Sensitivity of Antarctic fish to ocean warming – an energy budget approach

Tina Sandersfeld

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

Universität Bremen

This thesis was supported by a Ph.D. Scholarship of the
Helmholtz Graduate School for Polar and Marine Research

The cover picture was kindly granted by T. Lundälv, UGTO and modified by E. Wurz.

Sensitivity of Antarctic fish to ocean warming – an energy budget approach

Der Einfluss von Ozeanerwärmung auf die Energiebilanz antarktischer Fische

Dissertation
zur Erlangung des akademischen Grades
- Dr. rer. nat. -

dem Fachbereich 2. Biologie/ Chemie
der Universität Bremen
vorgelegt von

Tina Sandersfeld
M. Sc. Biologie

Bremen, August 2015

1. **Gutachter: Prof. Dr. Claudio Richter**
   Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung
   Sektion Bentho-Pelagische Prozesse
   Am Handelshafen 12, 27570 Bremerhaven
   und Universität Bremen

2. **Gutachter: Prof. Dr. Myron Peck**
   Universität Hamburg
   Institut für Hydrobiologie und Fischereiwissenschaften
   Olbersweg 24, 22767 Hamburg

Contents

Summary

Like the Arctic, the Antarctic region hosts some of the hot spots of climatic change. At the western Antarctic Peninsula, alterations of air and water temperature, pH, salinity and sea-ice regime were reported and associated shifts in species abundance and changes in food web structure have already become evident. In contrast, for most high-Antarctic regions, no climate related changes have yet been found. However, future temperature increases are also projected for these areas. Ocean warming affects marine ectotherms by directly impacting their body temperature and thus physiology. Antarctic marine ectotherms, such as fish, are highly adjusted to the very cold and stable conditions of the Southern Ocean and are suggested to be highly temperature sensitive. Fish constitute an important link in Antarctic food webs by being prey and predator alike. While various studies focused on the impact of elevated temperature on lower organisational levels in Antarctic fish, trade-offs of increased temperature for the whole organism remain unclear, but are highly relevant from an ecological perspective.

Thus, this thesis aimed to assess the impact of increasing temperature on Antarctic fish at the whole-organism level from an energy budget perspective. The energy taken up by an organism can be allocated to different vital functions, such as routine metabolism, growth, reproduction and excretion. When routine metabolic costs are covered, energy can be allocated to growth and reproduction, the factors influencing a species abundance and population structure. In the first study of this thesis, energy allocation to routine metabolism as well as response patterns to an acute increase of temperature in the fish species *Lepidonotothen squamifrons, Trematomus hansoni* and *Lepidonotothen nudifrons* were analysed using oxygen consumption measurements. While metabolic responses to changing temperature were comparable in all species, metabolic costs of high-Antarctic fish were higher at habitat temperatures. Starting from higher metabolic rates at habitat temperature, it was hypothesised that high-Antarctic species might achieve critical thermal thresholds much earlier than low-latitude species when temperature increases. In the second study, temperature-dependent trade-offs at the whole-organism-level in Antarctic fish were analysed measuring different energy budget parameters. The results indicated a lower thermal tolerance of the high-Antarctic *Trematomus bernacchii* compared to the low-Antarctic *Lepidonotothen nudifrons*. After nine weeks of acclimation to elevated temperatures (4°C), routine metabolic rates of *T. bernacchii* returned to baseline levels (0°C). However, mass growth was reduced by 84% at 2°C, likely due to less efficient food assimilation. In nature, such severe reductions

in fish growth could delay sexual maturity and reduce production. In the third study, temperature-dependent growth rates of fish species from different latitudes were assessed. Polar and especially Antarctic species showed low growth and a narrow thermal tolerance window for growth performance compared to temperate species. A further climate induced reduction of already low growth rates could significantly affect population structures and abundances of polar fish.

In conclusion, this thesis indicates differences in energy allocation, such as potentially higher routine metabolism, among low- and high-Antarctic fish. These could contribute to a high thermal sensitivity of high-Antarctic species. On the whole-organism level, this thermal sensitivity was displayed by significant reductions of already low growth rates at elevated temperatures. Finally, these results suggest that ocean warming may have far-reaching consequences for Antarctic fish production and population structures with potential extensive implications for entire Antarctic ecosystems and food webs.

