for other physiological pathways (Crockett and Sidell, 1990).

Finally, at the level of performance of individual enzymes, it is clear that the functional properties of enzyme orthologs of notothenioids are highly cold adapted. These enzymes – for example, the glycolytic enzyme lactate dehydrogenase-A – exhibit substrate-binding abilities (as indexed by the Michaelis–Menten constant, K_m) at low, ambient temperatures that are the same as those found for orthologs of more warm-adapted species at their higher body temperatures (Fields and Somero, 1998). Substrate turnover number (k_{cat} values) shows striking, but not complete, compensation for temperature as well (Low et al., 1973; Fields and Somero, 1998).

Mitochondrial function

Adequate mitochondrial function, in particular maintaining the capacity to effectively generate ATP at ambient temperatures, plays an important role in determining the thermal limits of animals. Several studies have characterized cold adaptation in these organelles, including thermal sensitivity of mitochondrial function in Antarctic fish (Weinstein and Somero, 1998; Hardewig et al., 1999; O'Brien and Sidell, 2000; Mark et al., 2012). Results to date support that thermal compensation of mitochondrial activity may be driven by increased mitochondrial density and not by more efficient mitochondria in notothenioids, at least in oxidative muscle of some species (Johnston et al., 1994, 1998; O'Brien and Sidell, 2000). In addition, a high thermal sensitivity was detected in respiratory rates and coupling of electron transport to ADP phosphorylation in mitochondria isolated from *T. bernacchii* liver compared with other marine ectotherms, providing strong evidence for cold adaptation and stenothermy in mitochondrial function (Weinstein and Somero, 1998). Conversely, results describing oxidative phosphorylation in the liver of *Pleuragramma antarcticum*, a pelagic notothenioid, indicate that thermal sensitivity of mitochondria is not the driving force behind their organismal level stenothermy (Martinez et al., 2013). Whether this finding indicates a tissue- and/or species-specific response or extends more broadly to other notothenioids is not clear.

The mechanisms underpinning cold adaptation of mitochondrial function in Antarctic fish are not well understood. Much attention has recently been given to the mitochondrial NADH dehydrogenase subunit 6 gene (ND6), a protein that is crucial in the assembly and function of complex I of the mitochondrial electron transport chain and has been found to be translocated within the mitochondrial control region (Papetti et al., 2007; Zhuang and Cheng, 2010; Mark et al., 2012). The significance of this translocation is yet to be fully elucidated, but recent work by Mark and collaborators has shown that certain changes in amino acid composition of this protein may play a role in the overall thermal sensitivity of mitochondrial ATP synthesis (Mark et al., 2012). Considering the crucial role of mitochondria in various cellular processes, further studies are warranted in exploring thermal adaptations of mitochondrial function in Antarctic notothenioids.

Loss of functions permitted by life at stable cold temperatures
Loss of inducible HSR

