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High latitude fish in a high CO_2 world: Synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids

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ABSTRACT

Although the physiological response of teleost fishes to increased temperature has been well documented, there is only a small body of literature that examines the effects of ocean acidification on fish under ecologically relevant scenarios. Furthermore, little data exists which examines the possible synergistic effects of increased sea surface temperatures and pCO_2 levels, although it is well established that both will co-committedly change in the coming centuries. In this study we examined the effects of increased temperature, increased pCO_2, and a combination of these treatments on the resting metabolic rate (RMR) of four species of notothenioid fish, *Trematomus bernacchii*, *T. hansoni*, *T. newnesi*, and *Pagothenia borchgrevinki*, acclimated to treatment conditions for 7, 14 or 28 days. While most species appear capable of rapidly acclimating to increased pCO_2, temperature continues to impact RMRs for up to 28 days. One species in particular, *T. newnesi*, displayed no acclimatory response to any of the treatments regardless of acclimation time and may have a reduced capacity to respond to environmental change. Furthermore, we present evidence that temperature and pCO_2 act synergistically to further elevate the RMR and slow acclimation when compared to temperature or pCO_2 increases alone.

1. Introduction

When studying the effects of global climate change, the roles of temperature and CO_2 are invariably linked. In the last 50 years, the Earth's overall temperature has warmed by ~0.6 °C (Walther et al., 2002), representing nearly a 30% change relative to pre-industrial values (Vitousek et al., 1997; Caldeira and Wickett, 2003) which can be attributed to the recent exponential increase of atmospheric CO_2. This rapid increase has been partially offset by the absorption of CO_2 by the worlds' oceans; however, this is expected to lead to a co-committed drop in ocean pH and alteration of seawater chemistry termed "ocean acidification." Under the Intergovernmental Panel on Climate Change (IPCC) A1F1 prediction scenario, the world's oceans will experience an increase in pCO_2 levels upwards of 990 ppm by the year 2100 (IPCC, 2007). Furthermore, it is projected that changes in sea surface temperature and pCO_2 levels will impact higher latitudes to a greater extent than temperate regions (Walther et al., 2002; Orr et al., 2005; Fabry et al., 2008; Halpern et al., 2008) and is expected to occur on a faster time-scale (Turner et al., 2005; McNeil and Matear, 2008; McNeil et al., 2010). The Southern Ocean is particularly vulnerable due its unique biogeochemical processes and natural deep-water entrainment of CO_2 that occurs during the extended winter months. It is anticipated the Southern Ocean will experience detrimental impacts related to ocean acidification by the year 2050; 50 years sooner than the IPCC projected year of 2100 (McNeil and Matear, 2008; McNeil et al., 2010). Additionally, the mean surface temperature of Faraday Station, Antarctica has risen about 2.5 °C since the 1950s (Turner et al., 2005), compared to the 0.6 °C increase seen in the Earth's overall temperature (Walther et al., 2002). While these ecosystems as a whole may adapt to large-scale changes, it is unclear exactly how these changes will occur, and more importantly, at what cost. To understand this, we must address how key organisms will respond to global changes as well as investigate the costs associated with adaptation.

A major portion of the Southern Ocean fauna is comprised of fishes of the perciform suborder Notothenioidei (Gon and Heemstra, 1990; Eastman, 1993). These fishes began to radiate into Antarctic waters in the early Tertiary, gradually adapting to the progressive cooling, which set in after the opening of the Drake passage and the formation of the circumpolar current some 14–25 million years ago (Eastman, 1993). Isolation of the Antarctic continental shelf by the Polar Front has produced arguably the coldest, most oceanographically stable environment on the planet. However, in opposition to this highly stenothermic environment, the profound environmental extremes produced by the transition from 24 h of sunlight to complete darkness over the winter months results in significant variation in primary productivity. As a result, Antarctic marine organisms inhabiting these ice-laden waters face unique metabolic and physiological challenges for survival and persistence. The impacts of low temperatures and seasonally limited food availability have long been recognized as primary selective forces driving the evolution of the many endemic species found in

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Antarctica today (Clarke, 1992; Peck et al., 2004; Pörtner, 2006; Clarke et al., 2007; Peck et al., 2009). In addition to the high degree of endemism produced by food availability and temperature, a wide-array of specialized physiological adaptations, spanning multiple cellular pathways, have arisen in specific genera or families of Antarctic fish, including chaperonins (Pucciarelli et al., 2006), heat shock proteins (Hofmann et al., 2000; Place et al., 2004), heme proteins (O'Brien and Sidell, 2000; Sidell and O'Brien, 2006), tubulin kinetics (Detrich et al., 2000), and anti-freeze proteins (Devries, 1969; Cheng et al., 2006).