Zusammenfassung

Die Polarregionen gehören zu den sich am schnellsten erwärmenden Gebieten der Erde. An der antarktischen Halbinsel sind bereits Veränderungen der Luft- und Wassertemperaturen, des pH-Wertes, der Salinität sowie des Meereisregimes erkennbar, die mit Verschiebungen von Verbreitungsgrenzen von Arten und Veränderungen des Nahrungsnetzes einhergehen. Im Gegensatz dazu ist das Klima in den hochantarktischen Regionen bisher stabil. Modelle prognostizieren jedoch auch für diese Regionen zukünftig Temperaturanstiege. Die Erwärmung der Ozeane beeinflusst die Physiologie mariner ektothermer Organismen direkt durch Veränderungen ihrer Körpertemperatur. Als ektotherme Organismen sind antarktische Fische hoch angepasst an die kalten und stabilen Temperaturbedingungen des Südpolarmeeres und somit vermutlich sehr empfindlich gegenüber Temperaturveränderungen. Fische sind selbst Jäger und Beute zugleich und stellen somit eine wichtige Verbindung zwischen verschiedenen Trophiestufen dar. Während der Einfluss von Temperatur auf molekulare und zelluläre Ebenen in antarktischen Fischen häufig untersucht wurde, sind die Auswirkungen von steigenden Temperaturen auf das Ganztier unklar. Besonders die Ganztierebene ist jedoch aus ökologischer Sicht höchst relevant.

Der Fokus dieser Arbeit liegt daher auf der Untersuchung des Einflusses steigender Temperaturen auf die Ganztierebene antarktischer Fische, unter dem Aspekt der Energieallokation. Energie, die ein Organismus mit der Nahrung aufnimmt kann für verschiedene Funktionen, wie zum Beispiel für den Grundstoffwechsel, Wachstum und Fortpflanzung verwendet oder ausgeschieden werden. Erst, wenn ausreichend Energie für den Grundstoffwechsel vorhanden ist, kann in Funktionen wie Wachstum und Reproduktion investiert werden, die die Abundanz und Populationsstruktur einer Art beeinflussen.

Im ersten Teil dieser Arbeit wurde der Routinestoffwechsel der antarktischen Fischarten *Lepidonotothen squamifrons, Trematomus hansoni* und *Lepidonotothen nudifrons* bei Habitattemperatur und bei akuter Temperaturerhöhung mit Hilfe von Sauerstoffverbrauchsmessungen bestimmt. Hierbei zeigten alle Arten vergleichbare Stoffwechselreaktionen auf Temperaturerhöhung, jedoch wurden Unterschiede im Routinestoffwechsel bei Habitattemperatur deutlich. Durch bereits erhöhte Stoffwechselraten bei Habitattemperatur könnten hochantarktische Fische bei Erwärmung schneller kritische physiologische Zustände (kritische Temperaturen) erreichen als Arten nördlicherer antarktischer Regionen. Nachfolgende Experimente zur Energieallokation in *Lepidonotothen nudifrons* aus der nördlichen und *Trematomus bernacchii* aus der südlichen Antarktis

bestätigten eine geringere Temperaturtoleranz der hochantarktischen Art. Bei *T. bernacchii* führte bereits eine Temperaturerhöhung auf 2°C zu Wachstumseinbußen von 84%. Dies wurde vermutlich u.a. durch eine weniger effiziente Nahrungsverwertung verursacht. In der Natur könnte eine solch signifikante Reduktion des Wachstums das Erreichen der Reproduktionsreife verzögern und die Produktion beeinträchtigen. Eine anschließende Metaanalyse von temperaturabhängigen Wachstumsdaten aus der Literatur zeigte ein generell geringeres Wachstum, wie auch ein schmaleres Temperaturtoleranzfenster für polare Fischarten, im Vergleich zu Arten aus gemäßigten Breiten. Eine klimabedingte Temperaturerhöhung könnte die bereits geringen Wachstumsraten polarer Fische zusätzlich verringern und weitreichende Folgen für Populationsstrukturen und das Vorkommen einzelner Arten haben.

Zusammengefasst deuten die Ergebnisse dieser Arbeit auf Unterschiede in der Energieallokation, wie zum Beispiel im Grundstoffwechsel, zwischen Fischen aus nördlichen und südlichen antarktischen Breiten hin. Diese Unterschiede könnten zu einer geringeren Temperaturtoleranz hochantarktischer Fische beitragen. Bei zunehmenden Temperaturen wird die geringere Temperaturtoleranz hochantarktischer Fische besonders durch zusätzliche Reduktion der bereits geringen Wachstumsrate deutlich. Die Ergebnisse dieser Arbeit lassen darauf schließen, dass durch steigende Wassertemperaturen Produktionsraten, Populationsstrukturen wie auch die Verbreitung antarktischer Fische sehr stark beeinträchtigt werden könnten, mit potenziell weitreichenden Folgen für antarktische Nahrungsnetze und Ökosysteme.