The overall cellular stress response is thought to involve up-regulation of molecular chaperones, metabolic reorganization and, under severe stress, induction of apoptotic mechanisms and inhibition of cell cycle progression (Kültz, 2005). Molecular chaperones play an important role in protein homeostasis and are involved in biosynthesis, folding and compartmentalization (Basu et al., 2002; Clark and Peck, 2009; Tomanek, 2010). When exposed to a cellular stressor, including thermal stress, inducible chaperones prevent the aggregation of denatured proteins. While constitutive forms of molecular chaperones are found across all taxa, including Antarctic fish, notothenioids lack the capacity to up-regulate key chaperoning molecules such as heat shock proteins (HSPs) under temperature stress or other abiotic stressors, e.g. heavy metal exposure (Hofmann et al., 2000, 2005; Buckley et al., 2004; Place et al., 2004; Clark et al., 2008). Loss of an inducible HSR has been further substantiated at the molecular level in several species via the use of large-scale transcriptomic methods (Buckley and Somero, 2009; Thorne, 2010; Huth and Place, 2013; Bilyk and Cheng, 2013). The lack of an inducible HSR and other chaperones detected in *Pagothenia borchgrevinki* is especially intriguing, because this species has demonstrated an increased capacity to induce a thermal compensatory response at the whole organismal level with warm acclimation (Seebacher et al., 2005; Robinson and Davison, 2008). Interestingly, however, a recent transcriptomic analysis revealed an induced HSR in the blood of *Notothenia coriiceps*, though a
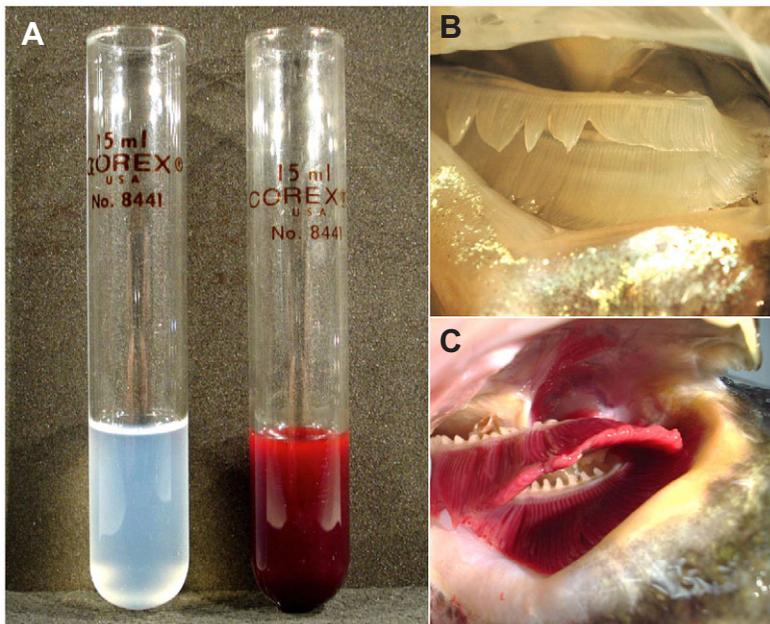
Fig. 3. Loss of Hb in the family Channichthyidae. (A) There is a startling contrast between the translucent white blood of Hb-lacking *Chaenocephalus aceratus* (left Corex tube) and that of the Hb-expressing notothenioid *Notothenia coriiceps* (right Corex tube). Similarly, icefish gills (B) are exceptionally pale in color compared with those of red-blooded species (C) that possess the oxygen-binding hemoprotein. Photo credit: J.M.B.

similar response was not confirmed at the protein level (Shin et al., 2014).

A high constitutive expression level of HSPs may be crucial to maintaining protein homeostasis in the cold because overall levels of damaged or misfolded proteins are higher in Antarctic fish compared with related species from temperate regions (Place and Hofmann, 2005; Todgham et al., 2007). The lack of HSP induction during stress thus might be a reflection of the relatively high constitutive expression of these molecules in notothenioids (Buckley et al., 2004; Todgham et al., 2007; Cuellar et al., 2014). Supporting this further, Cuellar and colleagues (2014) characterized the evolutionary adaptation of a constitutively expressed chaperone, CCT (chaperonin-containing t-complex polypeptide-1), a protein that plays a key role in cellular homeostasis by assisting folding of newly synthesized proteins (Pucciarelli et al., 2006; Valpuesta et al., 2002; Dekker et al., 2008). In the notothenioid *Gobionotothen gibberifrons*, the catalytic folding cycle of CCT is partially compensated for thermal effects at its habitat temperature, which helps at least in part to maintain efficient protein folding at cold temperatures (Cuellar et al., 2014).

In the absence of an inducible HSR, Buckley and Somero proposed that the notothenioid cellular stress response might reflect molecular changes underlying cell cycle arrest and programmed cell death (Buckley and Somero, 2009). Supporting this hypothesis, transcriptomic analyses of *T. bernacchii* and *P. borchgrevinki* exposed to 4°C over a short period of time displayed induction of genes involved in proteasomal degradation, cell cycle regulation and apoptosis (Buckley and Somero, 2009; Bilyk and Cheng, 2013). A more recent study further demonstrated that even at temperatures as low as 2°C, there are effects on cell cycle progression and proliferation, and an increase in apoptosis of *T. bernacchii* (Sleadd et al., 2014). Overall, these data suggest that lack of an inducible HSR under thermal stress might be causally linked to apoptotic cell fate. Sleadd and colleagues argue that pathways underlying programmed cell death might be favored in the absence of HSR in these fish, although such a process may not be evolutionarily advantageous. Considering that a similar cellular stress response was detected in *P. borchgrevinki* with short-term

exposure to a higher temperature, investigating the impact of long-term warm acclimation at the cellular level might provide further insights into thermal acclimatory capacity demonstrated by this organism (Seebacher et al., 2005). Nonetheless, these data clearly demonstrate that Antarctic fish are highly vulnerable to prolonged exposure to higher temperatures at the cellular and molecular level.