The Southern Ocean's rigid stability may have resulted in an ecosystem filled with endemic fauna that are poorly poised to deal with rapid climate variation (Peck et al., 2005; Clarke et al., 2007). For instance, extreme stenothermy has made Antarctic fishes very susceptible to stress induced by warming, with upper thermal limits reported around 6 °C in *Trematomus bernacchii*, *T. hansoni* and *Pagothenia borchgrevinki* (Somero and DeVries, 1967). Additionally, these fish have lost a ubiquitous cellular response to the cytotoxic effects of thermal denaturation of proteins that has been conserved across all taxa (Hofmann et al., 2000; Place et al., 2004; Place and Hofmann, 2005). While some cold-adapted species show an ability to alter their thermal sensitivities, significant inter-specific variation in these responses exist (Podrabsky and Somero, 2006). In addition to thermal tolerance, some notothenioids also display thermal flexibility in O_2 consumption (Robinson and Davison, 2008a,b). However, these changes in oxygen consumption do not appear to confer increased thermal tolerance (Podrabsky and Somero, 2006) and the thermal response of O_2 consumption also appears to be inter-specific (Somero et al., 1968). These previous findings have highlighted the need to take a multi-species comparative approach to understand the capacity of Antarctic notothenioids' ability to tolerate environmental variation. Lending urgency to the need to understand species-specific capacities is the likelihood that the Antarctic ecosystem will reach a critical tipping point far sooner than ecosystems north of the Antarctic Polar Front, perhaps as soon as 2050 (McNeil and Matear, 2008; McNeil et al., 2010). Thus, the Antarctic ecosystem may be particularly vulnerable to climate variations and it is imperative that we begin to understand the potential impacts on ecologically dominant species such as the notothenioids.

While numerous studies have been performed on the ability of notothenioid fishes to adapt to increases in temperature (Somero and DeVries, 1967; Forster et al., 1987; Davison et al., 1990; Pörtner, 2008; Robinson and Davison, 2008a,b); only a handful of studies to date have examined how fish respond to ecologically relevant increases in seawater pCO_2, and only one study has included species from the particularly vulnerable high latitude ecosystems. Studies have shown shunting energy to increase osmoregulation can affect basic processes such as growth and otolith formation (Ishimatsu et al., 2008; Munday et al., 2011), as well as various cellular processes (Langenbuch and Pörtner, 2003). Currently, few studies exist that examine the additive effects of temperature and ocean acidification on piscine species. The rate at which oceanic temperatures and pCO_2 levels are shifting leave little time for evolutionary change; thus species must rely on current physiological plasticity in order to adapt to increases in temperature and pCO_2 levels. Therefore, the question is raised if notothenioid fishes are reallocating energy stores in order to survive extreme cold temperatures, do they possess the metabolic scope necessary to respond to climate perturbations? In this study we examined the metabolic response of several notothenioid species to a multi-stressor scenario of increased temperature and pCO_2, and report how Antarctic fishes respond to the additive effect of global climate change factors under an ecologically relevant scenario.

2. Methods

2.1. Collection of study specimens

Benthic Antarctic notothenioids, *T. bernacchii* (Boulenger, 1902), *T. hansoni* (Boulenger, 1902), *T. newnesi* (Boulenger, 1902) and the cryopelagic Antarctic notothenioid, *P. borchgrevinki* (Boulenger, 1902), were collected in McMurdo Sound, Antarctica (77°53'S, 166°40'E), from October to November of 2011. *T. bernacchii*, *P. borchgrevinki*, and *T. newnesi* were caught by hook and line and *T. hansoni* fish were collected using baited fish traps set on the substrate at a depth of ~300 m. All fish were maintained in a 4100 L flow-through aquarium near ambient seawater temperatures (−1.5 °C) and acclimated for one week prior to being placed in experimental tanks. While in the acclimation tank, fish were fed frozen anchovy every other day.

2.2. Experimental design

While little to no information is available regarding changes to pCO_2 levels in McMurdo Sound during winter months, there appears to be little temporal change to pH or pCO_2 levels over the austral summer. High-frequency pH observations taken in McMurdo Sound during the summer of 2010 show relatively small fluctuations in pH over the course of hours to days (Matson et al., 2011). In 2011, Hofmann and colleagues estimated pCO_2 levels in McMurdo Sound based on observed pH, salinity and *in situ* temperature recorded at 1 h intervals from October to December and these measurements served as a target value for the control pCO_2 settings in our experimental tanks. At Hut Point, mean pCO_2 was estimated at 413 ± 8 ppm (± s.d.) with a range of 391 to 427 ppm and at Cape Evans, mean pCO_2 was estimated at 426 ± 16 ppm with a range of 358 to 450 ppm (Matson and Hofmann, unpubl. data). These data are representative of the values we recorded for incoming seawater in the aquarium facilities at McMurdo Station which held steady around 417 ppm (Table 1). We used four identical, 1240L experimental tanks maintained at different temperatures and/or pCO_2 to examine the combined effects of temperature and CO_2 on the resting metabolic rate of fishes acclimated from 7 to 28 days. The four target experimental treatments consisted of (1) a control treatment held near ambient seawater temperature and pCO_2 (−1 °C, 415 ppm), (2) an ambient low temperature + high pCO_2 treatment (−1 °C, 1000 ppm), (3) a high temperature + low pCO_2 treatment (+4 °C, 415 ppm), and a high temperature + high pCO_2 treatment (+4 °C, 1000 ppm). Fish (n = 4 fish per time point, per treatment) were placed in experimental tanks for a period of 7, 14 or 28 days. In these cold adapted species, food can impact resting metabolic rates for 14 days after the last meal (Davison et al., 1990) and previous work performed by Robinson and Davison (2008a,b) has shown withholding food for 28 days in Antarctic fish does not affect metabolic rate measurements. As fish were acclimated in treatment tanks for varying time periods, food was withheld from experimental tanks for the duration of the experiment to ensure the resting metabolic rates were not impacted by the organism's specific dynamic action. Given the extended influence of specific dynamic action of feeding on metabolic rates in these species, there was potential for our first experimental time point to be affected by fish which were fed prior to introduction to the experimental tanks. However, no significant differences were found between control fish across acclimation times, suggesting only resting metabolic rates of fish were measured.

