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RESEARCH ARTICLE

Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*

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ABSTRACT

As a response to ocean warming, shifts in fish species distribution and changes in production have been reported that have been partly attributed to temperature effects on the physiology of animals. The Southern Ocean hosts some of the most rapidly warming regions on earth and Antarctic organisms are reported to be especially temperature sensitive. While cellular and molecular organismic levels appear, at least partially, to compensate for elevated temperatures, the consequences of acclimation to elevated temperature for the whole organism are often less clear. Growth and reproduction are the driving factors for population structure and abundance. The aim of this study was to assess the effect of long-term acclimation to elevated temperature on energy budget parameters in the high-Antarctic fish *Trematomus bernacchii*. Our results show a complete temperature compensation for routine metabolic costs after 9 weeks of acclimation to 4°C. However, an up to 84% reduction in mass growth was measured at 2 and 4°C compared with the control group at 0°C, which is best explained by reduced food assimilation rates at warmer temperatures. With regard to a predicted temperature increase of up to 1.4°C in the Ross Sea by 2200, such a significant reduction in growth is likely to affect population structures in nature, for example by delaying sexual maturity and reducing production, with severe impacts on Antarctic fish communities and ecosystems.

KEY WORDS: Antarctica, Climate change, Teleost, Energy budget, Growth, Production, Thermal tolerance

INTRODUCTION

Changes in sea temperature can affect the ecophysiology of marine organisms, with outcomes including changes in fish productivity and distribution shifts. A model by Cheung et al. (2013) predicts a global decline in maximum fish body mass as a consequence of global warming. While the authors attribute half of this effect to direct impacts on physiology, the remainder has been attributed to indirect effects, such as abundance and distribution shifts (Cheung et al., 2013). Such models are validated by published observations describing a poleward shift of fish communities as well as a shift towards deeper water layers (Perry et al., 2005; Dulvy et al., 2008; Baudron et al., 2011).

In spite of the cold temperatures, the Southern Ocean is one of the hot spots of global warming. Data from Byrd Station on the West Antarctic ice sheet recorded a warming (air temperature) of 2.4±1.2°C between the years 1958 and 2010, making central West Antarctica one of the most rapidly warming places on earth (Bromwich et al., 2013). In the Ross Sea region, shelf water warming of 0.8–1.4°C is predicted by 2200 (Timmermann and Hellmer, 2013).

Fish make up a large part of the biomass in the Southern Ocean. Their fauna is highly endemic and mostly consists of the perciform suborder Notothenioidei, with the family Nototheniidae dominating coastal ecosystems (Eastman and Hubold, 1999; Donnelly et al., 2004). Fish play an important role in the Antarctic food web, as they link top predators such as birds and mammals with lower trophic levels. Living in an extremely cold and stable environment, Antarctic fish are highly stenothermal. Moreover, they exhibit several adaptations to the cold, such as a lack of heat shock response, expression of anti-freeze glycoproteins, reduction or loss of haemoglobin and myoglobin, higher mitochondrial densities as well as other compensatory adaptations to the heart and circulatory system (Coppes Petricorena and Somero, 2007; Cussac et al., 2009).

The acclimation capacity of fish and other Antarctic organisms has been the subject of many studies. While the capacity for thermal adjustment seems to be species specific (Bilyk and DeVries, 2011; Enzor et al., 2013; Strobel et al., 2013), the underlying mechanisms that allow metabolic shifts during temperature acclimation are still not completely understood. The concept of oxygen-limited thermal tolerance aims to explain the effect of temperature on body functioning (Pörtner, 2012). Increased temperature is suggested to increase metabolic demand and thus whole-animal metabolic rates. However, experimental data on cellular and enzymatic levels are often contradictory and trade-offs for the whole organism are in many cases unclear. For example, studies on *Trematomus bernacchii* (Boulenger, 1902), at the mitochondrial or enzyme level as well as on cellular stress responses, suggest the capacity to acclimate to increased temperature (Gonzalez-Cabrera et al., 1995; Brauer et al., 2005; Enzor and Place, 2014), while results on transcriptomic changes and whole-animal metabolic rates indicate a lack of or incomplete compensation for temperature (Robinson, 2008; Enzor et al., 2013; Huth and Place, 2013). Knowledge of the consequences of thermal acclimation for the whole organism is scarce, but is most relevant in an ecological context. The whole-animal level is crucial for the representation of an organism's fitness, i.e. its energy budget. The energy budget is defined by the energy intake in the form of food that can be allocated to different vital functions, such as routine metabolism, growth, reproduction, activity and excretion. Here, growth and reproduction of individuals are crucial factors for a population, shaping its structure, abundance and distribution. Basic energetic costs of maintenance (routine metabolism) have to be met before energy can be allocated to growth and reproduction. At a species' optimal temperature, low routine metabolic costs are suggested to be related to a higher energy allocation to growth (Koehn and Shumway, 1982; Wieser, 1994; Brodte et al., 2006).

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The Journal of Experimental Biology (2015) 218, 2373-2381 doi:10.1242/jeb.122804

List of symbols and abbreviations

DM	dry mass
FCR	food conversion ratio
FI	total food intake
K	Fulton's condition factor
M	body mass
M_1, M_2	body mass of the fish at start and end of experiment
M_{gain}	total mass gain
RMR	routine metabolic rate
SDA	specific dynamic action
SGR	specific growth rate
SL	standard length
t_1, t_2	time at start and end of experiment
TL	total length

Antarctic and especially high-Antarctic fish generally display slow growth, small body sizes and longevity (Kock and Everson, 1998; La Mesa and Vacchi, 2001). Usually, fish do not reproduce before having reached a certain size. While this is typically around 55–80% of their maximal size in Antarctic fish, some high-Antarctic species do not reproduce before having reached at least 70% of their maximal size (Kock and Kellermann, 1991). This implies that energy expenditure is clearly partitioned between growth and reproduction. Thus, factors influencing energy allocation and thereby growth in these species are likely to have far-reaching consequences for life history.