List of Figures

Manuscript IV

Discussion

List of Tables

List of Abbreviations

ANOVA	analysis of variance
ATP	adenosine triphosphate
CS	citrate synthase
CT_{max}	critical thermal maximum
DM	dry mass
FCR	food conversion ratio
FI	total food intake
HOAD	3-hydroxyacyl CoA dehydrogenase
K	Fulton's condition factor
LDH	lactate dehydrogenase
M	body mass
M_1, M_2	body mass of the fish at start and end of experiment
MCA	metabolic cold adaptation
M_{gain}	total mass gain
n	number of replicates
OCLTT	oxygen- and capacity-limited thermal tolerance
p	significance level
Q_{10}	temperature coefficient
RMR	routine metabolic rate
RV	research vessel
SDA	specific dynamic action
SGR	specific growth rate
SL	standard length
t_1, t_2	start and end of experiment
TL	total length

1. Introduction

The Antarctic region is one of the most remote and pristine regions on earth. It is surrounded by the Southern Ocean, which is inhabited by various unique marine species. Displaying fascinating adaptation to their extreme habitat, the Antarctic fish fauna shows a high degree of endemism (Kock, 1992; Eastman, 1993). While the Southern Ocean ecosystems are facing various challenges, such as the increase of human activities and illegal fishing, this thesis will focus on the impact of increasing temperature on Antarctic fish species, with respect to potential impacts of anthropogenic climate change.

1.1. The Antarctic environment and climate change

Antarctica is a geographically isolated place, by distances to neighbouring continents as well as by topography. The Southern Ocean encircles the continent, enclosing the waters from the Antarctic continental borders to the Polar Front. The eastward-flowing Antarctic Circumpolar Current is the most prominent circulation feature of the Southern Ocean and connects the Pacific, Indian and Atlantic Ocean. Part of the Antarctic Circumpolar Current is the Polar Front, which is characterised by an abrupt change in surface water temperature of 2 to 3°C to over 1000 m depth (Kock, 1992; Clarke et al., 2005). Forming a barrier to north-south water exchange, the Antarctic Polar Front represents a biogeographic border and promotes the presence of endemic species in Antarctic marine invertebrates and fish fauna (Kock, 1992; Arntz et al., 1997). The Southern Ocean is made up of a system of deep basins of 3000 to 5000 m depth, connected by oceanic ridges, which form the only shallow areas besides the shelf (Eastman, 1993). Global continental shelves host most productive fish grounds. While being broad and shallow (<130 m) in most parts of the world, Antarctic shelves are considered to be deep (~500 m) and also narrow, because large proportions are covered by ice shelves (Anderson, 1991; Eastman, 1993).

In the following, the area covered by ice almost throughout the year, adjacent to the Antarctic continent, will be referred to as high-Antarctic region (Kock, 1992; Hunt et al., 2003). In contrast, the term low-Antarctic will describe the seasonal pack-ice zone and adjoining northern Antarctic waters (cf. Kock, 1992; Barnes et al., 2006).

In the Antarctic regions, light conditions vary strongly within the seasons. In high-Antarctic areas, 24 hours daylight in summer contrast nearly complete darkness in the austral winter. Coupled to this is a very seasonal primary production with a short, but dense bloom starting in

mid-December that is followed by constant low production in the water column for the rest of the year (Clarke, 1988). In contrast, in the benthic realm, food availability is constant throughout the year (DeVries and Eastman, 1981; Kock, 1992).

Water temperatures below 0°C are encountered in large parts of the Southern Ocean, characterising it as one of the coldest and most stable marine habitats. In high-Antarctic areas, sea water temperature shows little seasonal variation of -1.9 to -0.35°C throughout the year, while in northern areas, such as South Georgia, seasonal temperatures vary by up to 5°C (Fig. 1; Hunt et al., 2003; Barnes et al., 2006).

However, Antarctica does host some of the most rapidly warming regions today. While global sea surface temperatures have risen around 0.1°C per decade since 1971 (Hoegh-Guldberg et al., 2014), surface water temperatures in some Antarctic areas like Potter Cove (King George Island, South Shetland Islands) have increased by up to 0.36°C per decade (Schloss et al., 2012). Surface waters around South Georgia have risen in temperature by 2.3°C within the last 81 years (Whitehouse et al., 2008) and water temperature increases of more than 1°C have been reported for the Western Antarctic Peninsula since the 1950s (Meredith and King, 2005). Reductions in sea ice habitat (Stammerjohn et al., 2008) have caused various changes in ecosystems and food webs at the Western Antarctic Peninsula, such as shifts in phytoplankton species (Moline et al., 2004), decreases in krill and increases in salp abundance (Atkinson et al., 2004), shifts in abundance and reductions of Adélie, gentoo and chinstrap penguins (Forcada et al., 2006; Trivelpiece et al., 2011; Turner et al., 2014) as well as shifts in seal populations (Costa et al., 2010). Moreover, the appearance of invasive species, such as king crabs in the Palmer Deep (Smith et al., 2011), is suggested to be connected to elevated temperatures. Additionally, warming is suggested to increase the frequency of ice scouring, with negative effects on benthic organisms (Smale et al., 2008).