2.3. Manipulation of seawater conditions

Temperature and pCO_2 levels were manipulated within the experimental tanks using a combination of thermostated titanium heaters (Process Technology, Brookfield, CT, USA) and a modified pCO_2 generation system first described by Fangue et al. (2010) to blend pure CO_2 with CO_2-free atmospheric air at precise pCO_2 concentrations. Briefly, atmospheric air was pumped through a chilled condensing coil and passed through drying columns filled with drierite to remove all moisture. Next, CO_2 was scrubbed from the air using a series of columns filled with Sodasorb®. CO_2-free air and pure CO_2 were then mixed to desired levels using digital mass flow controllers (Sierra Instruments, Monterey, CA, USA), and infused into seawater using venturri injectors.

Table 1
Mean measurements of total alkalinity, pH, pCO_2, dissolved oxygen and temperature ± SD over the course of the experiment.

Sample	Total alkalinity (μmol/kg sol'n)	pH	pCO_2 (ppm)	Oxygen (% air sat)	Temperature (°C)
Incoming seawater	2329.53 ± 9.34	8.015 ± 0.012	417.15 ± 12.26	90.926 ± 4.697	−1.24 ± 0.08
Low temperature + low pCO_2	2330.17 ± 8.96	7.944 ± 0.014	438.82 ± 16.08	93.80 ± 2.465	−0.61 ± 0.17
Low temperature + high pCO_2	2328.31 ± 11.04	7.685 ± 0.068	953.89 ± 50.38	93.635 ± 2.80	−0.45 ± 0.16
High temperature + low pCO_2	2342.81 ± 9.76	7.944 ± 0.015	525.11 ± 21.07	94.14 ± 2.20	4.02 ± 0.44
High temperature + high pCO_2	2341.87 ± 8.02	7.675 ± 0.036	1026.66 ± 9.03	94.983 ± 1.610	4.22 ± 0.56

Treated air was delivered directly into experimental tanks as well as 45-gallon header tanks used to equilibrate seawater and allow for continuous exchange of treated seawater within the experimental tanks.

Experimental tanks as well as incoming seawater were sampled daily to determine temperature, pH (total scale), salinity, total alkalinity (T_A) and oxygen saturation. Seawater parameters for the duration of the experiment are reported in Table 1. Additionally, experimental tanks were tested daily for water quality (ammonia, nitrite and nitrate levels) of which, no significant increase in any nitrogenous waste were noticed in any of the experimental tanks for the duration of the experiment (data not shown).

2.4. Evaluation of metabolic rates

Resting metabolic rates (RMRs) were determined using an automated intermittent respirometry system (Loligo Systems, Denmark). Respirometry chambers were housed in covered 99-L tanks receiving continuous flow of treated seawater directly from the experimental tanks to maintain consistency among acclimation conditions and conditions under which metabolic rates were recorded. In addition, the 99-L tanks used for metabolic measurements of warm acclimated fish were fitted with glass aquarium heaters to maintain constant acclimation temperatures while cold 99-L tanks were submerged within an 850-L sea table with continuous flow of ambient seawater to maintain lower temperatures. Fish were placed in respirometry chambers with flush pumps running for 10–12 h prior to determination of oxygen consumption rates. Oxygen consumption measurements were collected over a 20 min interval followed by a 5 min flush cycle to re-oxygenate the respirometry chamber. Following the adjustment period to the respirometry chamber, respiration rates ($\dot{M}O_2$) were measured continuously over a three-hour period at the same time each day. Once no discernible chamber effect could be observed, as indicated by no significant changes in oxygen consumption over a 1 h period and r^2 values of >0.95 for the slope describing the rate of oxygen consumption, mean $\dot{M}O_2$ values were calculated by averaging five sequential measurements. Additionally, values whose slope deviated from an r^2 of >0.95 were excluded, as they are likely indication of fish activity in the chamber during that particular measurement period. Mass specific oxygen consumption rates were standardized to a 100-g fish (Steffensen, 2005; Robinson and Davison, 2008a,b) using a mass exponent of −0.25 (Schmidt-Nielsen, 1984). As we did not have enough individuals to experimentally determine the mass-exponent for our study species, we chose to be conservative with our measurements and utilize the more general model of −0.25 as a mass exponent. A study performed by Clarke and Johnston (1999) indicated that a wide range of teleost fish, whether polar or temperate, scale similarly.

2.5. Statistics

We used two-way analysis of variance (ANOVA) to test for significant differences in the RMR of *T. bernacchii* acclimated to different treatments, length of acclimation (7, 14 or 28-days), and tested for an interaction between treatment and acclimation time. For inter-species comparisons, we again utilized two-way ANOVA to test for differences within treatment, within species and for an interaction between treatment and species for 7-day (*T. bernacchii*, *T. hansoni* and *P. borchgrevinki*) and 28-day (*T. bernacchii*, *T. hansoni*, *P. borchgrevinki*, and *T. newnesi*) acclimation times. For comparisons that revealed a significant difference in the RMR ($p<0.05$), we utilized a Tukey's HSD multiple comparison test to identify the means that significantly differed from each other ($p<0.05$).

