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SYMPOSIUM

Understanding the Metabolic Capacity of Antarctic Fishes to Acclimate to Future Ocean Conditions

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Synopsis Antarctic fishes have evolved under stable, extreme cold temperatures for millions of years. Adapted to thrive in the cold environment, their specialized phenotypes will likely render them particularly susceptible to future ocean warming and acidification as a result of climate change. Moving from a period of stability to one of environmental change, species persistence will depend on maintaining energetic equilibrium, or sustaining the increased energy demand without compromising important biological functions such as growth and reproduction. Metabolic capacity to acclimate, marked by a return to metabolic equilibrium through physiological compensation of routine metabolic rate (RMR), will likely determine which species will be better poised to cope with shifts in environmental conditions. Focusing on the suborder Notothenioidei, a dominant group of Antarctic fishes, and in particular four well-studied species, *Trematomus bernacchii*, *Pagothenia borchgrevinki*, *Notothenia rossii*, and *N. coriiceps*, we discuss metabolic acclimation potential to warming and CO_2-acidification using an integrative and comparative framework. There are species-specific differences in the physiological compensation of RMR during warming and the duration of acclimation time required to achieve compensation; for some species, RMR fully recovered within 3.5 weeks of exposure, such as *P. borchgrevinki*, while for other species, such as *N. coriiceps*, RMR remained significantly elevated past 9 weeks of exposure. In all instances, added exposure to increased PCO_2, further compromised the ability of species to return RMR to pre-exposure levels. The period of metabolic imbalance, marked by elevated RMR, was underlined by energetic disturbance and elevated energetic costs, which shifted energy away from fitness-related functions, such as growth. In *T. bernacchii* and *N. coriiceps*, long duration of elevated RMR impacted condition factor and/or growth rate. Low growth rate can affect development and ultimately the timing of reproduction, severely compromising the species' survival potential and the biodiversity of the notothenioid lineage. Therefore, the ability to achieve full compensation of RMR, and in a short-time frame, in order to avoid long term consequences of metabolic imbalance, will likely be an important determinant in a species' capacity to persist in a changing environment. Much work is still required to develop our understanding of the bioenergetics of Antarctic fishes in the face of environmental change, and a targeted approach of nesting a mechanistic focus in an ecological and comparative framework will better aid our predictions on the effect of global climate change on species persistence in the polar regions.

Importance of incorporating realistic environmental variability in estimates of vulnerability to ocean change

Animals live in dynamic, multi-variate environments where many environmental factors are changing simultaneously or in series. Anthropogenic impacts to natural populations are also arising due to concurrent changes in environmental conditions, such as ocean warming, reductions in dissolved oxygen and carbon dioxide (CO_2) acidification of marine environments (Hofmann and Todgham 2010). While reviews published in the ecological literature (e.g., Crain et al. 2008; Darling and Côté 2008) demonstrate that most stressors interact in unpredictable,

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non-linear ways (i.e., synergistic or antagonistic interactions that are not additive), our mechanistic understanding of how stressors interact at the molecular, biochemical, and physiological level is underdeveloped (Todgham and Stillman 2013; Gunderson et al. 2016). To increase our accuracy for predicting the effects of global change and its many physical drivers of change on biodiversity, we need a better understanding of the cellular modes of action of different co-occurring stressors and how interactions of these stressors at the cellular level affect organismal performance in the face of changing environmental conditions.

Evidence from temperate and tropical ecosystems indicates that temperature-linked shifts in organismal distribution have already occurred in response to a changing climate, resulting in the poleward migration of species to escape the warming of their native habitats (Walther et al. 2002; Root et al. 2003). Although rhetorical, this then leads to the question of "Where can polar species go if there is nowhere colder to migrate?" Polar organisms, living in the coldest marine ecosystems, are vulnerable to climate change specifically because of their adaptation to historically stable thermal regimes, the rapid speed at which polar marine environments are currently changing (i.e., three to four times the rate of warming/decade compared with global averages), and their slow population growth (Meredith and King 2005; Smetacek and Nicol 2005; Barnes et al. 2009, Bromwich et al. 2013; Convey and Peck 2019). Compared with fishes in other ecosystems, the range of temperatures over which Antarctic ectotherms can maintain physiological function is one of the narrowest, and adult fishes are thought to only be able to acclimatize over the long term to conditions 4.5°C warmer than their current mean maximum temperature (Somero and Devries 1967; Podrabsky and Somero 2006; Richard et al. 2012). Cold polar waters are also able to absorb more CO_2 than warmer waters (due to increased solubility of gases as temperatures decrease), making the Southern Ocean a prominent CO_2 sink and a potential "bellwether" of the effects of CO_2-acidification to marine life (McNeil and Matear 2008; Fabry et al. 2008; McNeil et al. 2010). As high latitude Antarctic species are limited in their ability to relocate to favorable conditions, and many Antarctic species have long generation times (years to decades), the primary strategies for coping with rapid environmental change are linked to understanding whether Antarctic species possess the physiological poise, or acclimatization potential, to compensate for the changing ocean conditions predicted over the next century.