Loss of major hemoproteins

Perhaps the most striking biochemical trait within the Notothenioid suborder is loss of Hb in icefishes from the family Channichthyidae (Ruud, 1954). They are the only known vertebrate animals to completely lack Hb in their blood as adults. Loss of this oxygen-transporting hemoprotein is visually very distinct when observing their translucent-white blood or pale-white gills (Fig. 3). All 16 species within the Channichthyidae family lack the Hb protein as a consequence of a multi-step mutational process that has resulted in loss of the β-globin gene and partial omission of the α-globin gene from the αβ-globin complex, thereby rendering the locus functionally inactive (Cocca et al., 1995; Zhao et al., 1998; di Prisco et al., 2002; Near et al., 2006). Interestingly, one of the 16 icefish species, the phylogenetically derived *Neopagetopsis ionah*, retains a complete, although non-functional, αβ-globin complex that is similar to the ancestral condition seen in Hb-expressing species from the sister family Bathydraconidae (Near et al., 2006).

Absence of a transport protein has caused oxygen to be carried strictly in physical solution of the blood and has resulted in an oxygen-carrying capacity in icefishes that is <10% of that in closely related red-blooded species (Holeton, 1970). Icefishes largely compensate for the lack of Hb by utilizing a suite of cardiovascular system modifications: large-bore blood vessels, which are present in high density in some tissues; large blood volumes; and large hearts containing more mitochondria than hearts of similar-sized Hb-expressing fishes (Hemmingsen and Douglas, 1970; Holeton, 1970; Hemmingsen et al., 1972; Fitch et al., 1984; O'Brien and Sidell, 2000; Wujcik, et al., 2007). There is some evidence to support the hypothesis that some of these characteristics or 'hallmark traits' of icefishes, such as enhanced vascular densities (Wujcik et al., 2007),

may have originated via pathways mediated by nitric oxide (Sidell and O'Brien, 2006; Beers et al., 2010; Borley et al., 2010). Whatever their mechanism of origin, integration of all of these features allows channichthyids to circulate large blood volumes at relatively high flow rates and, importantly, without excessive pressure development. Ultimately, the combination of high-throughput cardiovascular systems, low absolute metabolic rates and waters of high oxygen content enables these animals to deliver sufficient oxygen to their tissues (Hemmingsen, 1991).

In addition to the loss of Hb, many species within the Channichthyid family lack Mb, an intracellular oxygen-binding protein found in muscle. In total, six out of 16 icefish species lack Mb expression in their heart ventricles, a loss that has occurred through four different mutational events (Sidell et al., 1997; Grove et al., 2004; Borley and Sidell, 2011). Lack of Mb expression has important functional effects (Acierno, et al., 1997), but overall significance in the long term may be less than that of Hb loss because the Mb deficit has evolved repeatedly in teleost fishes, whereas it appears that Hb deletion has been restricted to notothenioids alone (Macqueen et al., 2014). However, reduction in Hb concentration is common in many teleost species, possibly as a strategy for reducing blood viscosity in the cold (an energetic benefit) and/or because of relaxed selection for Hb-mediated oxygen transport in typically well-oxygenated waters where diffusion-based processes can suffice (Bargelloni et al., 1998; di Prisco et al., 2002; Beers and Sidell, 2011). Nonetheless, functional disaptations (traits whose use to the organism is discernibly inferior to that of a phylogenetically antecedent trait) of the cardiovascular system have been suggested for genetic loss of Hb and Mb (Montgomery and Clements, 2000; Sidell and O'Brien, 2006; Garofalo et al., 2009; Tota et al., 2012), which may have far-reaching implications in the face of environmental change. Also along these lines, O'Brien and colleagues revealed that hearts of icefishes lack the mitochondrial isoform of creatine kinase, an enzyme that plays a pivotal role in energy flux by catalyzing the reversible transfer of phosphate between creatine and ADP (O'Brien et al., 2014). This presents another protein within the cardiovascular system that has disappeared over evolutionary time at cold and stable temperature, and which may contribute to a compromised ability to respond to warming oceans in the future.

What are the consequences of cold specialization in a warming world?

Biochemical gains related to cold adaptation have been clearly beneficial to notothenioids in most respects, whereas losses of function have come to be tolerated, in some cases through further adaptations, e.g. in cardiac capacity. How these highly stenothermic and cold-specialized fishes will endure in the face of contemporary environmental change remains to be seen, however. Antarctica and the surrounding Southern Ocean, like the rest of the planet, are undergoing dramatic climatic alterations (Petit et al., 1999; Gille, 2002; Turner et al., 2005; Steig et al., 2009; Lyman et al., 2010). The Western Antarctic Peninsula (WAP) region in particular is one of our planet's 'hot spots' of rapid warming as temperatures are increasing at a rate that is several times the global average (Vaughan et al., 2001, 2003). The oceanic environment has been heavily impacted as temperatures of the Upper Circumpolar Deep Water of the ACC have risen 0.17°C from the 1950s to the 1980s, and surface seawater temperatures have increased by 1°C over the past 50 years and are predicted to rise another 2°C in the coming century (Meredith and King, 2005;