3. Results

3.1. Seawater chemistry

During the course of the 3-month experimental run of the seawater system, we observed minor variation between the measured seawater parameters and the target treatment (Table 1). Although we observed temporal variation in the absolute temperature and pCO_2 values, the treatments remained significantly different from one another over the course of the experiment. One-way ANOVA found the two high temperature treatments significantly differed from the two low temperature treatments as well as the ambient incoming seawater over the course of the experiment ($p<0.05$).

The two high pCO_2 treatments also significantly differed from both low pCO_2 treatments as well as incoming seawater ($p<0.05$). Low treatment pCO_2 levels did not differ from those of incoming seawater ($p>0.05$). Significant differences were seen when comparing the high temperature + low pCO_2 treatment to the low temperature + low pCO_2 treatment and incoming seawater ($p<0.05$). This may have been a consequence of the reduced flow rates of ambient seawater necessary to maintain the elevated temperature and the increased respiration rates of fish in response to increased temperature. No significant differences were found between the pCO_2 levels for the high pCO_2 treatments.

3.2. Intra-species comparison

For *T. bernacchii* specimens, acclimation to high pCO_2 and high temperature resulted in elevated RMRs (Fig. 1). The response varied both between treatments and across acclimation periods, suggesting *T. bernacchii* may be differentially affected by pH and temperature changes in their environment (Fig. 1). Two-way ANOVA performed on the RMRs of *T. bernacchii* acclimated to different seawater treatments indicated there was an effect of treatment, acclimation time , and an interaction between treatment and acclimation time in this species (Table 2). Overall, RMRs in *T. bernacchii* acclimated to high temperature or high temperature + high pCO_2, were significantly higher than the RMRs in fish acclimated to either of the low temperature treatments (Table 3). Additionally, within a given acclimation period, fish acclimated to control conditions (low temperature + low pCO_2) displayed lower RMRs when compared to all other treatments (low temperature + high pCO_2, high temperature + low pCO_2, and high temperature + high pCO_2, see Table 3).

T. bernacchii displayed a relatively rapid compensation with respect to oxygen consumption when acclimated to high pCO_2 and high temperatures (Fig. 1). Fish acclimated to these treatments show a consistent decline in RMR with specific oxygen consumption rates becoming nearly indistinguishable from control fish within a 28-day acclimatory period (Fig. 1). After a 7-day acclimation period, all experimental treatments resulted in significantly elevated RMR (low temperature + high pCO_2 = 30.36 mg O_2/kg/h; high temperature + low pCO_2 = 39.56 mg O_2/kg/h;

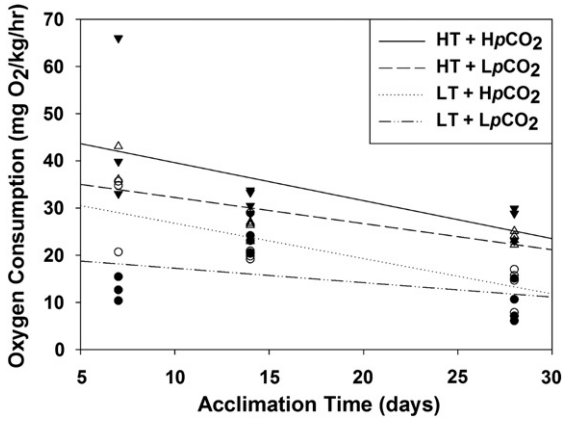

Fig. 1. Resting metabolic rates (scaled to a 100-g fish, mass exponent of −0.25) for *T. bernacchii* acclimated at 7, 14 and 28 days to treatments of ambient conditions, (low temperature + low pCO_2, LT + LpCO_2; indicated by closed circles), low temperature + high pCO_2 (LT + HpCO_2; open circles), high temperature + low pCO_2 (HT + HpCO_2; open triangles) and high temperature + high pCO_2 (HT + HpCO_2; closed triangles).

and high temperature + high pCO_2 = 46.28 mg O_2/kg/h) relative to control fish (12.80 mg O_2/kg/h; Fig. 1, Table 3). Within 14 days, there was no statistical difference between control (22.02 mg O_2/kg/h) and the low temperature + high pCO_2 treatment (22.21 mg O_2/kg/h), however after 28 days, the RMR of *T. bernacchii* acclimated to both high temperature treatments (high temperature + low pCO_2, 23.67 mg O_2/kg/h; and high temperature + high pCO_2, 27.26 mg O_2/kg/h) remained elevated above control values (9.72 mg O_2/kg/h; Table 3). These trends indicate that within 14 days, metabolic rates of fish acclimated to high pCO_2 seawater are indistinguishable from the metabolic rates of control fish, however elevated temperature continues to impact the RMR in *T. bernacchii* for upwards of a month after initial exposure.

P. borchgrevinki specimens also showed an effect of acclimation time and treatment when analyzed using two way ANOVA (Fig. 2, Table 2), but no interaction between acclimation time and treatment was found (Table 2). As with *T. bernacchii*, 7-day acclimated fish had elevated RMRs in the high temperature + high pCO_2 treatment (41.86 mg O_2/kg/h) when compared to the low temperature + high pCO_2 treatment (24.29 mg O_2/kg/h) and control treatment (25.64 mg O_2/kg/h; Fig. 2, Table 3). Fish acclimated in the high temperature + low pCO_2 treatment (38.84 mg O_2/kg/h) also showed elevated RMRs when compared to the low temperature + high pCO_2 treatment (24.29 mg O_2/kg/h; $p = 0.037$), however no difference was found in the oxygen consumption of high temperature-acclimated fish and those in the control treatment (Table 3). This finding may be a result of the low n-value for these fish as the difference found was just below the significance level. Fish acclimated for 28-days to experimental conditions showed elevated RMRs in the high temperature + high pCO_2 treatment (33.29 mg O_2/kg/h) when compared to control RMR values (19.57 mg O_2/kg/h; Fig. 3, Table 3). No differences were found in the RMRs between fish acclimated in the high temperature + low pCO_2 treatment (24.29 mg O_2/kg/h and fish in the control tanks (Table 3).