Central to an organism's capacity to respond to stressors is the capacity to mobilize the energy needed to recruit cellular stress response mechanisms, while balancing energetic demands to other critical biological processes, like growth and reproduction, and restoring homeostasis (Pörtner 2010; Sokolova 2013). Previous studies on the effects of ocean warming and CO_2-acidification on Antarctic fishes (Strobel et al. 2012, 2013a; Flynn et al. 2015; Davis et al. 2018) have provided numerous lines of evidence that while Antarctic fishes have the capacity to acclimate to elevated PCO_2 and elevated temperature as single stressors, capacity to acclimate is either delayed or attenuated when warming is coupled with elevated PCO_2. This is particularly true for early life stages (Flynn et al. 2015; Davis et al. 2018). Therefore, one of the generalized mechanisms animals can use to cope with chronic environmental change (i.e., acclimation/acclimatization) is compromised in Antarctic fishes under ecologically-relevant multiple stressor scenarios and may represent a weak link in an Antarctic fish's resilience to ocean change. The goal of this review is to examine our mechanistic understanding of metabolic plasticity in Antarctic fishes, with a focus on the capacity of these fishes to respond to environmental change through metabolic reorganization at molecular, biochemical, and physiological levels. There has been significant attention addressing the acclimation potential to warming in Antarctic fishes (e.g., Somero and Devries 1967; Peck 2002; Franklin et al. 2007; Bilyk and DeVries 2011; Robinson et al. 2011; Egginton and Campbell 2016), serving as an excellent foundation for guiding this review. The hypothesis is that metabolic capacity (i.e., the ability to match energy supply with energy demand) will be a limiting factor in the capacity of Antarctic fishes to acclimate to the simultaneous change in multiple environmental variables associated with global change in the Southern Ocean. If species are unable to undergo metabolic reorganization to reestablish homeostasis following exposure to multiple stressors, the energy demand required to cope with shifts in environmental conditions may outpace energy supply. As the energy budget becomes limited, basal maintenance will likely be prioritized over other functions, such as reproduction, development, growth, and activity, leading to a decline in physiological performance and likely organismal fitness (Sokolova 2013). Interacting stressors of elevated temperature and elevated PCO_2 will cause substantial energetic perturbations in the endemic fishes and species persistence will be determined by the acclimation potential to increase tolerance and the ability to restore metabolic balance

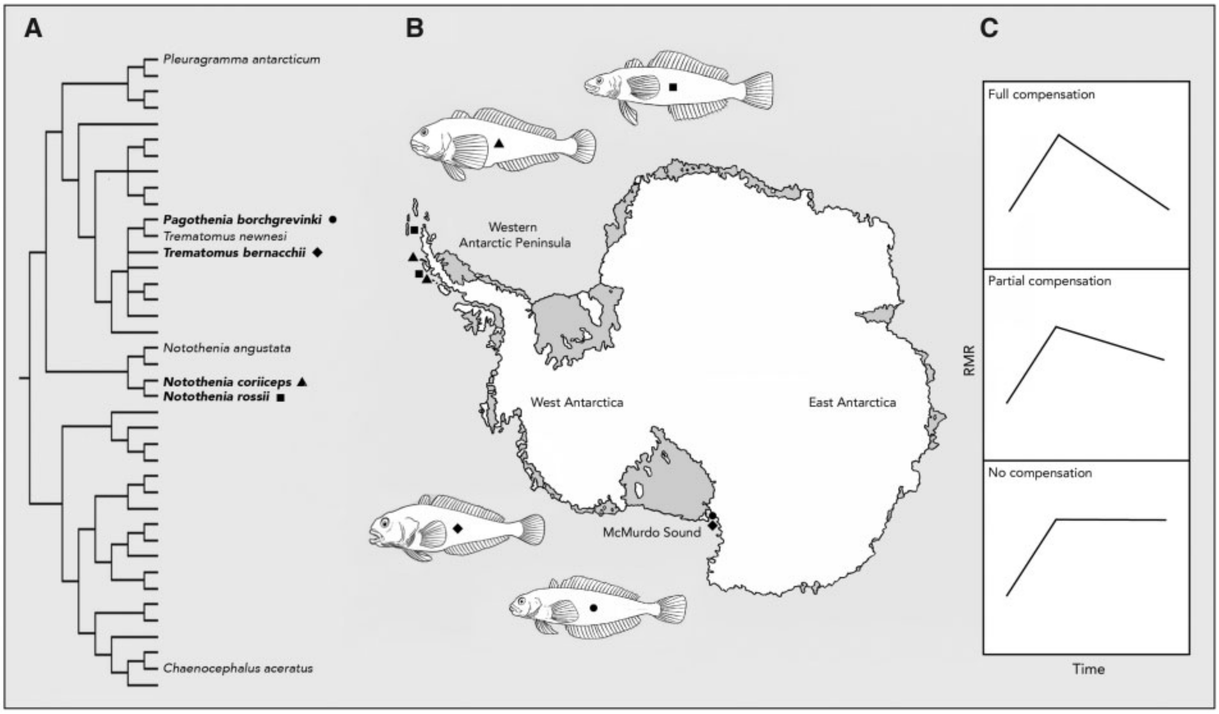

Fig. 1 Phylogenetic relationships of notothenioid fishes (**A**), geographic sampling of *P. borchgrevinki*, *T. bernacchii*, *N. rossii*, and *N. coriiceps* (**B**) and theoretical compensation graphs in response to environmental stressors (**C**). Phylogenetic tree of notothenioid species has been modified from (Near and Cheng 2008). While the geographic distribution of *P. borchgrevinki*, *T. bernachii*, *N. rossii*, and *N. coriiceps* are circum-Antarctic (Gon and Heemstra 1990), the species have been exclusively sampled either from the Western Antarctica Peninsula region or from McMurdo Sound for the research presented in this review. Theoretical compensation graphs of RMR in response to a change in environmental conditions, based on Peck (2002).

through physiological compensation of routine metabolic rate (RMR) (Sokolova et al. 2012; Sokolova 2013; Sandblom et al. 2016).