In contrast to already evident changes in some northern Antarctic regions, changes in water temperature have not yet been recorded for high-Antarctic areas. Overall, Antarctic sea ice extend was reported to increase since 1979, while causes of this development are unclear (Parkinson and Cavalieri, 2012; Fan et al., 2014; Gagné et al., 2015).

Recent models suggest a temperature increase of 0.6 to 0.9°C in the Southern Ocean until the year 2200, with high regional variations (Timmermann and Hellmer, 2013). Around the Western Antarctic Peninsula, warming is suggested to not exceed 2°C until 2100 (Timmermann and Hellmer, 2013; R. Timmermann personal communication 2015), while

scenarios for high-Antarctic seas vary widely. The Western Ross Shelf is predicted to experience minor warming of 0 to 0.4°C until 2100 (Timmermann and Hellmer, 2013). In contrast, high-Antarctic areas around the Filchner Trough in the Weddell Sea might warm by up to 2°C in the same period (Hellmer et al., 2012).

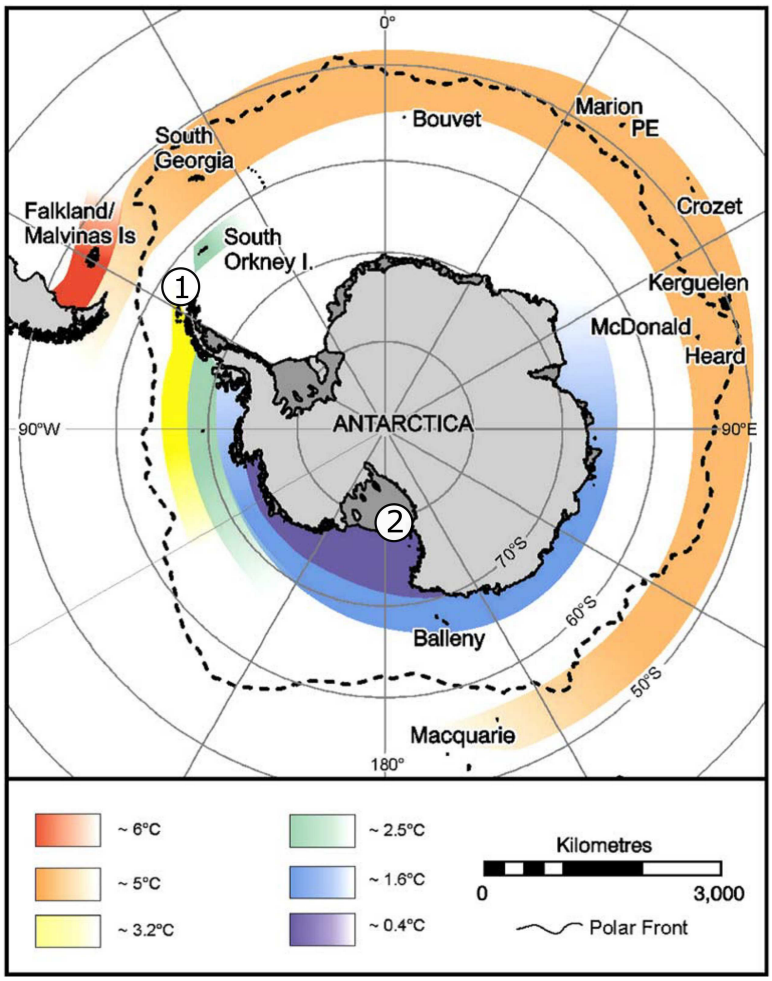

Fig. 1 Sea water temperature variation declines from low to high latitudes within Antarctic waters in 10-20 m depth (Barnes et al., 2006). Encircled numbers denote catch locations of model organisms *Lepidonotothen nudifrons* (1) and *Trematomus bernacchii* (2) as outlined in section 1.6.

1.2. The Antarctic fish fauna

The fish fauna shapes Antarctic coastal ecosystems by playing an important role in food webs. Adult life stages display various combinations of benthos, fish and plankton feeding, with shifts in diet composition being associated with ontogeny, season and local abundances of prey. Bivalves, amphipods, isopods and other crustaceans as well as polychaetes, algae and other small fish are used as food sources (Gon and Heemstra, 1990). Feeding strategies from bottom and ambush feeding, bottom slurping and grazing to water column feeding can be found (Daniels, 1982). However, ambush feeding is likely to be the most prevalent one.