Two-way ANOVA performed on the RMRs of *T. hansoni* showed only a treatment effect was present (Table 2). These fish followed the same trends as *T. bernacchii* and *P. borchgrevinki*; RMRs measured from the high temperature (38.35 mg O_2/kg/h) and high temperature + high pCO_2 (33.51 mg O_2/kg/h) treatments were elevated when compared to control values (18.65 mg O_2/kg/h) after 7 days of acclimation (Fig. 2, Table 3). Oxygen consumption rates of fish acclimated for 28-days did not differ from control values for any experimental treatment (Fig. 3, Table 3).

3.3. Inter-species comparison

Despite the phylogenetic distance between the species used in this study and the variety of habitat niches occupied by these fish, we found no species effect after 7 days of acclimation (Fig. 2, Table 4). However, resting metabolic rates did differ as a function of treatment (Fig. 2, Table 4). No interaction between species and treatment was observed (Table 4). As previously seen with *T. bernacchii*, RMRs for *T. hansoni* and *P. borchgrevinki* were significantly lower in the two low temperature treatments compared to the two high temperature treatments, regardless of pCO_2 levels (Fig. 2).

Unlike fish acclimated for shorter periods of time, a significant difference in the metabolic response was seen both between treatments and between species when acclimated for 28 days (Fig. 3). A two-way ANOVA revealed a significant difference between the mean RMR as a function of treatment (Table 4), while no significant differences were seen between fish acclimated to high temperature alone and the combined stress of high temperature + high pCO_2 (Fig. 3). However, RMRs in fish acclimated for 28 days were significantly different in control treatments when compared to the high temperature + high pCO_2 treatment, as well as high temperature + low pCO_2 treatments (Table 3). Additionally, there was a significant difference in RMR with respect to species (Table 4). Overall, RMRs of *T. bernacchii* (control = 9.72 mg O_2/kg/h, low temperature + high pCO_2 = 13.80 mg O_2/kg/h, high temperature + low pCO_2 = 23.67 mg O_2/kg/h, and high temperature + high pCO_2 = 27.26 mg O_2/kg/h) were lower when compared with the metabolic rates of *T. hansoni*, (control = 19.80 mg O_2/kg/h, low temperature + high pCO_2 = 33.35 mg O_2/kg/h, high temperature + low pCO_2 = 36.26 mg O_2/kg/h, and high temperature + high pCO_2 = 34.15 mg O_2/kg/h) and *T. newnesi* (control = 11.08 mg O_2/kg/h, low temperature + high pCO_2 = 31.76 mg O_2/kg/h, high temperature + low pCO_2 = 33.09 mg O_2/kg/h, and high temperature + high pCO_2 = 39.87 mg O_2/kg/h; Table 3), but when compared with *P. borchgrevinki*, (control = 19.57 mg O_2/kg/h, low temperature + high pCO_2 = 22.48 mg O_2/kg/h, high temperature + low pCO_2 = 24.29 mg O_2/kg/h, and high temperature + high pCO_2 = 33.29 mg O_2/kg/h), *T. bernacchii* RMRs were not significantly different (Fig. 3). No interaction was found between treatment and species (Table 4).

Across all species, *T. newnesi* exhibited remarkably different patterns of oxygen consumption after 28 days of acclimation to various pCO_2 and temperature treatments (Fig. 3). Unlike the other species tested, *T. newnesi* displayed elevated RMRs in response to all treatments relative to control values even after 28 days of acclimation (Fig. 3). A two-way ANOVA revealed fish acclimated to the control treatment (11.08 mg O_2/kg/h) had significantly lower mean RMRs when compared to the low temperature + high pCO_2 (31.76 mg O_2/kg/h), high temperature + low pCO_2 (33.09 mg O_2/kg/h), and high temperature + high pCO_2 treatments (39.87 mg O_2/kg/h; Table 3). These data suggest that unlike *T. bernacchii*, *T. hansoni* and *P. borchgrevinki*, *T. newnesi* cannot

Table 2
Results of 2-way ANOVA for *T. bernacchii* (acclimated at 7, 14 and 28 days), *P. borchgrevinki* (acclimated at 7 and 28-days), and *T. hansoni* (acclimated at 7 and 28 days). Values marked with an asterisk indicate a significant finding.

Species	Acclimation time	Treatment	Interaction
T. bernacchii	$F = 17.186$ df = 2 $p < 0.001$*	$F = 23.520$ df = 3 $p < 0.001$*	$F = 3.188$ df = 6 $p = 0.015$*
P. borchgrevinki	$F = 11.153$ df = 1 $p = 0.004$*	$F = 10.088$ df = 3 $p < 0.001$*	$F = 1.313$ df = 3 $p = 0.303$
T. hansoni	$F = 0.0177$ df = 1 $p = 0.8954$	$F = 5.188$ df = 3 $p = 0.007$*	$F = 0.0844$ df = 3 $p = 0.968$

Table 3
Results of Tukey's HSD test for *T. bernacchii*, *T. hansoni*, *P. borchgrevinki*, and *T. newnesi* acclimated at 7, 14 and 28 days. Treatment conditions of low temperature + high pCO_2 (LT + HpCO_2), high temperature + low pCO_2 (HT + LpCO_2) and high temperature + high pCO_2 (HT + HpCO_2) are compared with control conditions of low temperature + low pCO_2. Values marked with an asterisk indicate a significant finding.