The Antarctic fish fauna is dominated by the perciform suborder Notothenioidei (Eastman 2000). Notothenioids represent 55% of the fish species of the Southern Ocean and in many coastal shelf areas represent over 90% of the fish biomass. To formulate predictions on persistence of Antarctic species in face of future environmental change, it is imperative to examine the acclimatory capacity in a number of species, particularly those that belong to the suborder Notothenioidei. In this review, we have focused on four notothenioid species (*Trematomus bernacchii*, *Pagothenia borchgrevinki*, *Notothenia rossii*, and *N. coriiceps*) with the greatest comprehensive foundational literature on integrative responses to environmental change over both acute and chronic time scales. These notothenioid fishes are abundant and widely distributed in the Antarctic (Gon and Heemstra 1990; Eastman 1993). While these four species most likely have a circum-Antarctic distribution, sampling of these species for studies have been location-specific (Fig. 1B). Individuals of *N. rossii* and *N. coriiceps* have been exclusively caught in the Seasonal Pack Ice Zone (SPZ) of the Western Antarctic Peninsula (e.g., Strobel et al. 2012, 2013b; Joyce et al. 2018), where seasonal temperatures can increase to 2°C, while individuals of *T. bernacchii* and *P. borchgrevinki* have been caught in McMurdo Sound (e.g., Robinson and Davison 2008; Enzor et al. 2013; Sandersfeld et al. 2015; Davis et al. 2018), located at higher latitudes, termed the High Antarctic Zone (HAZ), where seasonal temperature can increase to −0.5°C. Thermal regime of the habitat is one important aspect to consider when examining multiple species and determining possible causes of species-specific differences in capacity for acclimation. Other important considerations include species relatedness or phylogenetic history, activity and vertical habitat distribution in the water column of each species (e.g., ecotype) (Fig. 1A). For example, a study taking into account phylogeny and ecotype, determined that species pattern of cardio-respiratory control in eight Antarctic species was primarily driven by ecotype with minor effect of phylogeny (Campbell et al. 2009). Predictions on acclimation capacity of Antarctic fishes will be better aided with a greater understanding of ecological, evolutionary, and physiological constraints.

Vulnerability of Antarctic marine ecosystems

High-latitude, polar oceans are predicted to undergo some of the greatest changes in pH and temperature over the next century (McNeil and Matear 2008; Ciais et al. 2013). Seawater pH may decrease as much as 0.3–0.5 units, while temperature is projected to increased 2–4°C (McNeil et al. 2010; Ciais et al. 2013) due to the continued increase of CO_2 emissions into the atmosphere ultimately driving ocean warming and CO_2-acidification of seawater (i.e., ocean acidification) (Caldeira and Wickett 2005; Feely et al. 2009; Gattuso et al. 2014). Within the Southern Ocean specifically, some of the fastest rates of change have been recorded (Vaughan et al. 2003; Turner et al. 2013). The Antarctic Peninsula has encountered increases in land and sea temperature at a rate of ca. 3.4–5.7°C per century, while McMurdo Sound in the Ross Sea has shown trends of 2.5–3.3°C per century (Vaughan et al. 2003). McMurdo Sound has also been shown to have near-shore coastal sites that experience seasonal variability in pH (Kapsenberg et al. 2015). Simulated models of continued ocean changes in McMurdo Sound suggest that the duration of low pH winter seasons may increase thus exposing marine organisms to deleterious levels of pH for longer periods into spring (Kapsenberg et al. 2015), when many early life history stages of fishes are in the water column.

Antarctic fishes provide some of the best examples of organisms that have fine-tuned their physiology to cope with living at extreme temperatures, evolving a series of physiological and biochemical adaptations to counteract the temperature constraints of inhabiting a sub-zero environment. Some of these adaptations include antifreeze protein production, elevated blood osmotic concentrations, decreases in hematocrit, mitochondrial proliferation, loss of the heat shock response, loss of hemoglobin and myoglobin expression, enzymes with properties that allow them to maintain function at sub-zero temperatures, large diameter muscle fibers, large hearts, and more extensive vasculature and thermal compensation of metabolic activity (for review see Eastman 1993; Cheng and Detrich 2007; Coppes Petricorena and Somero 2007; O'Brien 2016). While these adaptations provide Antarctic fish with an impressive resistance to cold, their cold-adapted physiology makes them susceptible to relatively small increases in environmental temperature (Somero and Devries 1967; Crockett and Sidell 1990; Peck et al. 2004; Podrabsky and Somero 2006; Peck et al. 2009), although there is some controversy over the degree of stenothermy in these fishes (Seebacher et al. 2005; Franklin et al. 2007; Robinson et al. 2011). For example, one of the most common strategies to maintain aerobic capacity, particularly in cold-adapted fishes, is to increase the volume of the cell taken up by mitochondria, to increase the concentration of aerobic enzymes and therefore ATP production and enhance oxygen diffusion (Egginton and Sidell 1989; Johnston et al. 1998; O'Brien 2011). There is a well-established link between aerobic metabolism and the creation of reactive oxygen species (ROS) (Abele et al. 2002). Oxidative stress occurs when antioxidant mechanisms are unable to defend against the damage from ROS. With an increase in ocean temperature, tissues with high mitochondrial density risk increased oxidative stress if antioxidant defenses are insufficient (Crockett 2011). Furthermore, Antarctic fishes have mitochondria and tissues that are high in polyunsaturated phospholipids to counteract the effects of subzero temperatures on membrane fluidity. Homeoviscous adaptation to cold makes Antarctic fishes particularly vulnerable to warming as this membrane composition promotes formation of ROS (Heise et al. 2003). The loss of the highly conserved heat shock response (Hofmann et al. 2000), a cellular defense mechanism to maintain protein homeostasis, further exacerbates the susceptibility of Antarctic fishes to environmental stressors that induce protein denaturation. Taken together, Antarctic fishes are predicted to be particularly sensitive to ocean change given their specialized phenotypes that have evolved over millions of years to allow these fishes to thrive under stable, cold ocean conditions.