Only a few authors have linked thermal tolerance limits determined in experiments with abundance in the field (Pörtner and Knust, 2007). Knowing that increasing temperature is likely to affect fish species production and distribution, knowledge on the effects of temperature on energy allocation and growth is essential to estimate future changes in Antarctic ecosystems.

Thus, we investigated the effect of acclimation to elevated temperature on the energy budget of the high-Antarctic fish *T. bernacchii* by measuring growth, routine metabolism, excretion and food consumption. *Trematomus bernacchii* is a commonly used model species for high-Antarctic fish and while a wealth of information is available on the thermal tolerance of this species from the molecular to the cellular level, the consequences of long-term acclimation to increased temperature for the whole animal are still unclear. Our aim was to identify these possible trade-offs for the whole organism, to assess the possible implications of global warming for high-Antarctic fish.

RESULTS

Temperatures above 1°C had a significant effect on *T. bernacchii* mortality, with 33% mortality in the experimental groups kept at 2 and 4°C compared with no mortality at 0 and 1°C (Fig. 1A). Most fish died during the first 4 weeks of the acclimation period. Fish at 2°C died on days 14, 16, 31 and 38 after the start of the acclimation period, while fish at 4°C died on days 11, 19, 22 and 28.

Both the condition factor (Fig. 1B) and liver lipid content (Fig. 1C) appeared to decrease with increasing temperature; however, this trend failed to reach statistical significance, potentially an outcome of the small sample size.

Individuals kept at 0 and 1°C showed comparable food intake, specific growth rate (SGR) and food conversion ratio (FCR) (Fig. 2, Table 1). A significantly lower food intake (ANOVA: $F_{3,36}$=4.858, P=0.006; Tukey: 0 versus 2°C, P=0.051; 1 versus 2°C, P=0.004) in combination with a significantly lower SGR for fish at 2°C (ANOVA: $F_{3,36}$=10.5, P<0.001; Tukey: 0 versus 2°C, P=0.005; 1 versus 2°C, P=0.007) resulted in a FCR close to zero with a high standard error (Fig. 2, Table 1). In contrast, fish at 4°C consumed intermediate amounts of food compared with fish kept at 0 and 1°C but showed significantly lower SGR (0 versus 4°C, P<0.001; 1 versus 4°C, P<0.001) and thus a FCR close to zero (ANOVA: $F_{3,36}$=6.037, P=0.002; Tukey: 0 versus 4°C, P<0.001; 1 versus 4°C, P=0.003; Fig. 2 and Table 1).

While growth in terms of body mass decreased with increasing temperature, growth in terms of body length was significantly higher at 1°C than at 2 and 4°C (ANOVA: $F_{3,36}$=6.418, P=0.001; Tukey: 2 versus 1°C, P=0.003; 4 versus 1°C, P=0.014; Table 1). Energy content of white muscle tissue as well as water content were comparable among fish at all temperature treatments (Table 1). Similarly, data on faecal excretion and ammonia excretion did not show any significant response to temperature (Table 1). No diurnal pattern was detected in ammonia excretion.

Costs for routine metabolism (routine metabolic rate, RMR) in *T. bernacchii* were significantly elevated after the acute temperature increase in the 4°C treatment (ANOVA: $F_{3,22}$=7.834, P=0.001; Tukey: 0 versus 4°C, P=0.003; 1 versus 4°C, P=0.01; 2 versus 4°C, P=0.002; Fig. 3; supplementary material Table S1), but after

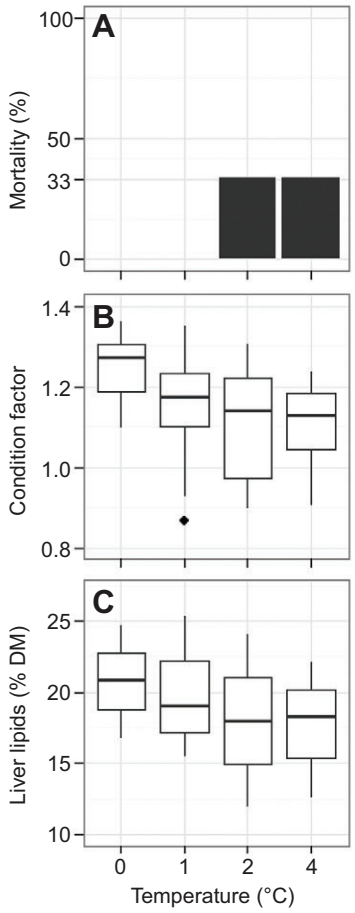

Fig. 1. Mortality and physiological condition of *Trematomus bernacchii* at different temperatures. The percentage mortality (A), condition factor (B) and liver lipid content (C) were measured in fish maintained in aquaria at 0, 1, 2 and 4°C (A and B, N=12 fish at 0 and 1°C; N=8 at 2 and 4°C; C, N=3 for all temperatures). In the box plots, the centre lines denote the medians, the upper and lower edges of the boxes the upper and lower quartiles. Values exceeding the upper or lower quartile by 1.5× interquartile range are displayed as points. DM, dry mass.