Murphy and Mitchell, 2005; Clarke et al., 2007). Needless to say, there has been considerable interest in how these rising temperatures will impact marine fauna (Pörtner et al., 2007; Somero, 2010; Patarnello et al., 2011; O'Brien and Crockett, 2013; Peck et al., 2014). Thus, we now consider how the highly specialized thermal adaptations of notothenioids might influence their vulnerability status and overall fitness in the context of global warming. We first discuss thermal tolerance 'windows', the entire range of temperatures that permit survival and a metric that provides insights into an animal's physiological plasticity in response to shifting temperature regimes. We then offer an assessment of the ability of Antarctic notothenioids to 'keep up' with climate change via another important indicator of function and potential for adaptation—acclimation capacity.

Thermal tolerance

The first report of reduced heat tolerance of notothenioids demonstrated that the upper incipient lethal temperatures of *P. borchgrevinki*, *T. bernacchii* and *T. hansoni* are between 5 and 7°C (Somero and DeVries, 1967). These were the lowest upper thermal limits recorded for an animal at the time. Since then, the breadth of thermal tolerance studies has extended considerably by including numerous new species from a diversity of biogeographic locations and habitats, ranging from McMurdo Sound to the Antarctic Peninsula region (Table 1) (Podrabsky and Somero, 2006; Franklin et al., 2007; Beers and Sidell, 2011; Bilyk and DeVries, 2011; Jayasundara et al., 2013). Whole-organismal temperature limits are described in this subsection, but it should be noted that thermal tolerance research has expanded to incorporate all levels of biological organization, some examples of which will be presented in the following discussion.

An extensive whole-organism thermal tolerance study was completed recently by Bilyk and DeVries (2011), in which they examined the critical thermal maximum (CT_{max}) of 11 Antarctic notothenioid species, ranging in location from McMurdo Sound to the Seasonal Pack-ice Zone of the Antarctic Peninsula (WAP region). CT_{max} is a commonly used method to assess upper thermal tolerance limits by monitoring an animal's ability to retain its righting response. Bilyk and DeVries (2011) found that CT_{max} ranged from 11.95 to 16.17°C and further noted that there was a significant regional split in the results, with higher CT_{max} values observed in species from the more northerly and variable WAP region (Table 1). In a subsequent study, the phenomenon of heat hardening, the short-lived increase in heat tolerance following a sub-lethal exposure to extreme heat shock, was demonstrated in six notothenioids (Bilyk et al., 2012). Increased tolerance of high temperatures following short-term heat exposure was also observed by Podrabsky and Somero (2006) for some, but not all notothenioid species examined. These studies suggest that notothenioids have retained a certain amount of plasticity in their response to heat stress.

In related research, Beers and Sidell (2011) quantified for the first time temperature sensitivities of the Hb-less icefishes. They compared the CT_{max} of two icefish species, *Chaenocephalus aceratus* and *Chionodraco rastrospinosus*, and three Hb-expressing notothenioids, and found that icefishes were more sensitive to rising temperature than their red-blooded relatives (Table 1). Notably, their results indicated that thermal tolerance is correlated to oxygen-carrying capacity of the blood, as indicated by the amount of Hb in circulation (Fig. 4) (Beers and Sidell, 2011). The CT_{max} values for icefishes are not as low as some of those reported for red-blooded species from McMurdo Sound,