Different species were observed to be ‘sit and wait predators’, waiting motionless on the ground for prey organisms to approach (Daniels, 1982; Hubold, 1992). In turn, fish are preyed upon by various predators, such as seals, seabirds, whales, squid as well as by larger fish species (Kock, 1992). Thus, they constitute an important link between lower and higher trophic levels in Antarctic food webs (Hureau, 1994; Kock et al., 2012).

The pelagic fish fauna in the Southern Ocean is poorer in diversity and density than the benthic fish fauna, which is widely distributed on the shelves and upper slopes (Kock, 1992). This coastal fish fauna is dominated by the endemic perciform suborder Notothenioidei, in terms of numbers as well as biomass. The notothenioids radiated into eight families, the Bovichtidae, Pseudaphritidae, Eleginopsidae, Nototheniidae, Harpagiferidae, Artedraconidae, Bathydraconidae and Channichthyidae (Eastman and Eakin, 2000), making up for 91 to 98% of species sampled by trawling in coastal regions (Eastman, 1993; Mintenbeck et al., 2012). Most notothenioids display a primarily benthic lifestyle, and thus lack a swim bladder. Only few species have adapted secondarily to the pelagic habitat (Kock, 1992; Eastman, 1993).

1.3. Adaptation to the Antarctic environment

Regarding the Antarctic fish fauna, the Nototheniidae are the most and best studied group. In recent years, they allowed researchers to gain a glimpse on a range of adaptations to one of the coldest and most stable marine environments on earth.

Multiple processes are suggested to be slower in Antarctic fish, compared to temperate species. Antarctic fish generally reach a high age, show comparatively slow growth, small body size and reach sexual maturity late in their life cycle (DeVries and Eastman, 1981; Kock and Kellermann, 1991; Kock and Everson, 1998; La Mesa and Vacchi, 2001). However, while these traits can display eco-physiological adaptations, it is unclear whether they are caused by genetic or ecological factors.

Generally, polar fish are suggested to have higher metabolic rates compared to temperate and tropical fish when extrapolated to the same temperatures, as hypothesised by the theory of metabolic cold adaptation (MCA) (Scholander et al., 1953; Wohlschlag, 1960). Since its introduction, the MCA concept has been intensely debated. Today, it is widely agreed upon MCA at the enzyme level (Crockett and Sidell, 1990; Kawall et al., 2002; White et al., 2012; Magnoni et al., 2013), while the existence of metabolic cold adaptation at the whole-organism

level is still controversially discussed (Holeton, 1974; Clarke and Johnston, 1999; Jordan et al., 2001; White et al., 2012).

Low temperature associated with ice coverage is one of the biggest challenges of the Southern Ocean environment, requiring resistance to freezing. Sea water freezes at temperatures below -1.86°C, due to its high ion concentration. The ionic content of blood in marine teleosts is typically one third of that of sea water, resulting in a freezing point of -0.8°C (Kock, 1992). Having adapted to their extreme habitat, Antarctic fish possess antifreeze proteins that prohibit the build-up of ice and contributes to a temperature tolerance down to -2°C (DeVries, 1971). Special antifreeze glycoproteins bind to ice crystals forming in or entering the organism and depress the growth of ice by causing a temperature difference between the melting and freezing point of ice (hysteresis) (Celik et al., 2013).

Moreover, an increased mitochondrial density was found in Antarctic fish red muscle, which is suggested to enhance the aerobic capacity in the cold (Archer and Johnston, 1991; Johnston et al., 1998; O'Brien et al., 2003). Besides, unusually large fibre diameters, but small fibre numbers have been reported for some notothenioids (Battram and Johnston, 1991; Johnston et al., 2003). Other adaptations at the molecular level include membranes that contain high proportions of unsaturated fatty acids to maintain a fluid state at low temperatures (Morris and Schneider, 1969; Macdonald and Wells, 1991) as well as tubulins of Antarctic fish cytoskeletons that polymerise into microtubule at much lower temperatures than commonly found in other organisms (Detrich III, 1991). Furthermore, high lipid contents in Antarctic fish tissue serve as an important energy store and contribute to buoyancy in some species (Eastman and DeVries, 1981; Sidell et al., 1995).