The Journal of Experimental Biology (2015) 218, 2373-2381 doi:10.1242/jeb.122804

Table 1. Condition, energy conversion and growth parameters for *Trematomus bernacchii* at different temperatures

	0°C	1°C	2°C	4°C
Mortality (%)	0 (*N*=12)	0 (*N*=12)	33.3 (*N*=12)	33.3 (*N*=12)
Condition factor	1.25±0.03 (*N*=11)	1.15±0.04	1.11±0.05	1.11±0.04
Food intake (J g^{-1} *M* day^{-1})	34.13±2.42^{a}	39.32±4.38^{a}	19.85±3.58^{b}	28.39±3.92^{a,b}
SGR (% *M* day^{-1})	0.25±0.02^{a}	0.31±0.05^{a}	0.04±0.05^{b}	0.05±0.03^{b}
Growth (% SL day^{-1})	0.022±0.006^{a,b}	0.047±0.008^{b}	0.012±0.004^{a,c}	0.019±0.004^{a,c}
FCR	0.32±0.02^{a}	0.35±0.06^{a}	–0.02±0.016^{a,b}	0.03±0.06^{b}
Energy content white muscle (J g^{-1} DM)	24,309±166 (*N*=3)	24,419±65 (*N*=3)	24,557±74 (*N*=3)	24,556±76 (*N*=3)
Water content white muscle (%)	80.93±0.27 (*N*=3)	81.25±0.35 (*N*=3)	81.34±0.14 (*N*=3)	81.34±0.30 (*N*=3)
Lipid content liver (% DM)	20.71±2.29 (*N*=3)	19.93±2.90 (*N*=3)	17.95±3.51 (*N*=3)	17.62±2.80 (*N*=3)
Faeces nitrogen (% N g^{-1} *M* g^{-1} FI day^{-1})*	0.562±0.195 (*N*=10)	0.261±0.054 (*N*=8)	1.268±0.615 (*N*=7)	0.711±0.352 (*N*=7)
NH_4 excretion (µmol g^{-1} *M* h^{-1})	0.40±0.07 (*N*=6)	0.31±0.09 (*N*=7)	0.16±0.05 (*N*=6)	0.22±0.03 (*N*=8)

Data are means±s.e.m.; number of replicates (*N*)=12 for 0 and 1°C, and *N*=8 for 2 and 4°C, if not stated otherwise. SGR, specific growth rate; SL, standard length; FCR, food conversion ratio; *M*, body mass; DM, dry mass; FI, total food intake.
*Related to mean daily food intake during experiment.
Different superscript letters denote significant differences between measurements.

acclimation, RMR decreased to a comparable level to that of groups kept at 0, 1 and 2°C (Fig. 3; supplementary material Table S2).

Energy allocation in joules per gram fish mass per day as well as in percentage of total energy intake is presented in Table 2 and Fig. 4. Energy intake in the form of food, energy lost in ammonia excretion and RMR showed no significant differences, but a significantly smaller fraction of energy was allocated to growth at 2 and 4°C compared with that at 0 and 1°C (ANOVA: $F_{3,23}$=6.872, *P*=0.002; Tukey: 0 versus 2°C, *P*=0.025; 0 versus 4°C, *P*=0.024; 1 versus 2°C, *P*=0.016; 1 versus 4°C, *P*=0.014).

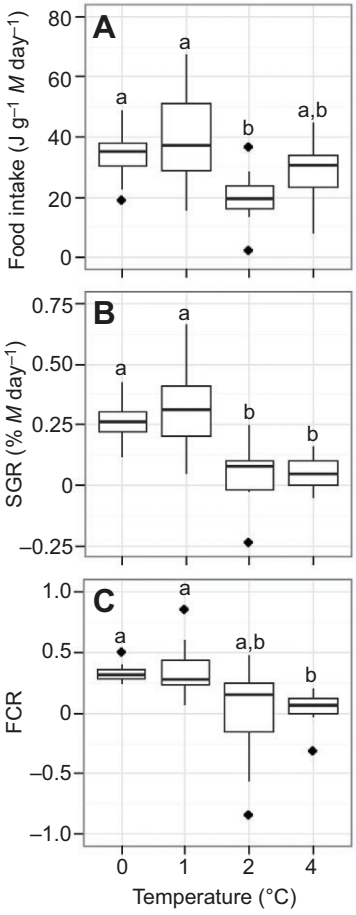

Fig. 2. Food consumption and growth of *T. bernacchii* at different temperatures. Food intake (A), specific growth rate (SGR, B) and food conversion ratio (FCR, C) were measured in fish maintained at 0, 1, 2 and 4°C (*N*=12 at 0 and 1°C, *N*=8 at 2 and 4°C). Different letters above boxes denote significant differences between temperatures.

DISCUSSION

Experimental evidence for low and high temperature tolerance at the molecular and cellular level in Antarctic fish has been vigorously discussed in the recent literature (Seebacher et al., 2005; Strobel et al., 2013; Enzor and Place, 2014). However, little is known about the ecologically relevant whole-organism level. This study attempts to close this gap by providing the first estimates for a temperature-dependent energy budget in a high-Antarctic fish species.