Table 1. Summary of upper thermal limits measured for Antarctic notothenioids

Organism	Habitat[a]	Method	Thermal limit at habitat temperature (°C)	Acclimation		Study
				Temperature and duration	Thermal limit (°C)	
Chaenocephalus aceratus[c]	Demersal	CT$_{max}$ (3.6°C h^{-1})	13.9±0.38			Beers and Sidell, 2011
Gobionotothen gibberifrons	Demersal	CT$_{max}$ (18°C h^{-1})	16.11±0.99	4°C for 7 days	17.90±1.08	Bilyk and DeVries, 2011
		CT$_{max}$ (3.6°C h^{-1})	15.5±0.21			Beers and Sidell, 2011
Lepidonotothen nudifrons	Demersal	CT$_{max}$ (18°C h^{-1})	15.06±0.56	4°C for 21 days		Bilyk and DeVries, 2011
Notothenia coriiceps	Demersal	CT$_{max}$ (18°C h^{-1})	16.17±1.40	4°C for 14 days	17.39±1.2	Bilyk and DeVries, 2011
		CT$_{max}$ (3.6°C h^{-1})	17.1±0.20	4°C for 7 days	17.00±0.1	Beers and Sidell, 2011
Notothenia rossii	Demersal	CT$_{max}$ (18°C h^{-1})	16.16	4°C for 21 days		Bilyk and DeVries, 2011
Trematomus bernacchii	Demersal	Cardiac arrhythmia (5°C h^{-1})	11.67±0.77	4.5°C for 14 days	12.13±0.96	Jayasundara and Somero, 2013
		CT$_{max}$ (18°C h^{-1})	13.62±1.32	4°C for 21 days	15.02±1.06	Bilyk and DeVries, 2011
		Upper incipient lethal temperature[b]	5–6			Somero and DeVries, 1967
Trematomus hansoni	Demersal	CT$_{max}$ (18°C h^{-1})	13.09±0.69	4°C for 21 days	15.42±0.55	Bilyk and DeVries, 2011
		Upper incipient lethal temperature[b]	5–6			Somero and DeVries, 1967
Trematomus pennellii	Demersal	CT$_{max}$ (18°C h^{-1})	12.28±0.74	4°C for 21 days	15.42±0.85	Bilyk and DeVries, 2011
Lycodichthys dearborni[d]	Bathydemersal	CT$_{max}$ (18°C h^{-1})	13.44±0.82	4°C for 21 days	15.37±0.84	Bilyk and DeVries, 2011
Pachycara brachycephalum[d]	Bathydemersal	CT$_{max}$ (18°C h^{-1})	14.54±0.61	4°C for 21 days	17.15±0.66	Bilyk and DeVries, 2011
		CT$_{max}$ (0.042°C h^{-1})[e]	10.00			Van Dijk et al., 1999
Chionodraco rastrospinosus[c]	Benthopelagic	CT$_{max}$ (3.6°C h^{-1})	13.3±0.15			Beers and Sidell, 2011
Lepidonotothen squamifrons	Benthopelagic	CT$_{max}$ (18°C h^{-1})	15.38±1.02	4°C for 21 days		Bilyk and DeVries, 2011
		CT$_{max}$ (3.6°C h^{-1})	14.2±0.36			Beers and Sidell, 2011
Pagothenia borchgrevinki	Cryopelagic	CT$_{max}$ (18°C h^{-1})	11.95±0.90	4°C for 21 days	15.19±1.28	Bilyk and DeVries, 2011
		Upper incipient lethal temperature[b]	5–6			Somero and DeVries, 1967

Podrabsky and Somero (2006) quantified time to mortality at 14°C for *T. bernacchii* (<20 min), *T. hansoni* (<20 min), *P. borchgrevinki* (<15 min) and *T. pennellii* (<35 min), and for zoarcid *L. dearborni* (<40 min) acclimated to −1.8°C. With acclimation to 4°C, time to mortality increased significantly except in *P. borchgrevinki* and *L. dearborni*.
[a]Habitat information based on www.fishbase.org; [b]Highest temperature at which 50% of fish survived ≥1 week; [c]Channichthyids that lack hemoglobin; [d]Antarctic eelpout from Zoarcidae (all other species from suborder Notothenioidei); [e]Temperature increased 1°C per night over 12 days up to 10°C.

but this is likely explained by their inhabitance of a more northerly (i.e. relatively warm) and variable environment in the WAP compared with the Ross Sea. Thus, among notothenioids from common latitude, icefishes may be the most temperature-sensitive group and, consequently, the most susceptible to global warming.

The finding that thermal tolerance correlates with oxygen-carrying capacity in notothenioids may be one of the best examples to date to support the concept of oxygen- and capacity-dependent thermal tolerance, which has been used to explain climate-induced effects of rising temperatures in numerous other marine species (Mark et al., 2002; Pörtner and Knust, 2007; Pörtner, 2010). However, as intriguing as this relationship is, we advise that it should be taken with some caution, particularly in the context of chronic temperature stress, because of the complexities of the icefish cardiovascular system and likelihood that multiple factors could be contributing to the enhanced thermal sensitivity of this group. Among these other potential factors are a diminished ability of icefish hearts to induce an antioxidant defense (Mueller et al., 2012) and the absence of other key proteins involved in cardiovascular function from the icefish genome (O'Brien et al., 2014). Additionally, it has recently been suggested that a better approach to testing the upper thermal limits of metabolism is to assess the correlation of thermal tolerance to systemic oxygen transport (SOT), the product of cardiac output and blood oxygen content (Buckley et al., 2014). Using this type of SOT analytical method, Buckley and colleagues showed that the mutation resulting in the Hb-less condition would be metabolically prohibitive at elevated temperatures (Buckley et al., 2014). Finally, there are also data to support the hypothesis that cardiac Na$^+$ ion channels may be the 'weakest link' and potentially a limiting factor for upper thermal tolerance in fish (Vornanen et al., 2014), though whether this holds true for notothenioids is not yet known.