	LT + HpCO_2	HT + LpCO_2	HT + HpCO_2
T. bernacchii			
7-day acclimation	q = 5.229 p <0.005*	q = 7.128 p <0.001*	q = 9.969 p <0.001*
14-day acclimation	q = 0.0627 p = 1.00	q = 1.281 p = 0.802	q = 3.287 p = 0.115
28-day acclimation	q = 1.402 p = 0.756	q = 4.794 p = 0.010*	q = 5.582 p = 0.002*
T. hansoni			
7-day acclimation	q = 2.997 p = 0.178	q = 4.642 p = 0.017*	q = 3.501 p = 0.092
28-day acclimation	q = 2.607 p = 0.281	q = 3.167 p = 0.144	q = 2.761 p = 0.236
P. borchgrevinki			
7-day acclimation	q = 0.342 p = 0.995	q = 3.850 p = 0.063	q = 4.732 p = 0.018*
28-day acclimation	q = 0.900 p = 0.919	q = 1.462 p = 0.733	q = 4.541 p = 0.024*
T. newnesi			
28-day acclimation	q = 5.079 p = 0.005*	q = 5.406 p = 0.003*	q = 7.072 p <0.001*

adjust physiologically to increased levels of pCO_2 within the 28-day acclimation period used in this study.

4. Discussion

Isolation of the Antarctic Polar Front has resulted in the evolution of marine organisms in an extremely stable environment that are often considered to be limited in their capacity to respond to environmental change (Peck et al., 2005; Clarke et al., 2007). Consequently, these organisms are often perceived as highly vulnerable to the predicted changes in environmental parameters as a result of anthropogenic disturbances. Waters of the Southern Ocean are expected to experience some of the earliest impacts of global climate change (Orr et al., 2005; McNeil and Matear, 2008). Therefore, inhabitants of this unique ecosystem are considered potential forecasters of biological impacts of climate change and thus we set out to examine the metabolic response of several species of the suborder Notothenioidei, the dominant fish fauna of the Antarctic ecosystem (Eastman, 1993), to ecologically relevant perturbations of seawater pCO_2 and temperature.

Control RMRs from fish in this study are representative of oxygen consumption rates previously measured. The measurements of RMRs in *T. bernacchii* (12.80 mg O_2/kg/h), *T. hansoni* (18.65 mg O_2/kg/h), and *P. borchgrevinki* (25.64 mg O_2/kg/h), were similar to those found by Steffensen; 17.3, 22.4 and 28.2 mg O_2/kg/h, respectively (2005). Davison and colleagues have reported slightly higher RMRs for *P. borchgrevinki* (32.8 mg O_2/kg/h, 1990), however these values were obtained at 0 °C and this may account for the small difference between our measurements. Our resting measurements of oxygen consumption in *T. newnesi* under ambient conditions (11.07 mg O_2/kg/h) were much lower than previously unpublished values of 41 mg O_2/kg/h (referenced in Steffensen, 2005), however we cannot speculate as to the differences between these two studies. The idea of metabolic cold adaptation has been long-debated in regard to Antarctic species. These data follow previously established findings that notothenioid resting metabolic rates routinely fall below values reported by Wohlschlag (1960, 1964) that helped establish the theory of metabolic cold adaptation in these fish. Our data find no evidence that would suggest metabolic cold adaptation of resting metabolic rates has occurred in these species and offers no further empirical support for Krogh's initial predictions (1914, 1916).

As environmental temperature increases, oxygen demand also increases and organisms must increase oxygen consumption in order to compensate for this increased demand. If demand is not met, tissues become hypoxic, causing protein synthesis to slow, ultimately halting growth and reproduction (Fry, 1947; Pörtner et al., 2005). The use of anaerobic metabolism has been documented as a common tool to combat the physiological stress that accompanies increased environmental temperature (Pörtner et al., 2005). Given the limited glycolytic capacity

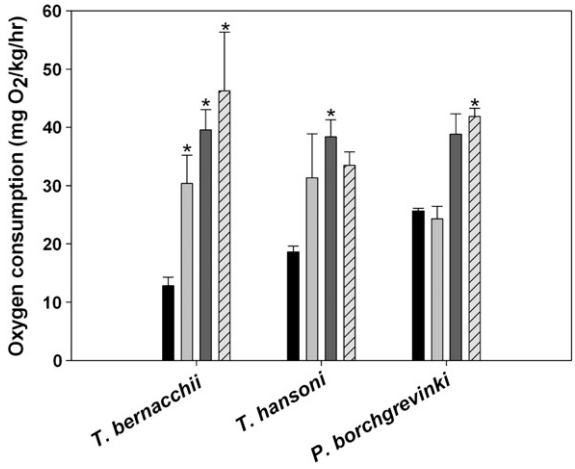

Fig. 2. Resting metabolic rates (±SE; scaled to a 100-g fish, mass exponent of −0.25) for *T. bernacchii*, *T. hansoni*, and *P. borchgrevinki* acclimated at 7 days to treatments of ambient conditions, (low temperature + low pCO_2; black bars), low temperature + high pCO_2 (light grey bars), high temperature + low pCO_2 (dark grey bars) and high temperature + high pCO_2 (light grey bars with crosshatch's). Treatments found to be significantly different from control values are marked with asterisks.