Mortality and physiological condition

Mortality of 33% at 2 and 4°C in a controlled laboratory environment suggests a deleterious impact of temperature in spite of *ad libitum* feeding. Moreover, the surviving fish in these high-temperature

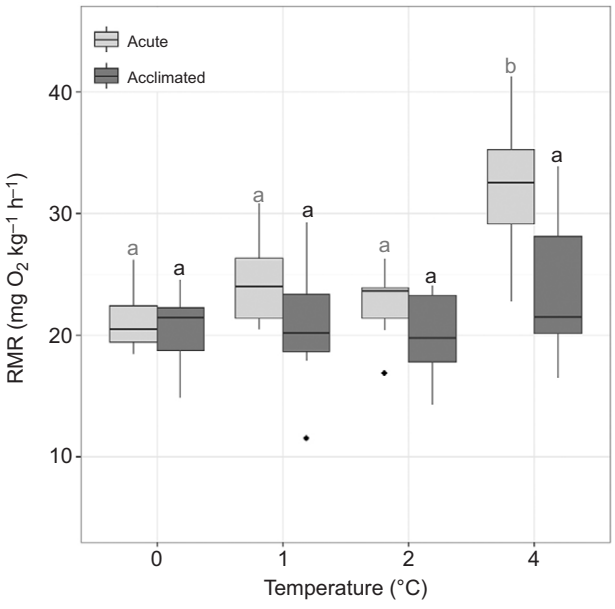

Fig. 3. Routine metabolism of *T. bernacchii* after acute temperature increase and temperature acclimation. Routine metabolic rate (RMR) was measured in fish following acute exposure to 0, 1, 2 and 4°C and after acclimation to these temperatures (acute: *N*=4 at 0°C, *N*=8 at 1 and 2°C, *N*=6 at 4°C; acclimated: *N*=7 at 0°C, *N*=8 at 1, 2 and 4°C). Different letters above boxes denote significant differences between measurements.

The Journal of Experimental Biology (2015) 218, 2373-2381 doi:10.1242/jeb.122804

Table 2. Energy budget of *T. bernacchii* at different temperatures

	0°C	1°C	2°C	4°C
N	6	7	6	8
Consumed energy	32.3±3.1	39.5±6.1	22.7±3.5	28.4±3.9
Growth	11.5±1.5^{a} (35.1±2.7%)	11.8±2.2^{a} (29.1±2.8%)	1.8±3.3^{b} (2.4±20.6%)	2.3±1.3^{b} (2.9±6.2%)
Excretion (ammonia)	4.0±0.7 (13.2±2.4%)	3.1±0.9 (9.0±2.4%)	1.8±0.5 (9.2±3.1%)	2.4±0.3 (11.0±3.8%)
RMR	6.6±0.5 (20.8±1.4%)	6.6±0.7 (20.5±5.3%)	6.7±0.5 (32.0±3.6%)	7.7±0.8 (33.7±8.5%)
Total energy expenditure	22.1±2.2^{a} (69.1±4.5%)	21.5±2.7^{a} (58.6±7.3%)	10.3±3.8^{b} (43.5±21.6%)	12.4±2.0^{a,b} (47.6±7.6%)
FCR	0.31±0.02	0.26±0.03	0.22±0.19	0.03±0.06

All energy budget parameters are given in J g^{-1} *M* day^{-1} (means±s.e.m.). Values in parentheses represent energy investment as a percentage of food energy consumed. Acclimated routine metabolic rate (RMR) was used. Only animals for which a complete energy budget was determined are included. Different superscript letters denote significant differences between measurements.

treatments showed negative, albeit insignificant, trends in condition factor and liver lipid content. The condition factor is an estimate of the overall condition of the animal, while liver lipids are an important energy store for Antarctic fish. A negative trend in these parameters could suggest a decreasing capacity for protein turnover and a mobilisation of energy stores with increasing temperature, as proposed by Huth and Place (2013).

Food consumption

The basis for energy allocation within an animal is the energy supply in the form of food. Food consumption, SGR and thus FCR were comparable for fish at 0 and 1°C. In contrast, FCRs close to zero correspond with significantly lower SGRs at 2 and 4°C. A lower food conversion efficiency implies that a larger amount of food or more energy-rich food would be needed to support the same growth performance. However, it must be emphasised that these fish were offered an unlimited amount of food, which they refused, so it is not just a simple case of food availability.

Growth

Growth performance in this experiment compares well with previous estimates of field growth for *T. bernacchii* in McMurdo Sound of about 1.25 cm per year (La Mesa et al., 1996). A higher growth rate than that observed in nature might have been expected from an experiment with excessive food availability (Fischer, 2003), although differences between provided food and natural prey items are likely to cause differences in energy supply and thus growth. Temperature had a clear effect on mass growth performance even though tissue energy content did not change significantly among treatments. This is consistent with the results of Buckley and Somero (2009), who found indicators of growth and cell cycle arrest at the molecular level in *T. bernacchii* after exposure to 4°C. A growth (mass) reduction of 80–84%, as observed at 2 and 4°C, is likely to impact life history parameters. In contrast to decreasing mass with increasing temperature, growth in terms of length was not negatively influenced by temperature. While sexual maturity is attained late in the life cycle of high-Antarctic fish, the strategy allows the juveniles to build up energy stores for adult reproduction (Hubold, 1992). *Trematomus bernacchii* was reported to spawn only when having reached 65% of its maximum length (Kock and Kellermann, 1991; La Mesa et al., 1996). At elevated temperature, decreasing mass growth could be associated with the depletion of energy stores as suggested by negative trends in liver lipid content and condition factor, as was suggested by other authors (Huth and Place, 2013), possibly affecting reproductive tissue and reproductive success.

Maximal length growth and highest SGR at 1°C suggests a growth optimum for *T. bernacchii* above 0°C in this experiment. Highest growth performance above habitat temperature has also been reported for the Antarctic eelpout (Brodte et al., 2006). However, the reasons for these findings are unclear. Optimal temperature for growth was shown to decline with fish body mass and age (Björnsson and Tryggvadóttir, 1996; Björnsson et al., 2001). As the current study used juvenile fish, a decrease of optimal growth temperature may be associated with development to the adult stage of the fish. Temperature-dependent growth rates of adult *T. bernacchii*, however, are not available yet.