Acclimation capacity

The limited upper thermal tolerance of most notothenioids is associated with a reduced acclimatory capacity – the ability to adjust thermal optima of physiological processes at a new temperature. Recent studies have demonstrated a limited increase in upper thermal limits following warm acclimation in several notothenioid species (Table 1) (Podrabsky and Somero, 2006; Bilyk and DeVries, 2011). Bilyk and DeVries showed that the magnitude of warm acclimation-induced thermal tolerance is highly species specific, with *P. borchgrevinki* significantly increasing its upper thermal limits compared with others when acclimated to 4°C (Bilyk and DeVries, 2011). In contrast, Podrabsky and Somero (2006) failed to observe a significant increase in acute heat tolerance in this species following acclimation to 4°C. The basis of this inter-study disagreement is unclear, but it emphasizes the potential contributions of variation in physiological state and experimental technique to observed thermal tolerance limits.

It is important to keep in mind that acclimation capacity also depends on the biological organization level under examination; assessing the capacity of systems other than the whole organism often yields additional key insights. As shown by Mark and colleagues, thermal range of notothenioids at the whole-organismal level is determined by circulatory limitations rather than apparent failure in ATP-generating mechanisms (Mark et al., 2002, 2005). However, thermal range and upper limits of mitochondrial function fall well below values predicted based on temperate organisms, indicating that stenothermy and cold specialization detected in Antarctic fish are reflected at the cellular and molecular level (Somero et al., 1998). Therefore, examining thermal limits and
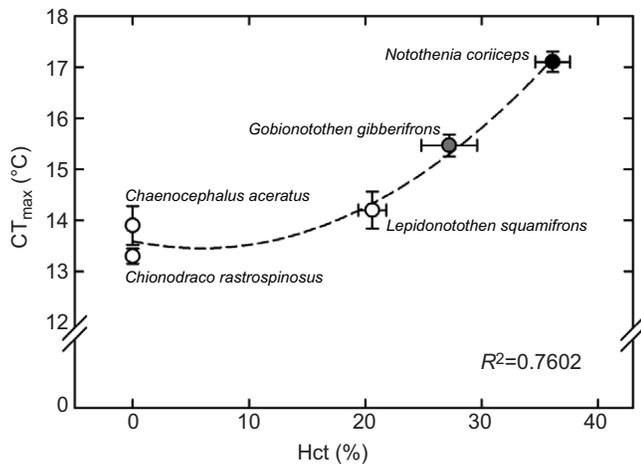
Fig. 4. Thermal tolerance correlates with oxygen-carrying capacity of the blood in Antarctic notothenioids. Temperature was elevated acutely from ambient ($0\pm0.5°C$) at a constant rate ($3.6°C\ h^{-1}$), and the critical thermal maximum (CT_{max}) was defined as the temperature at which animals lost their righting response. There is a strong curvilinear relationship between CT_{max} and hematocrit (Hct), an indicator of the oxygen-carrying capacity of the blood. The second-order polynomial ($y=0.0044x^2-0.0569x+13.581$) and R^2 were calculated from a plot of all individual data points. Values are presented as means $\pm$s.e.m.; $N=8$ for each species. Significant differences in CT_{max} between species are denoted by a contrast in symbol shade (from Beers and Sidell, 2011).

plasticity of bioenergetic processes provides important insights into cold adaptation and stenothermy of these fishes.

With long-term exposure to higher temperatures, several notothenioids demonstrate changes in overall cellular metabolic processes, indicating some level of capacity for warm acclimation. For instance, *T. bernacchii* and *P. borchgrevinki* have been shown to decrease their serum osmolality when acclimated to elevated temperatures for several weeks, suggesting that changes in osmoregulation reflect their ability to successfully acclimate to new temperatures via phenotypic plasticity (Lowe and Davison, 2005; Hudson et al., 2008). This example is interesting given that notothenioids maintain a serum osmolality nearly double that of temperate fishes and only upon warm acclimation do their serum levels resemble those of warmer water species. In other illustrations, warm-acclimated *T. bernacchii* displayed significant changes in CS and LDH activities in heart and skeletal muscle (Jayasundara et al., 2013), while *P. borchgrevinki* showed an increase in LDH and cytochrome *c* oxidase activity in skeletal muscle, possibly attributable to an increase in metabolic activity that reflects higher energetic costs associated with living at warmer temperatures (Windisch et al., 2011). A decrease in lipid metabolic capacity with warm acclimation was detected in *T. bernacchii* and *Pachycara brachycephalum* (Windisch et al., 2011), suggesting a potential shift towards carbohydrates as the primary source of metabolic fuel at higher temperatures. This shift is possibly to support carbohydrate-based anaerobic metabolism that may occur under increased hypoxemic conditions or, alternatively, it is possible that the reduction in ATP-generation potential, particularly in *T. bernacchii*, is a consequence of compromised functions at warmer temperatures. Overall, current research suggests some capacity for metabolic reorganization with warm acclimation in Antarctic fish, but further work is warranted to distinguish compensatory versus pathophysiological effects of higher temperatures.