Despite the different physiological strategies that have evolved over millions of years to survive subzero conditions, we hypothesize that the capacity to cope with rapidly changing environmental conditions associated with global climate change will rest in a species' capacity to balance energy supply and demand in the face of multiple stressors. In this review, we will discuss acclimatory capacity of Antarctic fishes to single and multiple stressors, from whole organism to sub-cellular levels, focused on the integrative capacity to restore RMR following chronic acclimation to environmental change and return to a state of energy balance (Fig. 1C).

Warm temperature acclimation increases thermal tolerance

Antarctic notothenioids are known for extreme stenothermy or narrow thermal limits, with the lowest recorded upper incipient lethal temperatures of around 5–7°C for a fish (Somero and Devries 1967). Despite the narrow thermal range, there is intraspecific variation in thermal tolerance in Antarctic notothenioids, as assessed by critical thermal maximum (CTMax), broadly correlating with their geographic distribution (Bilyk and DeVries 2011). Species in the HAZ, where summer water temperatures peak at −0.5°C showed a lower CTMax than the species inhabiting the SPZ, where summer water temperatures increase up to 2°C (Bilyk and DeVries 2011). Notothenioids have been shown to significantly increase their upper thermal tolerance following a period of warm temperature acclimation (Podrabsky and Somero 2006; Bilyk and DeVries 2011); however, it is not yet clear if regional differences between HAZ and SPZ contribute to species-specific capacities to alter heat tolerance (Bilyk and DeVries 2011). Antarctic notothenioids, despite having evolved in a stable environment, appear to have the capacity to acclimate by adjusting upper thermal tolerance following a period of warm temperature acclimation.

In the eurythermal European perch (*Perca fluviatilis*) living in waters warmed by a nuclear power plant with thermal fluctuations, there was little to no thermal compensation in heat tolerance limits, but basal energy requirements were found to be thermally plastic (Sandblom et al. 2016). The authors termed this "plastic floors with concrete ceilings" and argued for the need of understanding both upper critical heat limits and basal metabolic functions in order to capture the species' sensitivity to global warming. In the case of the European perch, the limited capacity for upper thermal adjustment may be a limiting factor for these species, as tolerance may not be sufficient to withstand transient heat waves predicted to increase with global warming (Sandblom et al. 2016). In the case of Antarctic species, warm acclimation increased CTMax significantly to an average of 15°C across different species (Bilyk and DeVries 2011), which is well above the predicted increase in temperature in the next 80 years (Gattuso et al. 2014). Unless Antarctic waters become susceptible to excessive heat waves, Antarctic species, unlike the European perch, may not be limited by the "ceiling" or their upper thermal tolerance but may instead be constrained by the plasticity of the "floor" or maintaining energetic needs of basal functions. The persistence of Antarctic species in warming and acidifying oceans may depend on a return to energetic equilibrium in order to balance sufficient energy for basal functions as well as other biological processes contributing to fitness.

Degree of compensation of RMR as indicator of acclimation potential

Routine metabolic rate is a measure of O_2 consumed to fuel basic functions, such as ion and acid–base regulation, ventilation, and excretion. In response to an increase in temperature, routine metabolic rate (RMR) increases (e.g., Robinson and Davison 2008; Joyce et al. 2018), reflecting a greater energetic cost of functions associated with maintenance (for review see Sokolova et al. 2012). Over time, the severity of stress and the capacity of an individual to return to a metabolic equilibrium will determine the extent of the effect an environmental stressor has on fitness. In the case when the environmental stressor is extreme, achieving favorable energetic balance is unlikely and the probability of survival is low; however, when environmental stressor is low or moderate, survivability will be dependent on the degree of deviation from the optimum and an individual's ability to reduce the negative impact of stress (Sokolova et al. 2012; Sokolova 2013). As an indicator of energetic imbalance, RMR can be tracked over time to determine the physiological capacity for compensation to changes in temperature and/or PCO_2 (Enzor et al. 2017). In response to an environmental stressor, after the initial rise, RMR may remain at the new rate, termed no compensation, or may partially or fully return to pre-stress levels, termed partial or full compensation, respectively (Fig. 1C; Peck 2002). Survival in the warming and acidifying Southern Ocean will likely dependent on the species' ability to achieve full physiological compensation in order to return to a state of energetic balance. It is also possible that a new physiological set-point may be achieved whereby RMR remains high yet energetic balance exists and sufficient energy is supplied to all required biological functions. Therefore, our ability to predict survival and persistence of Antarctic species will be dependent on understanding if the magnitude of increase in temperature and PCO_2 is in the low or moderate range for different Antarctic species allowing for full compensation and whether such full compensation is actually necessary for survival.

To understand the acclimation potential of Antarctic species, we look to RMR data collected across species in different studies. However, lack of methodological consistency often creates difficulties in cross-study comparisons, and the experimental protocols for determination of metabolic rate varied significantly among the studies included in this review. One potentially problematic inconsistency is that although all refer to the O_2 consumption