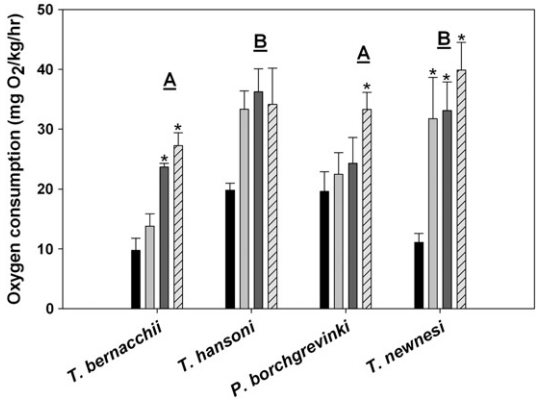

Fig. 3. Resting metabolic rates (±SE; scaled to a 100-g fish, mass exponent of −0.25) for *T. bernacchii*, *T. hansoni*, *P. borchgrevinki*, and *T. newnesi* acclimated at 28 days to treatments of ambient conditions, (low temperature + low pCO_2; indicated by black bars), low temperature + high pCO_2 (light grey bars), high temperature + low pCO_2 (dark grey bars) and high temperature + high pCO_2 (light grey bars with crosshatch's). Letters indicates a significant species effect. Asterisks indicate a significant difference between the treatment and control values within a given species.

Table 4
Results of 2-way ANOVA for fish species (*T. bernacchii*, *P. borchgrevinki* and *T. hansoni*) acclimated for 7-days and species (*T. bernacchii*, *P. borchgrevinki*, *T. hansoni* and *T. newnesi*) acclimated for 28-days. Values marked with an asterisk indicate a significant finding.

Acclimation time	Species	Treatment	Interaction
7-day acclimation	$F=0.274$ df $=2$ $p=0.762$	$F=13.352$ df $=3$ $p<0.001^*$	$F=1.322$ df $=6$ $p=0.282$
28-day acclimation	$F=7.336$ df $=3$ $p<0.001^*$	$F=13.920$ df $=3$ $p<0.001^*$	$F=1.203$ df $=9$ $p=0.319$

of the notothenioids, (Dunn and Johnston, 1986; Forster et al., 1987; Davison et al., 1988) there can be little doubt that the initial stress response involves restructuring of energy stores, and hence, an increase in metabolic rate, until cellular homeostasis can again be achieved. Overall, each species examined in this study displayed an increase in resting metabolic rate above control conditions after a 7-day exposure to experimental treatments. These data are consistent with previous studies that have identified energetic shifts in cellular processes as a result of increased temperature in Antarctic notothenioids. Studies performed on the thermal tolerance of *P. borchgrevinki* acclimated to 4 °C for short periods of time shows a marked increase in RMR (Wilson et al., 2002; Robinson and Davison, 2008a,b). In addition, work performed by Somero and DeVries (1967) illustrated that oxygen consumption of brain tissue from *T. bernacchii* increased by almost 2-fold at 4 °C.

Similarly, it has been shown that elevated pCO_2 environments require fish to spend more energy on physiological responses such as acid–base regulation and ventilation rates (Perry and Gilmour, 2002; Evans et al., 2005; Perry and Gilmour, 2006). The cost of baseline osmoregulation in fishes is estimated to be approximately 6-15% of resting metabolic rate (Kidder et al., 2006); therefore energy spent on increased acid–base regulation is likely to shunt energy away from growth and otolith formation (Ishimatsu et al., 2008; Munday et al., 2011), as well as other cellular processes such as protein synthesis (Langenbuch and Pörtner, 2003). In our study, both elevated temperature and pCO_2 levels alone resulted in an upward shift in RMR within the first 7 days of exposure, suggesting a potential short-term energetic cost for physiological adaptations needed to restore proper cellular homeostasis.

Given the higher cost of ventilation amongst aquatic breathers (Dejours, 1981), is it thought fish will exhibit little to no respiratory acclimation when confronted by high pCO_2 environments. Indeed, Atlantic salmon display significantly elevated ventilation rates for upwards of 62 days when exposed to high pCO_2 environments (Fivelstad et al., 1999; Hosfeld et al., 2008). This increase in RMR is likely an attempt to balance cellular energetics resulting from hypercapnic effects on respiratory gas exchange and acid–base balance in marine fish (for reviews see Perry and Gilmour, 2006; Brauner and Baker, 2009). Unlike Atlantic salmon, Antarctic notothenioids displayed a rapid acclimatory response when exposed to elevated pCO_2 levels for an extended duration. Respiration rates quickly leveled off to those of control values within 14 days of exposure to hypercapnic conditions. Some of the discrepancy in the acclimatory response may in-part lie with the significant difference in the pCO_2 levels fish were exposed to in the studies by Fivelstad et al. [12,000 μatm] (1999) and Hosfeld et al. [7800 μatm] (2008). Recently, Esbaugh et al. (2012) reported a similar rapid physiological compensation for blood plasma acidosis resulting from low level hypercapnia in another marine fish, *Opsanus beta*, and suggested the potential impacts of ocean acidification on marine teleosts may not necessarily stem directly from acidosis, but the energetic costs associated with chronic exposure. Alternatively, short term studies performed on juvenile tropical (Munday et al., 2009) and coral reef fish (Nowicki et al., 2012), as well as juvenile Atlantic cod, *Gadus morhua*, (Melzner et al., 2009), have shown little to no detrimental effects when fish are reared in a hypercapnic environment at levels predicted for 2100. Taken together, these studies highlight the variability of sensitivities among species and across developmental stages. The longer acclimation times and ecologically relevant pCO_2 levels used in this study may suggest many of these unique species of fish have the necessary metabolic scope to compensate for the expected shift in seawater pH and pCO_2 levels. However, it remains to be determined if the rapid acclimatory response to elevated pCO_2 is indicative of a return to an optimal physiological status, or a result of energetic tradeoffs associated with a continued defense of acid–base regulation that could have potential long-term impacts on growth and fecundity.