Quantifying the cardiorespiratory response to long-term exposure to higher temperatures is another useful metric to determine acclimatory

capacity, given the profound effects of temperature on cardiac performance and oxygen delivery to tissues (Farrell, 2002; Mark et al., 2002; Lannig et al., 2004; Pörtner and Farrell, 2008). Previous studies have shown that *P. borchgrevinki* can, in fact, compensate for temperature effects on aerobic swimming performance, factorial cardiac scope, and overall cardiac contractile machinery following long-term exposure to 4°C (Seebacher et al., 2005; Franklin et al., 2007). This species also demonstrated a capacity to alter oxygen consumption and ventilation rates with warm acclimation, potentially optimizing performance at higher temperatures (Robinson and Davison, 2008). Conversely, similar attempts to characterize heart and ventilation rates in warm-acclimated *T. bernacchii* did not demonstrate a significant capacity for acclimation (Jayasundara et al., 2013). Interspecific differences in ecological habitat and locomotory demands of *T. bernacchii* and *P. borchgrevinki* might explain the differential acclimatory capacities detected in these two species. *Trematomus bernacchii* is a shallow-water benthic notothenioid that resides in the High-Antarctic Zone, whereas *P. borchgrevinki* is a cryopelagic circum-Antarctic species that potentially encounters a wider range of temperatures (Gon and Heemstra, 1990). Despite these differences observed in thermal limits and acclimatory capacity of notothenioids inhabiting different habitats and with distinctive locomotory demands, as a lineage, Antarctic fish remain highly sensitive to changes in temperature compared with their temperate counterparts. Indeed, the reduced capacity to induce a thermal acclimatory response is particularly evident when comparing cardiac responses to an acute temperature increase in *T. bernacchii* with those of a temperate eurythermal teleost, such as the goby *Gillichthys mirabilis* (Fig. 5) (Jayasundara and Somero, 2013; Jayasundara et al., 2013). The comparisons shown in Fig. 5 illustrate the wide range of thermal tolerance in cardiac function of the eurythermal goby species, and its capacity to shift the thermal responses of cardiac function through acclimation, relative to the stenothermal notothenioid. In contrast, there is little evidence for thermal compensation of cardiac activity, as indexed by heart rate (beats min^{-1}), in the cold-adapted stenotherm.

Concluding remarks
How notothenioids will fare in the rapidly changing Southern Ocean remains an open question. It is interesting to speculate what will become of the biochemical adaptations to low, near-freezing temperatures, such as the AFGPs, which may no longer be needed in a warmer ocean. Indeed, production and maintenance of these proteins are likely to be energetically costly and may be selected against over time (Cheng et al., 2003). Reduction and loss of AFGPs has already been documented in notothenioids that have migrated to warmer latitudes from endemic waters with seemingly no negative repercussions (Cheng and Detrich, 2007). However, compared with the rate of temperature change during past periods of genetic divergence, modern climatic warming is proceeding at an unprecedented rate. This rate of environmental change is going to be critically important in determining how species, and their associated traits, respond to future warming conditions. Equally intriguing to think about is how the loss of genetic information, such as that needed for temperature-induced expression of HSPs and for production of hemoproteins, will impact notothenioids in coming years. The loss of oxygen-binding proteins in icefishes may prove to be the most costly trait of all, as the high thermal sensitivity of this group may poise them to be one of the first impacted by rising temperatures (Beers and Sidell, 2011; O'Brien and Crockett, 2013). Thus, in the context of these 'losses' and 'gains' and their
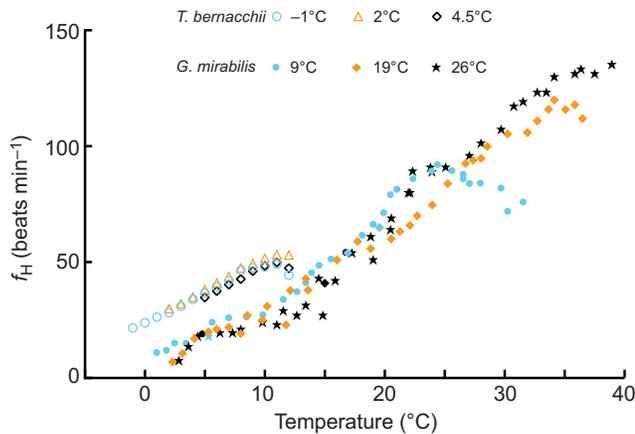
Fig. 5. Comparison of heart rates between a cold-adapted stenothermal Antarctic notothenioid and a temperate eurythermal goby. Heart rate (f_H) was measured during an acute increase in temperature for Antarctic notothenioid *Trematomus bernacchii* acclimated to −1, 2 and 4.5°C and for the eurythermal estuarine goby *Gillichthys mirabilis* acclimated to 9, 19 and 26°C. The data demonstrate a significant effect of acclimation on maximum heart rate in *G. mirabilis*, which was not detected in *T. bernacchii.* However, cold adaptation of cardiac function, i.e. increased heart rate at colder temperatures in Antarctic fish compared with temperate species, is clearly evident (from Jayasundara and Somero, 2013; Jayasundara et al., 2013).