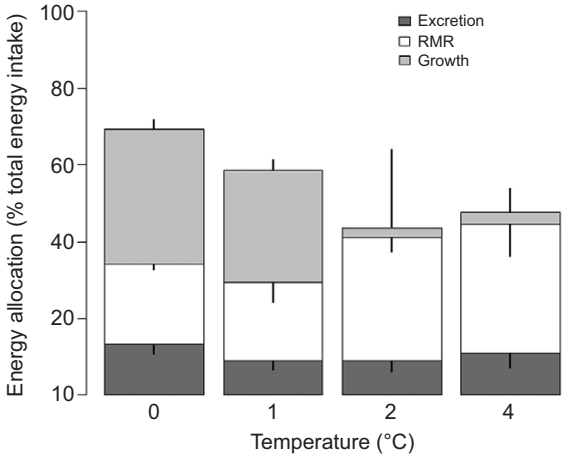

Fig. 4. Energy budget for *T. bernacchii* at different temperatures. Energy allocation to ammonia excretion, routine metabolism (RMR) and growth is given as a percentage of energy taken in as food (means±s.e.m.). Data are for fish maintained in aquaria at 0, 1, 2 and 4°C for which a complete energy budget was determined (*N*=6 at 0°C, *N*=7 at 1°C, *N*=6 at 2°C, *N*=8 at 4°C).

Routine metabolism

A RMR of 21.4±1.7 mg O_2 kg^{-1} h^{-1} measured under control conditions at 0°C is comparable with literature values for this species recorded at −1°C of 27.4±6.9 mg O_2 kg^{-1} h^{-1} (Steffensen, 2005) and 12.8 mg O_2 kg^{-1} h^{-1} (Enzor et al., 2013). Significantly elevated RMR was measured at 4°C after an acute temperature increase; however, this decreased to control levels after an acclimation period of 9 weeks. In contrast, Robinson (2008) measured the acclimation capacity of *T. bernacchii* at 4°C and reported a greatly elevated RMR on day 5 and 100% mortality on day 6 of the acclimation period. Similarly, Enzor et al. (2013) found that *T. bernacchii* did not acclimate to 4°C within 28 days. These results suggest that acclimation in *T. bernacchii* occurs between 4 and 9 weeks after exposure to higher temperatures (Podrabsky and Somero, 2006) and imply that experimental studies with this species should be carried out after acclimation periods longer than 4 weeks.

This agrees well with low acclimation capacities reported for Antarctic marine ectotherms and acclimation times of 21–36 days reported for other Antarctic fish species (Peck et al., 2014).

Excretion

Excretion is not easily measured in fish and estimates need to be treated with caution because of potential interference with toxic excretory products, leaching of faecal pellets and high dependency on consumed food rations, food type and feeding time (Cockcroft and Du Preez, 1989; Dockray et al., 1996; Wood, 2001). Animals in this experiment were fed *ad libitum*, which resulted in a large variance in rations between individual fish (due to differences in feeding activity), which is likely to have affected variance of ammonia as well as faecal excretion in turn. Published rates of ammonia excretion of Antarctic fish are scare (Boyce and Clarke, 1997; Boyce, 1999; Brodte et al., 2006). The most comparable data are those for the energy budget of the Antarctic eelpout (*Pachycara brachycephalum*), where ammonia excretion is higher than in this study, accounting for about 20% of energy expenditure (Brodte et al., 2006). Absolute ammonia excretion data measured in this experiment agree well with literature data for various marine teleosts in a fed state (Handy and Poxton, 1993). While no significant differences in ammonia excretion were detected among temperature treatments, a trend towards higher nitrogen content in faeces of fish at 2 and 4°C was observed. This supports the suggestion that a lower FCR is associated with a larger amount of food energy being excreted and not used for metabolism and tissue assimilation at warmer temperatures.

Energy budget

When presented as a percentage of energy intake (Fig. 4), it is apparent that the largest fractions of energy available to the organism are allocated to growth and routine metabolism. Routine metabolic costs include all energy-demanding processes that are necessary to keep an organism alive. Only after these basal costs have been met can energy be allocated to somatic growth or reproductive tissue.

The energy budget of this study is comparable with those reported for other teleost fish (Fang et al., 2010; Xie et al., 2011). The most similar data set is the energy budget of the Antarctic eelpout, *P. brachycephalum*, reported by Brodte et al. (2006). While routine metabolic costs usually make up about 50% of total energy expenditure in most fish (Brodte et al., 2006; Fang et al., 2010; Xie et al., 2011), the relatively small proportion of energy allocated to routine metabolism (20.8±1.4% at 0°C) in *T. bernacchii* is noteworthy. Importantly, the measured RMRs in *T. bernacchii* agree well with recent literature data (Enzor et al., 2013), making an underestimation of metabolic rate seem unlikely.

The adjustment of routine metabolic costs after long-term exposure to 4°C suggests acclimation of metabolic processes in routine metabolism, including protein turnover, ion pump activity, circulation and others (Clarke, 1980, 1993). This might be connected to compensatory responses, as, for example, for Na^+/K^+-ATPase in osmoregulatory tissue (Gonzalez-Cabrera et al., 1995; Brauer et al., 2005), compensation of oxidative damage and antioxidant responses (Enzor and Place, 2014). Similarly, very little temperature compensation in growth rates (this study) corresponds with evidence of lowered protein turnover and mobilisation of energy stores at elevated temperatures as suggested by Huth and Place (2013). Acclimation to elevated temperature resulted in a significant decrease of liver lipid content in the Antarctic eelpout (Brodte et al., 2006). For this species, a shift from lipid- to carbohydrate-based metabolism as a response to warm acclimation was suggested by Windisch et al. (2011). Similarly, we found a trend towards decreasing lipid content in liver of *T. bernacchii* with increasing temperature.