The low temperatures of the Southern Ocean not only increase the viscosity of sea water, but also of other fluids such as blood. Higher viscosity increases the work of the heart and circulatory system by affecting vascular resistance (Macdonald and Wells, 1991). Lower haematocrit, i.e. a lower erythrocyte fraction, measured in different Antarctic species is thought to mitigate the effect of increased viscosity due to low temperature (Eastman, 1993; Egginton, 1996). Besides, the lack of the oxygen-binding protein haemoglobin, in all species, and myoglobin, in some species, of the notothenioid family Channichthyidae (‘icefishes’) is a remarkable adaptation (Ruud, 1954; Sidell et al., 1997; Wittenberg and Wittenberg, 2003). While haemoglobin is involved in the transport of oxygen via blood from the respiratory organs to the tissue, myoglobin is suggested to play a critical role for storage as well as for diffusion of oxygen in muscle tissue. As a consequence, oxygen merely goes in solution in

icefish blood, resulting in an oxygen carrying capacity of less than 10% compared to that of red-blooded notothenioids, but a low blood viscosity (Holeton, 1970; Egginton, 1996). For compensation, icefish possess large hearts, enabling a several-fold increased cardiac output (Hemmingsen et al., 1972), large blood volumes and capillaries of large diameter (Fitch et al., 1984). In spite of lacking haemoglobin and myoglobin, the combination of high oxygen concentration in cold Antarctic waters and the aforementioned arrangements allow a sufficient oxygen supply for the whole organism (Sidell and O'Brien, 2006).

Some of their adaptations to the cold, such as higher mitochondrial densities, high proportions of unsaturated fatty acids in membranes and high lipid contents, have been suggested to render Antarctic fish more susceptible to oxidative and thereby any physiological stress (including thermal stress) compared to lower-latitude species (Abele, 2002; Abele and Puntarulo, 2004).

Additionally, some Antarctic fish species were found to have lost the heat shock response (Hofmann et al., 2000; Place and Hofmann, 2005), which was thought to be a nearly universal stress response among organisms (Lindquist, 1986). Usually, a heat shock response is triggered e.g. by thermal stress. Heat-induced chaperones bind to denatured proteins, preventing their aggregation and support their refolding into the native functional state, when temperatures normalise (Parsell and Lindquist, 1993). Evolution of Antarctic fish in such a very stable and cold environment might have permitted the loss of this functional trait to respond to temperature changes. It is paralleled by a very low thermal tolerance in these fish, which will be discussed in the next paragraph.

1.4. Thermal tolerance

Temperature is an abiotic key factor in the marine realm. In ectotherm organisms, such as fish, body temperatures are driven by ambient temperatures, impacting metabolic processes and shaping distribution limits (e.g. Pörtner and Knust, 2007).

In recent years, shifts in fish species distribution as a response to increasing water temperatures have been reported (Murawski, 1993; Perry et al., 2005; Dulvy et al., 2008). Recent model predictions suggest a global reduction in fish body weight of 14 to 24% within 50 years under a high-emission scenario. While half of this effect is caused by shifts in distribution and abundance of local species assemblages, the other half is assumed to be caused by changes in physiology (Cheung et al., 2013).

Within the physiological tolerance range of an organism, i.e. its thermal window, an acute increase of temperature leads to an exponential increase of metabolic rate. Usually, a 10°C increase of temperature results in a two to three-fold rise of metabolism, generally called the Q_{10} (Jensen et al., 1993). A change in metabolic rate of an organism displays a variation in tissue oxygen demand for ATP production, where oxygen is needed for the oxidation of substrates gained from food consumption (Jobling, 1994). Consequently, oxygen consumption can serve as an indirect estimate of metabolic rate and a measure for the impact of temperature on an organism's performance.

The concept of oxygen- and capacity-limited thermal tolerance (OCLTT) explains how reduced oxygen supply can limit the aerobic capacity (i.e. aerobic scope, the difference between standard and maximum metabolic rate) of an organism at both sides of the thermal window (Pörtner, 2010). The thermal window of an organism is defined by the optimal temperature, at which an organism meets best condition, enhancing growth and reproduction (Fig. 2). If temperature increases or decreases, it can enter the upper or lower pejus temperature range, where the performance decreases due to reduced aerobic scope, leading to reduced oxygen availability to tissues. At upper and lower critical temperatures, oxygen supply can no longer match the increasing demand and metabolism reverts to anaerobic pathways (Pörtner, 2010). The OCLTT concept has been supported by various studies at different organisational levels in marine invertebrates and fish from various latitudes. While OCLTT implies that aerobic scope drives most other physiological performances, such as growth, digestion, reproduction etc., alternative concepts suggest the existence of different optimal temperatures for the performance of different functions. The idea of 'multiple performances – multiple optima' (MPMO) suggests aerobic scope to be one function out of many others, without hierarchical order (Clark et al., 2013). The response to increasing temperature is likely to vary between species as well as between populations, with life stage and ecotype and depends on the rate of temperature change (Pörtner and Farrell, 2008; Clark et al., 2013).