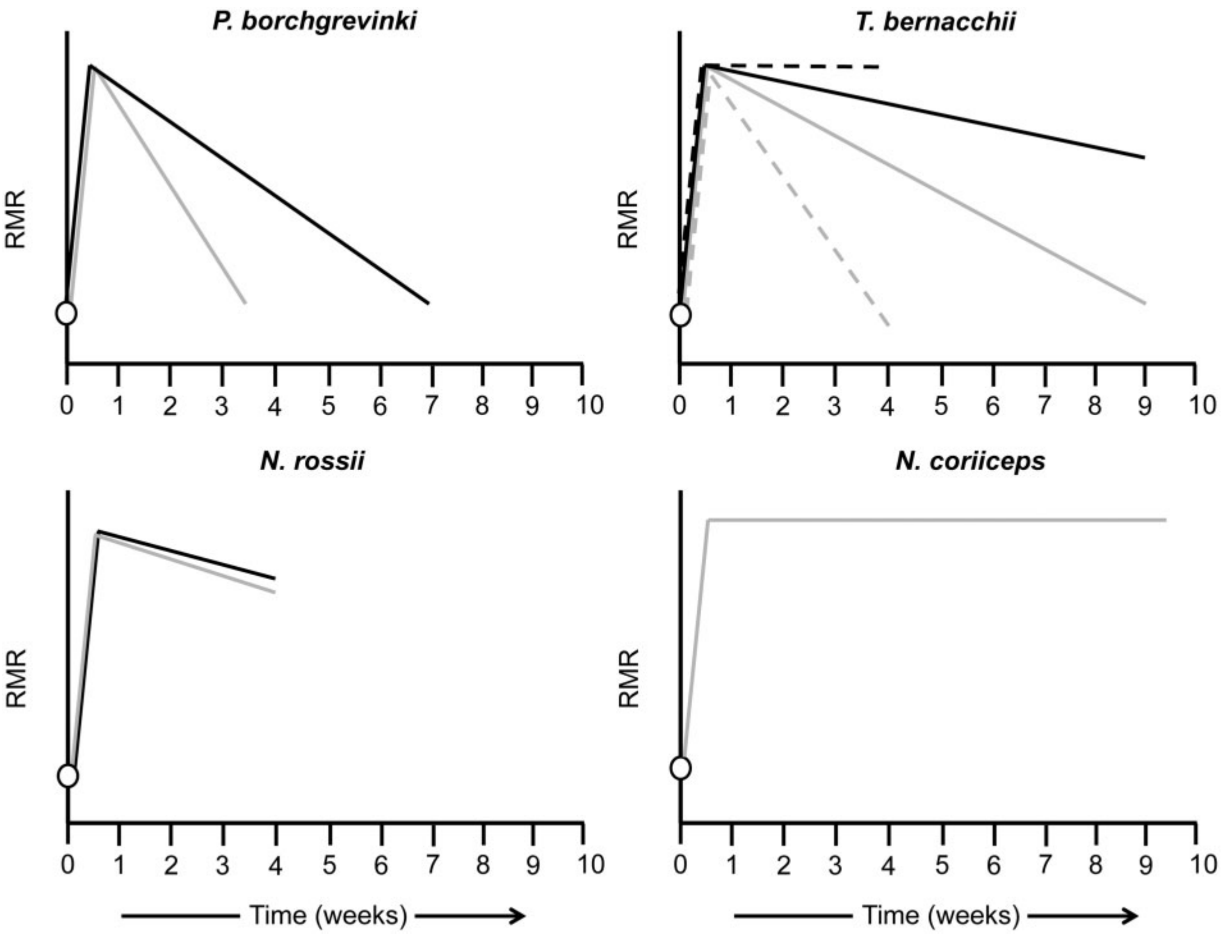

Fig. 2 Representative schematics of RMR compensation in response to warming (gray lines) and warming in combination with high PCO_2 (black lines) in *P. borchgrevinki*, *T. bernacchii*, *N. rossii*, and *N. coriiceps*. The white circle represents RMR prior to exposure to environmental stressor(s), solid lines represent adult fish data, while dotted lines represent juvenile fish data. In *P. borchgrevinki*, the warming compensation curve is based on data from Robinson and Davison (2008) (Temp = 4°C), warming and high PCO_2 from Enzor et al. (2017) (Temp = 4°C; $PCO_2 = 1000\ \mu$atm). In *T. bernacchii* compensation curves for adult fish data are from Enzor et al. (2017) (Temp = 4°C; $PCO_2 = 1000\ \mu$atm), while juvenile fish data are from Davis et al. (2018) (Temp = 4°C; $PCO_2 = 1200\ \mu$atm). In *N. rossii* compensation curves are based on data from Strobel et al. (2012) (Temp = 7°C; $PCO_2 = 1970\ \mu$atm). In the *N. coriiceps* warming compensation curve is based on data from Joyce et al. (2018) (Temp = 5°C).

measurements as routine metabolic rate, due to differences in fasting periods (no fasting, Joyce et al. 2018; 10 day fasting period, Strobel et al. 2012), there is a possibility that the species under comparison were not in a similar metabolic state. The species with longer fasting periods may have been closer to standard metabolic rate rather than RMR. Limited information is available on the metabolic response to feeding, or specific dynamic action, in Antarctic fishes, but data from a few species indicate that it can be long lasting when compared with temperate species (Sandblom et al. 2012), posing substantial difficulty in determining the appropriate fasting period for respirometry experiments. While ensuring consistency among studies is not trivial, there is a real need to standardize protocols in order to achieve greater potential for assessing species patterns across studies. Despite the possible shortcomings of a species comparison of this sort, examining metabolic rate change over time in response to environmental perturbation provides valuable information about species acclimation potential.