The energy budget shows that the parameters measured in this experiment do not add up to 100% of the energy taken up by the organisms (Fig. 4). This indicates that either energy intake was overestimated or parameters to which energy was allocated by the organisms were not assessed. An overestimation of energy intake is likely, as determination of energy intake is based on food consumption and energy content of food rather than on the digestible energy content of food. Regarding feeding efficiency and energy consumption, the determination of digestible energy is likely to give the most reliable information, as it only takes into account the energy that can be physiologically used by the organism. Measurements of digestible energy are usually based on experimental diets containing marker substances. However, the success of this experiment was based on growth performance and, hence, food intake of fish. As fish were found to be feeding very selectively, a natural food that was found to be accepted very well by most fish was chosen to avoid problems with feeding and insufficient energy supply to organisms. Another reason for the mismatch between energy intake and energy expenditure might be that some parameters of energy allocation are not included in this energy budget, such as a part of spontaneous activity or faecal excretion. Spontaneous activity is considered to be low in *T. bernacchii*. During the experiment, fish were typically resting on pelvic fins, as also observed during measurements of RMR. Thus, spontaneous activity was suggested to be included in routine metabolic costs. Only during feeding were fish more active and this activity could not be accounted for in any measurement, possibly contributing to the observed bias. In addition, costs of faecal excretion were not included in the energy budget. Here, the small size of faecal pellets did not allow determination of energy content and therefore were not included in energy budgets.

Ecological context

When discussing acclimation capacities at the whole-organism level, ontogenetic changes in the thermal tolerance of an organism play a crucial role. Usually, the earliest life stages are more temperature sensitive, while juveniles and growing adults can exploit the largest range in thermal habitats. In reproductively mature adults, thermal tolerance decreases again, as oxygen has to be supplied to eggs and sperm, lowering thermal capacity (Pörtner and Farrell, 2008; Pörtner and Peck, 2010; Peck et al., 2013). Because of the size and maturation stages of *T. bernacchii* used in this experiment, animals can be considered to be juveniles. Consequently, our results are most likely to be conservative, overestimating the thermal capacities of populations of this species.

Generally, a high energy demand due to a lower conversion efficiency could be compensated for by consumption of more energy-rich food in nature. The main food source of *T. bernacchii* in the western McMurdo Sound is the Antarctic scallop *Adamussium colbecki* (La Mesa et al., 2004), although in eastern McMurdo Sound (the source of our fish), there are few *A. colbecki* and the fish's diet consists of other invertebrates (Foster and Montgomery, 1993; Kiest, 1993). However, this scallop was reported to be extremely temperature sensitive and unable to acclimate to 4°C (Bailey et al., 2005). While little information is known about alternative food choices in Antarctic fish (Montgomery et al., 1993),

The Journal of Experimental Biology (2015) 218, 2373-2381 doi:10.1242/jeb.122804

changes in prey composition could occur in the future and further influence energy budgets of the fish.

Increasing sea temperatures will almost certainly affect the physiology of fish, causing changes in production as well as shifts in abundance and distribution (Pörtner and Peck, 2010; Cheung et al., 2013). Generally, fish communities are suggested to migrate to colder regions and higher latitudes (Perry et al., 2005; Dulvy et al., 2008). Such distribution shifts could imply that sub-Antarctic species might intrude into high-Antarctic waters, thereby increasing competition for endemic Antarctic species. This would be an additional challenge for Antarctic fish species, possibly within a warming Southern Ocean.

When it comes to resource competition, the capacity to adapt to achieve more efficient food conversion and growth at higher temperatures might be of similar importance to, for example, the ability to adjust to alternative food sources. Comparative energy budget studies offer valuable insight into possible advantages and disadvantages for individual species in a changing physical and ecological framework. The importance of such studies is apparent considering that processes within Antarctic ecosystems including predator–prey relationships, inter-species interactions and competition are poorly understood and are likely to become even more variable and difficult to predict within a changing Southern Ocean.

Conclusions

Even though some studies indicate a compensatory capacity for increased temperature at the molecular to organism level in Antarctic fish (Franklin et al., 2007; Strobel et al., 2013; Enzor and Place, 2014), negative trade-offs on the whole-organism level were found in this study, indicating an overall insufficient compensation. While we found complete adjustment of routine metabolism to increased temperature, growth performance declined by up to 84% after long-term acclimation to 2 and 4°C in *T. bernacchii*. *Trematomus bernacchii* belongs to the family Nototheniidae, which makes up a large part of the biomass in coastal ecosystems, such as the Ross Sea (Donnelly et al., 2004). An 84% reduction in growth of *T. bernacchii* would result in a decrease of production of a similar magnitude for this species in the Ross Sea (using growth estimates of Hureau, 1970). As a consequence, a temperature increase of 0.8–1.4°C as predicted for the Ross Sea region by 2200 (Timmermann and Hellmer, 2013) could potentially cause large decreases in production and changes in the fish community, with possible implications for the whole ecosystem.

While these findings have important implications for polar fish responses to warming, it will be important to consider long-term adaptations over life cycles and associated tolerance shifts across generations, which could mitigate some of the outcomes of warming oceans (Suckling et al., 2015).

MATERIALS AND METHODS

All work was carried out under the University of Canterbury, New Zealand, animal ethics approval 2011/08R. Fish were collected in accordance with the Antarctic Marine Living Resources Act 1981 (permit no.: AMLR13/R03/Lamare/K068).