The thermal window of an organism is shaped by the temperature conditions experienced in its natural environment. While species from temperate regions usually display a broader thermal tolerance, species from the more stable high-latitudes show a lower thermal tolerance range (Fig. 2; Somero and DeVries, 1967; Van Dijk et al., 1999; Brodte et al., 2006a). Antarctic marine ectotherms, including fish, were found to be very stenotherm (Peck et al., 2014). Antarctic fish display low critical thermal maxima, compared to temperate or tropical

species (Bilyk and DeVries, 2011). However, acclimation to increased temperature was shown to elevate critical thermal maxima in various Antarctic species (Bilyk and DeVries, 2011). Similarly, some Antarctic fish are able to acclimate their metabolic rate to increasing temperature, compensating for thermal effects on metabolic processes (Seebacher et al., 2005; Franklin et al., 2007). For example, *Pagothenia borchgrevinki* was found to compensate for elevated temperature completely after 28 days at 4°C (Robinson and Davison, 2008). In contrast, other notothenioid species could not be acclimated to this temperature (Robinson, 2008).

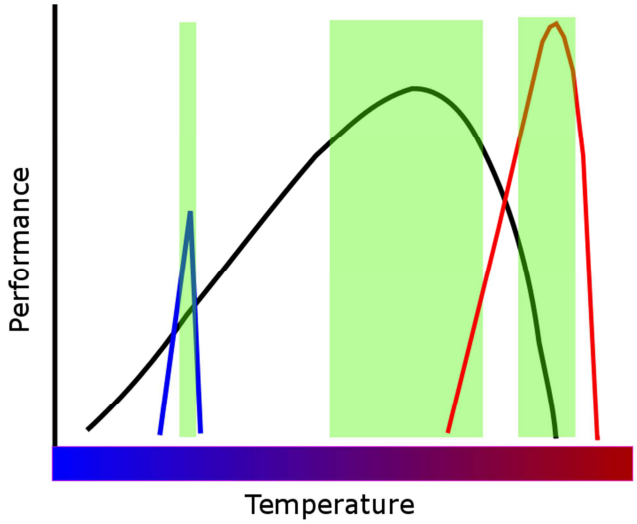

Fig. 2 Schematic temperature dependent performance curve (adapted from Pörtner and Farrell, 2008). The thermal window is narrow for polar stenotherm (blue) and for tropical organisms (red), while being broader for temperate eurytherms (black). Areas shaded in green denote optimal temperatures for physiological performance, such as growth.

Even though the thermal tolerance of Antarctic fish has been the subject of various studies, the underlying physiological mechanisms are still not completely understood. Studies on different species showed deviations between thermal limits for different organisational levels (Gonzalez-Cabrera et al., 1995; Mark et al., 2005; Robinson, 2008; Strobel et al., 2013; Enzor and Place, 2014). Performance limitations might become evident at the most complex organisational level first, but specialisation of molecules could also have forced an interdependency of thermal tolerance limits of different organisational levels (Pörtner et al., 2007). While studies on lower organisational levels, such as molecules or enzymes, are important to understand physiological mechanisms, the thermal response of whole-organism functions, such as growth and reproduction, are most essential in an ecosystem context.

1.5. Energy budgets

Various studies show relations between temperature and organismic traits, such as growth and metabolism (Fonds et al., 1992; Brodte et al., 2006a; Hildebrandt et al., 2011). Besides, shifts in distribution of fish species due to increasing temperature have been reported with progressing climate change (Murawski, 1993; Perry et al., 2005; Grebmeier et al., 2006; Dulvy et al., 2008). However, studies linking experimental results on thermal tolerance to production and abundances in the field are rare. Pörtner and Knust (2007) showed agreement of thermal limits being effective in the field with lab-determined thermal tolerance. They found temperatures at which declining growth rates, accumulation of anaerobic metabolites and increasing oxygen consumption were measured in the lab, to comply well with declining abundances in the field (Pörtner and Knust, 2007).

The abundance of a population is driven by natality and mortality. While both factors vary with the Darwinian fitness of the single individuals of a population, natality moreover depends on individual fecundity, and mortality is regulated by individual growth and predation pressure in the community. Thus, the fitness of an individual, by determining fecundity and growth, impacts natality and mortality within a population and shapes population structures and abundances. This fitness is influenced by the energy budget.

According to Jobling (1994), the energy budget can be explained by the following equation:

$$R = P + M + U + F$$

The energy that is taken up by an organism in form of food (R) can be allocated to different vital functions, such as body growth or reproduction (P), basal/routine metabolism including cots of digestion and activity (M), general excretion (U) and faecal excretion (F) (Jobling, 1994). The routine or basal metabolic rate covers the vital functions to keep an organism alive. Only when these energetic costs have been met, energy can be allocated, e.g. to growth and reproduction (Wieser, 1994; Sokolova, 2013).