The degree of compensation of RMR following exposure to environmental stress in Antarctic notothenioids is dependent upon the type of environmental stressor, species, and acclimation time. A rise in temperature caused a significant increase in RMR in four notothenioid species, *T. bernacchii*, *P. borchgrevinki*, *N. rossii*, and *N. coriiceps*, yet thermal compensation of RMR differed markedly among the species (Fig. 2). A complete recovery of RMR with the shortest period of acclimation occurred in

P. borchgrevinki after 25 days of exposure (4°C, Robinson and Davison 2008), followed by *T. bernacchii* at 8–9 weeks of acclimation (4°C, Enzor et al. 2017). There was only a partial compensation of RMR in *N. rossii* after 4 weeks of acclimation time (7°C, Strobel et al. 2012), while no compensation was shown by *N. coriiceps* following 6 weeks (5°C, Egginton and Campbell 2016) or 9.5 weeks of acclimation to warm temperature (5°C, Joyce et al. 2018). Most of the Antarctic notothenioids appear to at least partially restore RMR to the original set point in a warming environment, given sufficient time. It is possible that if the time-scale of experiments were lengthened it would capture the eventual return of RMR to baseline in *N. rossii* and *N. coriiceps*. In contrast, when a rise in temperature was followed by a concomitant rise in PCO_2, RMR remained elevated for a significantly longer period of time when compared with treatments with elevated temperature alone, in species for which information is available (Fig. 2; Strobel et al. 2012; Enzor et al. 2013, 2017; Davis et al. 2018). Experimental exposure to multiple stressors is an important consideration given that both temperature and PCO_2 are expected to increase in the polar waters of Antarctica. Simultaneous exposure to both stressors decreased the degree of compensation of RMR, potentially compromising the acclimatory capacity of Antarctic fishes. If an elevated RMR represents an energetic imbalance, the combination of the capacity to achieve full compensation with the duration of time it takes to do so will likely have significant impact on persistence of the different Antarctic notothenioids in a changing environment.

Early life stages may be the most vulnerable to ocean warming and acidification (Pankhurst and Munday 2011) and a few studies have examined the effect of elevated temperature and PCO_2 on embryonic and juvenile stages of Antarctic fishes to determine the acclimation potential of early life stages and if they differ from that of the adults. In subadult *T. bernacchii*, RMR rose significantly with elevated temperature and similar to the adults, RMR returned to control levels after 9 weeks of acclimation (Sandersfeld et al. 2015). Interestingly, a different study on younger juvenile *T. bernacchii* (~18 months old) found that RMR returned to resting levels within 4 weeks of acclimation time exposed to the same increase in temperature, demonstrating that juvenile fish can more rapidly compensate for thermal stress than adults (Davis et al. 2018). However, in combination with high PCO_2, there was no compensation and RMR remained significantly elevated for 4 weeks of experimental time, similar to the adults (Fig. 2; Davis et al. 2018). While it appears that the acclimation potential in juvenile *T. bernacchii* may be equal or better than that of the adults, there was a significant reduction in basal swimming activity (Davis et al. 2018) and growth declined by up to 84% with exposure to elevated temperature (Sandersfeld et al. 2015). The significant decrease in growth performance could impact timing to sexual maturity and spawning, which only occurs once an individual has reached 65% of its maximum length (Kock and Kellermann 1991). Little is known about the impact of elevated temperature and PCO_2 on Antarctic fish embryos; however, a decrease in survival and development was found early in embryogenesis in the naked dragonfish, *Gymnadraco acuticeps*, when exposed to a combination of the two stressors (Flynn et al. 2015). While it appears from a few studies that early life stages possess an acclimation potential similar to the adult fish, there is energetic trade-off that, if persistent, will affect the population and species dynamics in the warming and acidifying oceans.

It is important to consider the possibility that an elevated RMR, which does not return to baseline over an extended period of time, such as in *N. coriiceps*, indicates a new stable energetic state rather than one of energetic imbalance. The cost of basal functions may be increased as a consequence of living at a higher temperature; however, if sufficient energy is available it is possible that the species may be able to attain a new sustainable homeostatic equilibrium. In this case, elevated RMR does not provide significant indication of whether there is an energy disturbance that may be limiting animal performance. Evidence suggests, however, that it is unlikely that a persistent elevated RMR is an indication of a new sustainable equilibrium. In *N. coriiceps*, in conjunction with a lack of compensation in RMR in individuals exposed to elevated temperature and PCO_2, there was a significant decrease in fish condition factor (Strobel et al. 2012). Similarly, in *T. bernacchii*, over the period when RMR was elevated due to exposure to warm temperature, there was a significant decrease in condition factor and growth rate (Enzor et al. 2017). Low condition factor and growth rate may be a result of reduced food assimilation rates, potentially limiting energy supply (Sandersfeld et al. 2015). If energy supply is limited and basal maintenance, which is incurring a greater energetic cost during times of stress, is prioritized over functions such as growth and development, this will have important implications for the fitness of the species. Additionally, if RMR remains chronically high, to cover the increased cost of basal functions, increased food intake would be required and consequently there would be an increase in costs associated with foraging and hunting (Joyce et al. 2018), further off-setting the energetic balance. Therefore, it is likely that a full compensation of RMR is necessary for the persistence of Antarctic species. For the species not capable of restoring RMR, indicating energetic imbalance, mechanistically the limitation may be found at the level of the mitochondria.

Biochemical constraints underlying the ability to compensate RMR in face of environmental stress

Acclimation time and degree of compensation in RMR suggest differences in acclimation potential among the Antarctic species. This is dictated by a return to metabolic equilibrium, or matching of energy demand with energy supply, which can be achieved via cellular, biochemical, and physiological changes. Limitations at any of these biological levels can impact the ability of an animal to respond to environmental stressors and limit acclimation potential. *Pagothenia borchgrevinki* shows very few metabolic changes, as assessed by enzyme activities, to elevated temperature and $P\text{CO}_2$ (Enzor et al. 2017) and a muted coordinated cellular stress response that peaks early in the acclimation period (between 7 and 14 days) and is followed by a quick decline (Huth and Place 2016a). This could potentially indicate a reduced capacity to respond to environmental stressors, or it could indicate a rapid entry into a stable metabolic state (Huth and Place 2016a). The latter is a perhaps a more likely scenario and it is possible that changes at the physiological level, such as thermal compensation of cardiac function (Seebacher et al. 2005; Franklin et al. 2007), are sufficient to allow for compensation in RMR and a return to energetic equilibrium. More than other notothenioid species, *P. borchgrevinki* may be more physiologically robust and less sensitive to environmental variation (Seebacher et al. 2005; Huth and Place 2016a). Indeed, in other notothenioid species, reliance on anaerobic metabolism, significant depletion of energy stores and reduced mitochondrial capacities likely underlie the limited potential for compensation and acclimation to environmental stressors.