Animals

Specimens of *T. bernacchii* were collected in the Ross Sea, Antarctica, at different shallow sites around McMurdo Sound in October and November 2013 by SCUBA diving as well as by fishing with lines and baited barbless hooks. Only animals <20 cm total length were collected to avoid any influence of different states of sexual maturity. After capture, animals were transported in insulated containers to Scott Base (New Zealand Antarctic Programme), where they were kept in flow-through aquaria at −0.5 to +0.5°C until transport by air to the University of Canterbury's aquarium facilities in New Zealand. Subsequently, fish were held in a cooled seawater system at temperatures between 0 and 0.5°C until the start of experiments.

Growth

For the somatic growth experiments, groups of 12 fish were held in four separate aquarium systems at 0, 1, 2 and 4°C. All aquaria were closed systems, in which water parameters were monitored and water was exchanged regularly to maintain water quality. Fish were kept separately in individual cages to allow monitoring of food consumption and faecal excretion. When placed together, fish were observed to show aggressive interaction, affecting stress levels and possibly growth, which was avoided by separation. Cages allowed good water circulation and were not observed to restrict movement of fish.

Before the start of the temperature acclimation, body mass, total length and standard length of each fish were recorded. For all fish, standard length varied from 6.9 to 15.1 cm, total length from 7.9 to 17.0 cm and body mass from 4.3 to 58.7 g, with no significant difference among the different temperature groups (ANOVA, $P>0.05$). For the measuring procedure, fish were anaesthetised with tricaine methane-sulphonate (MS-222, 55 mg l^{-1}) for several minutes. Subsequently, individuals were allowed to recover from the measuring procedure for at least 24 h before the start of the experiment. For temperature incubations, the aquarium systems were heated at a rate of 1 K per 12 h until respective temperatures were reached. After this acute temperature increase, the first set of respiration measurements was obtained.

A 24 h light regime was maintained for the duration of the experiment, to simulate summer light conditions in McMurdo Sound. At the end of the experiment, a second set of respiration measurements was carried out. Fish were then anaesthetised with MS-222 and killed by a cut through the spine. Mass and length data were recorded. Tissue samples of all animals were collected and stored at −80°C until analysis.

Food consumption

During the acclimation period, fish were fed every second day individually *ad libitum* with small pieces of monkfish fillet (*Kathetostoma giganteum*). Amounts of daily food rations as well as left-overs collected from the cages after feeding were recorded. Left-overs and non-fed food were oven dried for 24 h at 55°C to determine dry mass. Control samples to determine wet mass to dry mass conversion factors were determined regularly, to allow calculation of consumed food.

Respiration

The experimental setup was composed of nine acrylic respiration chambers of about 1.8 l volume submerged in tanks at the respective temperature treatment, allowing simultaneous measurement of eight fish and a blank control. Measurements were performed using automated intermittent-closed box respirometry. An aquarium pump ensured a constant mixing and circulating water flow within the respirometer, while water exchange of the respirometer and the ambient water was controlled by a flush-pump. During measuring periods, the water exchange between the chamber and the ambient water was interrupted and water was circulated within the chamber. At the end of the measuring period, the flush-pump replenished oxygen saturation in the chamber to 100%. Oxygen concentration in the chamber was measured using optical oxygen probes and a 10-channel oxygen meter (PreSens-Precision Sensing GmbH, Hamburg, Germany). Before each experimental run, oxygen probes were calibrated against a sodium sulphite–seawater solution (20 mg l^{-1}) and fully aerated water from the respective aquarium system. Intervals of flush and measuring periods were adjusted to each fish's oxygen consumption, so that the oxygen saturation in the chamber never fell below 85%. For calculation of oxygen consumption rates, the volume of the fish was subtracted from the volume of the respirometer.

Before transfer to the respiration chambers, fish were fed individually *ad libitum* with monkfish fillet. Measurements were conducted with fed fish to include metabolic elevation due to specific dynamic action (SDA) at a similar degree to that during the growth experiment. Moreover,

measurements included spontaneous activity, although this is typically low in *T. bernacchii*. Therefore, measured metabolic rate was assumed to resemble metabolic costs during the experiment most accurately. Because of time limitations it was not possible to determine SDA in this experiment. Besides, metabolic elevation due to SDA was observed to take a minimum of 72 h in *T. bernacchii* (W.D., unpublished), and starving the fish for such a long time would potentially stunt growth. Metabolic rates including SDA and spontaneous activity are referred to as RMR in this study.

After transfer to the respiration chamber, fish were allowed to recover within the chamber for 24 h, followed by another 24 h measuring period. Means of the 24 h measuring period were used for RMR calculations. Measurement of oxygen consumption was conducted on eight fish per treatment after the acute temperature increase at the beginning of the experiment as well as after 59–70 days of acclimation at the end of the experiment. For the first set of respiration experiments at the beginning of the growth experiment, fish were fed and transferred to the respiration chambers immediately after target temperatures for the respective groups were reached, and transferred back to the cages in the holding system after the end of the measurement.

Ammonia excretion

Sampling for ammonia excretion measurements was combined with the second set of respirometry at the end of the experiment. Samples were taken during oxygen consumption measurements from the respiration chamber after the fish were acclimated to the respirometers. Thus, eight individuals were sampled per treatment. For each individual, a water sample was drawn from the respiration chamber at the end of the flushing period to attain an initial sample. After flushing, the respiration chamber was closed for the respiration measurement, thus no water exchange happened during this time. The circulation pump of the respirometry setup ensured mixing of the water within the chamber. The second water sample was taken just before the next flushing period started. Ammonia excretion was determined from the difference between the two samples. To control for a diurnal rhythm in excretion, excretion of the fish in the 0°C temperature treatment (the control), was sampled three times per day at 09:00 h, 14:00 h and 19:00 h. Fish in temperature treatments at 1, 2 and 4°C were only sampled at 14:00 h. Water samples were stored at −20°C until analysed for ammonia concentration following Holmes et al. (1999, protocol B).