implications in a changing world, we present several focal areas to consider for future studies.

Experimental designs and analyses

What should be readily apparent when examining the summary of upper thermal limits of notothenioids (Table 1) is that the experimental methods used vary broadly. In particular, there have been many different acclimation durations used to evaluate acclimatory capacity and, among CT_{max} experiments, the rate of temperature change differs widely between studies. There is an inherent difficulty when it comes to logistics of polar fieldwork and it is this factor that often dictates acclimation periods and precise details of CT_{max} heat ramps. Nonetheless, a concerted effort from polar researchers to standardize thermal tolerance studies would provide more meaningful insights into stenothermy of notothenioids. Comparison across Antarctic and sub-Antarctic species is another key consideration, as this approach is valuable for uncovering many evolutionary facets of notothenioid cold adaptation (Hofmann et al., 2005; Coppes Petricorena and Somero, 2007; Detrich et al., 2012; Bilyk and DeVries, 2011). This method may be particularly useful for elucidating cold-adaptive mechanisms within the Channichthyidae, as one species, *Champsocephalus esox*, is distributed almost entirely north of the APF (Egginton et al., 2002). Additionally, future studies will benefit by designs that take life stages into account because little is known about the influence of ontogeny on thermal tolerance of these animals.

Integration across subdisciplines

With the advent of high-throughput technologies, there has been an explosion of molecular-based studies revealing new insights into the genomic basis of notothenioid cold adaptation. It is well beyond the limits of our review to cover this emergent area of research; therefore, we provide a number of recent references for further reading (Papetti et al., 2012; Bilyk and Cheng, 2013; Coppe et al., 2013; Marino et al., 2013; Rizzello et al., 2013; Bilyk and Cheng, 2014). While these studies lend a great deal of insight into the

molecular underpinnings of notothenioid physiology, it is important to incorporate these findings with functional studies including post-translational modification analyses to obtain a comprehensive understanding of biochemical traits in these fish. It is also essential to evaluate processes underlying rates and patterns of adaptive responses, e.g. genome modification and epigenetic regulation (Varriale and Bernardi, 2006; Near et al., 2012), that are crucial in determining the capacity of notothenioids to respond to future environmental challenges.

Multiple stressors

Temperature does not act in isolation; thus, it is necessary to consider the effects of all potentially stressful abiotic factors when evaluating susceptibility to climate change. This is particularly important for the Southern Ocean and its endemic fauna because, in addition to the expected continual increase in temperature, changes in other oceanic parameters, including pH, dissolved oxygen content and salinity, are predicted (Fabry et al., 2008). Assessing impacts of multiple stressors in practice is quite challenging. Studies in this area are in the infancy stages, but already have yielded interesting results that include synergistic effects from dual stressors (temperature and pH) that alter metabolic rates and acclimation capacity (Strobel et al., 2012; Enzor et al., 2013; Enzor and Place, 2014). What appears evident even early on is that multiple stressor outcomes may be complex and species specific.

After more than a half-century of research focused on the physiological and biochemical adaptations of notothenioids, there are many questions that remain to be answered. Forthcoming investigations will continue to uncover the unique characteristics of this remarkable group of animals and their response to changes in environmental conditions. It will be fascinating to see what emerges from this area of study in coming years.

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