In contrast to *P. borchgrevinki*, there is metabolic reorganization in *T. bernacchii* in response to a change in environmental conditions. Under ocean warming, in *T. bernacchii* there was a shift from lipid to carbohydrate metabolism in skeletal muscle with a significant rise in anaerobic potential and a decrease in ATP-generating capacity in the heart tissue, coinciding with a lack of thermal compensation in cardiac function (Jayasundara et al. 2013). A similar increased reliance on carbohydrate metabolism was seen in the liver and gill of *T. bernacchii* when exposed to combined effects of high temperature and $P\text{CO}_2$. Following the initial rise in activity and gene expression of carbohydrate enzymes, there was an increase in anaerobic dependence as time of exposure to the multiple stressors progressed (Huth and Place 2016b; Enzor et al. 2017). Lack of complete compensation of RMR and increased reliance on anaerobic metabolism suggest an energy-limited tolerance (Sokolova 2013), indicating a limitation to the capacity to acclimate of *T. bernacchii* to multiple stressors (Huth and Place 2016b; Enzor et al. 2017). Although not directly tested in this species, the energy limitation may be at the level of the mitochondria as an early study in *T. bernacchii* found no evidence of acclimation capacity of mitochondrial function in response to elevated temperature (Weinstein and Somero 1998).

In *N. rossii*, partial RMR compensation in response to elevated temperature and the combination of elevated temperature and $P\text{CO}_2$ may be attributed to the capacity limits of mitochondrial function (Strobel et al. 2012). In isolated liver mitochondria, there was no compensation of mitochondrial respiration as indicated by a lack of difference in maximal mitochondrial respiration between the warm-acclimated and control group, while a decrease in maximal mitochondrial respiration and activity of cytochrome c oxidase (COX) below that of the control group were seen in individuals exposed to both high temperature and high $P\text{CO}_2$. The limits in liver mitochondrial function while exposed to environmental variation may indicate decreased tissue aerobic capacity, which can have at least partial effects on whole animal performance (Strobel et al. 2012, 2013a). In the heart, combination of elevated temperature and $P\text{CO}_2$ resulted in a decrease in enzyme activities of citrate synthase (CS) and COX, suggesting an inability for further increase in aerobic capacity, an important limitation given the high energy demand of this aerobic tissue (Strobel et al. 2013b). On the other hand, red muscle was found to show large increases in aerobic capacities in response to warming and elevated $P\text{CO}_2$, indicating that *N. rossii*'s capacity for metabolic reorganization is tissue-specific (Strobel et al. 2013b). Despite at least partial metabolic compensation of some tissues to changes in temperature and $P\text{CO}_2$, there was a significant depletion of energy stores. Reduced energy stores will have long-term energetic impacts and likely cause a reduction of available energy for fitness-related functions such as growth and reproduction (Strobel et al. 2012, 2013b). In response to elevated temperature, mitochondrial respiration and enzymatic function capacities of *N. coriiceps* increased until close to 9°C, after which there were decreased membrane potentials and a lack of increase in respiration rate, indicating a thermal limitation (Mark et al. 2012). Similarly, mitochondrial state III respiration rate was not impaired at temperatures up to 10°C in cardiac tissue of *N. coriiceps* (O'Brien et al. 2018). No study to date has examined the simultaneous effect of temperature and PCO_2 on mitochondrial function of *N. coriiceps*, and a combination of the two stressors may further limit the cellular metabolic capacity of this species.

Distinct species-specific responses to increases in temperature and PCO_2 exist among the notothenioid fishes, contributing to differences in their acclimation potential to changing environmental conditions. Reduced acclimation potential is likely a result of a limitation of the metabolic capacity, and the failure to return to energetic equilibrium by matching energy supply with energy demand. Pinpointing the source of the metabolic limitation is difficult and likely differs among the species, although in *N. rossii* and *N. coriiceps* that have low degree of compensation of RMR to environmental variation, it appears to be at the level of the mitochondria (Strobel et al. 2012, 2013a). If mitochondrial plasticity is limited in response to warming or CO_2-acidification, this would reduce capacity of ATP generation via oxidative phosphorylation. As a consequence, over time, as noted in *T. bernacchii*, energy production would shift to an increased reliance on anaerobic metabolism. Relying on anaerobic metabolism is not sustainable as a long-term strategy due to finite substrate source of anaerobic metabolism, leading to rapid decrease in energy reserves, greater requirement for food assimilation, increasing energy expenditure, and ultimately leading to a decrease in growth rate.

Where do we go from here?

Environmental stress can tip the homeostatic balance of an animal causing a disruption in energy supply, constraining the capacity of an animal to perform energy-demanding functions. The extent of homeostatic displacement and the ability for eventual reestablishment of an energetic equilibrium can determine the impact an environmental stress has on an individual and ultimately the population and the species. In Antarctic notothenioids there are species-specific differences in the extent of recovery kinetics of RMR following exposure to a single stressor, ranging from full homeostatic compensation to a lack of one. Simultaneous exposure to multiple stressors further compromised the ability of the three species tested to date to return to energetic equilibrium (e.g., Strobel et al. 2012; Enzor et al. 2017; Davis et al. 2018). Species with an inability to regain homeostasis or those that require a long acclimation period to do so are marked by significant energetic disturbance and metabolic reorganization (e.g., Huth and Place 2016b). This comes at a cost, and energy is shifted away from important biological functions such as growth (e.g., Sandersfeld et al. 2015; Enzor et al. 2017; Davis et al. 2018), potentially creating fitness-related consequences. Future environmental changes in temperature and CO_2-acidification will likely have significant repercussions on species' persistence and Antarctic notothenioid biodiversity.