Faecal excretion

Towards the end of the acclimation period, faeces were collected from all fish. For this purpose, cages were cleaned at the starting time and subsequently checked regularly for produced faeces. Faeces were collected by siphoning into a beaker and filtering onto pre-weighed, organic-free glass fibre filters together with a volume of 100 ml water from the sampling beaker. To account for particulates in the water, a sample from the collected water was filtered as a blank. Filters were stored at −20°C until analysis. For analysis, filters were oven dried at 57°C and dry mass was determined. Faecal quantities were determined gravimetrically and analysed for carbon (C) and nitrogen (N) using a Euro EA Elemental Analyser (Hekatech GmbH, Wegberg, Germany). Sizes of faecal pellets were not sufficient for calorimetric analysis.

Tissue sample analysis

For determination of lipid and energy content as well as CN composition, tissue sample dry mass was determined after lyophilisation for 48 h. Lipid content was determined for liver tissue of *T. bernacchii* and monkfish food. Lipids were extracted using dichloromethane:methanol (2:1 by volume). Lipid mass was determined gravimetrically according to Folch et al. (1957) adapted according to Friedrich and Hagen (1994). CN content was determined for muscle tissue of *T. bernacchii* and monkfish fillet using the Euro EA Elemental Analyser. For energy content determination of muscle tissue of *T. bernacchii* and monkfish fillet, samples were homogenised by a ball mill, re-dried for 12 h at 60°C and analysed by an IKA C2000 bomb calorimeter (IKA GmbH & Co KG, Staufen, Germany).

Stoichiometric analysis

Fulton's condition factor (K) was calculated according to Ricker (1975):

$$K = \frac{M}{\mathrm{TL}^3} \times 100, \quad (1)$$

where M is body mass (g wet mass) and TL is total length (cm).

FCR was calculated as:

$$\mathrm{FCR} = \frac{M_{\mathrm{gain}}}{\mathrm{FI}}. \quad (2)$$

where M_{gain} is total mass gain (g wet mass) and FI is total food intake (g wet mass).

Specific growth rate (SGR) was calculated as per cent per day according to Eqn 3:

$$\mathrm{SGR} = 100 \times \frac{\ln(M_2) - \ln(M_1)}{t_2 - t_1}, \quad (3)$$

where M_1 and M_2 are body mass of the fish at times t_1 and t_2 (g wet mass), respectively, and t_1 and t_2 are the start and end times of the experiment (days), respectively.

For energy budget calculations, the conversion factor of 5.94 cal mg^{-1} to convert ammonia nitrogen into energy units according to Elliott and Davison (1975) was used. Analysis of the food showed a composition of 88.7% protein, 9.8% carbohydrate and 1.5% lipid and an energy content of 24.4 kJ g^{-1} dry mass of monkfish fillet. For the conversion of rate of oxygen consumption to the rate of heat production, an oxycaloric coefficient of 13.53 J mg^{-1} was calculated using conversion factors by Elliott and Davison (1975).

Statistical analysis

All data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Bartlett test). When these criteria were met, a one-way ANOVA and Tukey's *post hoc* test ($P \leq 0.5$ significance threshold) was performed. When criteria were not met, the max-t method accounting for heteroscedasticity in unbalanced designs was used (Hothorn et al., 2008; Herberich et al., 2010). Statistical analysis was performed using R statistical language (R Core Team, 2014; version 2.1.51).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

R.K. and T.S. conceived the experiments. M.L., W.D. and C.R. provided logistical support. M.L. and T.S. collected experimental animals. T.S. designed and implemented the experiments. W.D. aided in experiment implementation. T.S. prepared the manuscript and figures. R.K., M.L., W.D., C.R. and T.S. revised the manuscript.

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Supplementary material

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Table S1. Routine metabolic rates of *T. bernacchii* after acute temperature increase

Fish no.	Temperature [°C]	RMR [mg O_2 kg^{-1} h^{-1}]
1	0	19.72
2	0	21.17
3	0	26.17
8	0	18.44
13	2	21.72
14	2	23.58
15	2	23.61
16	2	24.90
17	2	20.34
18	2	16.87
19	2	26.28
20	2	23.59
25	4	22.72
26	4	36.10
27	4	32.81
30	4	41.22
31	4	32.18
32	4	28.14
37	1	24.81
38	1	30.82
39	1	26.26
40	1	26.56
41	1	21.67
42	1	23.15
43	1	20.42
44	1	20.70

Table S2. Routine metabolic rates of *T. bernacchii* after acclimation to increased temperature

Fish no.	Temperature [°C]	RMR [mg O_2 kg^{-1} h^{-1}]	Acclimation time [d]
1	0	23.02	70
3	0	17.18	70
4	0	14.84	65
5	0	21.45	67
6	0	21.42	67
7	0	20.21	67
8	0	24.56	65
13	2	24.02	65
14	2	14.55	65
15	2	18.90	65
16	2	20.12	63
17	2	23.17	65
20	2	19.37	63
21	2	14.24	59
23	2	23.66	59
25	4	16.47	69
26	4	33.86	69
28	4	32.41	69
30	4	21.27	63
32	4	16.78	65
33	4	21.33	65
35	4	21.66	65
36	4	26.66	65
37	1	20.84	62
38	1	18.90	62
39	1	11.55	62
40	1	23.88	62
41	1	29.27	63
42	1	19.50	63
43	1	17.88	63
44	1	23.14	63