As seawater temperature and pCO_2 are expected to concomitantly change, we also assessed the potential synergistic impact these combined changes will have on oxygen consumption rates. The combination of these two experimental treatments resulted in further elevation of the RMR in most individuals; yet, we found no statistically significant interaction between elevated pCO_2 and temperature. This implies that these two environmental stresses may potentially be additive in their effect, however, our data suggest temperature may be the major driver of metabolic rates in these fish. For instance, *T. bernacchii* appears to quickly acclimate to increased levels of pCO_2, with fish exposed to high pCO_2 seawater at ambient temperatures showing no significant difference in RMR compared to control fish within a 14-day acclimation period. Temperature on the other hand, continues to impact RMRs in these fish even after a 28-day acclimation period. The trends identified for *T. bernacchii* held true for both *T. hansoni* and *P. borchgrevinki*, suggesting acclimation to elevated SST may come at a higher energetic cost in these notothenioids. Work performed by Robinson and Davison (2008a,b) illustrated *P. borchgrevinki* is capable of acclimating to 4 °C within one month; we identified a similar trend in our study with respect to temperature effects on the RMR of this species. Oxygen consumption was initially elevated in fish acclimated to 4 °C followed by a steady decrease over the 28-day acclimation period. When increases in SST and pCO_2 are co-varied in an ecologically relevant manner, the RMR of *P. borchgrevinki* is further elevated, and in contrast to temperature alone, *P. borchgrevinki* continued to display significantly elevated RMRs after a 28-day acclimation period. The additive effects of elevated pCO_2 and temperature appear to slow the acclimatory response in *P. borchgrevinki*, and much like *T. bernacchii*, the RMR of fish in this treatment continue to expend relatively more energy than fish acclimated under control conditions. It was hypothesized by Pörtner (2008) that elevated CO_2 levels may heighten an organisms' response to thermal stress; thus, the physiological stress of adapting to increased temperature and pCO_2 concurrently could explain the deviation from the previously observed metabolic compensation in *P. borchgrevinki* (Franklin et al., 2007; Robinson and Davison, 2008a,b). These data provide some of the first evidence that changes in these two seawater parameters may act synergistically to impact the performance of marine teleosts for extended durations.

Unlike the other species used in this study, *T. newnesi* continues to display elevated RMRs after acclimation to increased pCO_2, temperature, and the combined treatment of temperature and pCO_2, even after 28 days. These data suggest this species may be particularly vulnerable to changes in seawater conditions and further investigation is needed to examine if *T. newnesi* requires a longer acclimation time or if they are not capable of acclimating to these experimental conditions at all. Several key differences between *T. newnesi* and other members of the family Nototheniodae have been noted in previous comparative studies, such as differences in morphology (Balushkin, 1984) and hemoglobin components (di Prisco et al., 1991). These differences caused Balushkin and others to reconsider

the evolutionary relationship of *T. newnesi* to other members of the Notothenioidae family. Recent studies, however, have placed *T. newnesi* squarely within the Trematomid genus (Sanchez et al., 2007) and this physiological deviation in the acclimation response of *T. newnesi* from the other notothenioids tested may be more representative of the rapid radiation and high plasticity of the Trematominae.

Although both temperature and hypercapnia have both received a significant amount of attention with respect to physiological impacts on marine teleosts, the current results are among a small handful of studies that demonstrate the potential synergistic impacts these linked environmental parameters will have on marine fish. Furthermore, this study demonstrates ecologically relevant changes in seawater temperature and pCO_2 can significantly impact the physiological response of the highly endemic fishes of the Southern Ocean. Our results suggest that while these animals appear to have the necessary scope to adjust to near future changes in seawater temperature and pCO_2, there is an additive energetic cost to maintaining homeostasis in a high CO_2 world evident by the extended acclimation time required by *P. borchgrevinki* under a realistic multi-stressor scenario. What is unclear from these initial studies is whether or not these physiological adjustments come in the form of energetic trade-offs with respect to other cellular functions. Notably, a particularly important determinant of what form these energetic costs may take is whether or not the availability of food is also impacted. As sufficient food availability may provide a means to potentially offset the energetic costs, it will be critical to have a better understanding of the impact climate variation will have on key species within these food webs as well. These studies have highlighted the need to further understand the energetic costs of long-term acclimation to ecologically relevant environmental changes in order to predict the potential downstream impacts of chronic elevation of atmospheric CO_2.

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