The underlying cause to species-specific differences in metabolic compensation to environmental stressors is still not well understood. Important factors to consider include habitat, species relatedness, and life history traits. Species found at colder, higher latitudes, such as in the HAZ, would be expected to have a lower acclimation potential than the species living at warmer, lower latitudes, such as the SPZ. Interestingly, *P. borchgrevinki* and *T. bernacchii*, both exclusively sampled in the HAZ, had greater capacity for RMR compensation than the SPZ species, *N. rossii* or *N. coriiceps* (Fig. 2), suggesting an absence of local adaptation to warmer temperatures. Species trait patterns may be dictated by phylogenetic history and species more closely related may share similar responses to environmental variation as a result of phylogenetic constraint (Felsenstein 1985). This may explain the trends in RMR among the notothenioid species as *P. borchgrevinki* is more closely related to *T. bernacchii* than to *N. rossii* or *N. coriiceps* (Fig. 1). However, *T. newnesi*, a sister species to *P. borchgrevinki* had little to no compensation of RMR in response to warming and CO_2-acidification (Enzor et al. 2017), indicating that the acclimation potential in *P. borchgrevinki* may have evolved independently. Life history traits may also play a role in shaping the species response to a changing environment. The benthopelagic (e.g., *T. newnesi*, *N. rossii*) and cryopelagic species (e.g., *P. borchgrevinki*) are more active swimmers than the strictly benthic notothenioids (e.g., *T. bernacchii*, *N. coriiceps*; Eastman et al. 2011), which may impose different constraints and trade-offs on acclimation capacity. Notothenioids have offered a powerful comparative model for understanding cold adaptation and testing hypotheses regarding physiological limitations related to gene loss (Cheng and Detrich 2007; Coppes Petricorena and Somero 2007; O'Brien 2016). Future work should continue to take advantage of this phylogenetic framework, focusing on integrating bioenergetics in an ecological context, to gain a greater understanding of the vulnerability of notothenioid species to future climate change. Conducting multiple stressor experiments at remote field stations, particularly those in the Antarctic is hard; however, the data does not support running a number of single stressor experiments and extrapolating the consequences of exposure to multiple stressors. The majority of the stressors studied to date interact in non-additive and non-predictable ways (e.g., Strobel et al 2012; Flynn et al. 2015; Davis et al. 2018); therefore, combining the results from single stressor studies will be inaccurate in predicting vulnerability of species to ocean change.

Antarctic biologists have only started to scratch the surface in terms of understanding the metabolic capacity to acclimate to multiple stressors and there is a lot of research still needed to understand how acclimation capacity varies between species and across ontogeny within a particular species. That said, we don't have endless amounts of time to uncover the weak links leading to sensitivity to future ocean conditions given the rapid pace of environmental change in the Southern Ocean. A more strategic and targeted approach is needed where phylogenetic relationships, differences in local environmental conditions and life history are considered in species choices (Fig. 1A). The Antarctic silverside, *Pleurogramma antarcticum*, is a species of notothenioid that is relatively distantly related to the four species from the family Nototheniidae highlighted in this review and therefore would allow for testing of unifying principles of metabolic capacity to acclimate broadly across this suborder of fishes. Furthermore, being able to collect multiple life history stages of this species as well as the critical role this species plays in the Antarctic food web make it a strong species choice for understanding ontogenic differences in metabolic plasticity as well as indirect effects of future oceans on higher trophic levels, like penguins. While we have only touched on the large body of foundational work examining the differential sensitivity to warming between white and red-blooded Antarctic fishes (e.g., O'Brien 2016), adding an icefish (e.g., *Chaenocephalus aceratus*, which lacks both hemoglobin and myoglobin) to the comparative framework would be essential in terms of understanding the role of oxygen/energy supply in the capacity to full compensate RMR. There are a number of notothenioids that are not found in the Antarctic but rather along the coasts of New Zealand and South America. The New Zealand black cod, *Notothenia angustata*, is thought to be an Antarctic escapee, having been relocated to New Zealand about 5 mya when the Antarctic Polar Front shifted northward (Cheng et al. 2003). *Notothenia angustata* shares many of the cold-adapted physiological and biochemical traits as other fishes in the family Nototheniidae that are exclusively found at high latitudes, such as functional antifreeze genes (Cheng et al. 2003), conserved sequence and diversity of hemoglobins (D'Avino and di Prisco 1997) and a kidney with an intermediate structure with respect to glomeruli (i.e., pauciglomerular, Eastman and DeVries 1986). These similarities in physiology and biochemistry could provide evidence of an evolutionarily conserved intrinsic mechanism of energy metabolism. Lastly, we feel additional comparative work between *T. newnesi* and its sister species *P. borchgrevinki* will provide a framework for understanding whether the capacity to metabolically acclimate has evolved independently in the family Nototheniidae. We appreciate that attaining the fish numbers, diversity of species, and different life stages for a particular species of Antarctic fish is not as straightforward as it is for many non-Antarctic species we work on at our home institutions and so our suggested future directions may be a lofty goal. Antarctic scientists like a challenge and so as an international community, we see a lot of potential for collaborative projects to uncover whether there is a unifying bioenergetic framework underlying sensitivity to complex ocean change that can be used to more broadly predict how ocean warming and CO_2-acidification could affect Antarctic fish biodiversity.

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