Energy allocation can be influenced by abiotic factors, such as temperature, as well as by physiological adaptations to the environment. This is demonstrated for example by differences in length at first spawning that can indicate differences in energy allocation to growth and reproduction. For instance, some Antarctic species reproduce only after having reached up to 80% of their final size, suggesting that energy allocation to growth and reproduction is clearly separated (Kock and Kellermann, 1991). Such traits are likely to

resemble adaptations to enhance fitness in a specific environment. Furthermore, differences in and impacts on energy allocation to growth and reproduction can affect population structures. Therefore, energy budgets are a useful tool to analyse whole-organism performance, the impact of elevated temperature as well as potential consequences for abundance structures.

1.6. Objectives of this thesis

In the framework of progressing climate change, the understanding of thermal response patterns of Antarctic organisms becomes more and more important. Fish play a crucial role in Antarctic food webs. The objective of this thesis was to analyse the thermal tolerance of Antarctic fish at the whole-organism level.

The natural environment of an organism is thought to influence its thermal tolerance (e.g. Stillman 2003; Tewksbury et al. 2008). While Antarctic fish in general are suggested to be highly stenothermal (Somero and DeVries, 1967; Somero, 2010), differences between Antarctic species have rarely been investigated with respect to habitat conditions (Bilyk and DeVries, 2011). As shown in Fig. 1, the Southern Ocean comprises different thermal habitats, possibly influencing thermal tolerance. Therefore, the correlations between thermal tolerance and habitat temperature conditions were investigated in this thesis.

Moreover, various studies on thermal tolerance of lower organisational levels, such as cells and enzymes of Antarctic fish are available, while little is known about thermal response of the whole animal to warming (e.g. Mark et al., 2005; Jayasundara et al., 2013; Strobel et al., 2013; Enzor and Place, 2014). Thus, another objective of this thesis was to analyse the impact of elevated temperature on the whole-organism performance parameters and potential consequences for population structures and abundances.

The following working hypotheses were posed:

1. **Due to life in different thermal regimes, thermal tolerance of Antarctic fish varies depending on habitat conditions. High-Antarctic fish display a lower thermal tolerance compared to low-Antarctic species.**

2. **Cold adaptation is paralleled by high thermal sensitivity. Increasing temperature causes energetic trade-offs at the whole-organism level in Antarctic fish.**

3. **Elevated temperature impacts energy allocation patterns to vital functions, such as growth. General growth performance and the temperature dependence of growth differ in species from different latitudinal ranges, influencing a species' sensitivity to ocean warming.**

With respect to the first hypothesis, metabolic rates at habitat temperatures as well as metabolic responses to acute temperature changes of the Antarctic fish species *Lepidonotothen squamiforns*, *Lepidonotothen nudifrons* and *Trematomus hansoni*, from different thermal environments were compared (manuscript I). While metabolic rate at habitat temperature displays the routine energetic cost in terms of an organism's energy budget, the assessment of the acute thermal tolerance served as a schematic approach to compare response capacities.

To assess potential trade-offs caused by elevated temperature at the whole-organism level in Antarctic fish, as posed in the second hypothesis, energy allocation experiments were carried out. Energy budget parameters, such as food intake, growth, routine metabolism and reproduction were measured after long-term acclimation to increased temperatures in fish with low- and high-Antarctic distribution (manuscript II & III). Here, the yellowfin nothie, *Lepidonotothen nudifrons,* served as a model species for fish with low-Antarctic distribution (Fig. 3). *L. nudifrons* occurs in the Scotia Arc, from the Antarctic Peninsula and associated islands to South Georgia in the north (Gon and Heemstra, 1990). The specimens worked with in this thesis, were caught around Elephant Island (cf. Fig 1). The emerald rockcod, *Trematomus bernacchii*, served as a model organism for high-Antarctic fish (Fig.4). It shows a circum-Antarctic distribution and is a very common species of the high-Antarctic Ross Sea shelf (cf. Fig 1), where animals were caught for this study (Gon and Heemstra, 1990). Both species are primarily benthic and prey on various epifaunal organisms, such polychaetes,

gammarids, isopods, amphipods and also on fish eggs, bivalves or small crustaceans (Gon and Heemstra, 1990; Montgomery et al., 1993; La Mesa et al., 2004).

For both species, general energy allocation patterns, as well as the impact of temperature on energy allocation, were determined and assessed with regard to potential ecological consequences.

Considering the third hypothesis, temperature dependent growth rates of fish species from different latitudes, including results from manuscript II, as well as literature data, were analysed in manuscript IV. These results were assessed with regard to the knowledge gained from the investigations of the first and second hypotheses. Therefore, general differences in growth rates as well as differences in thermal tolerance of growth performance were discussed in an energy allocation framework, including potential implications of these traits for a species' sensitivity to ocean warming.

Fig. 3 *T. bernacchii* resting on ice (copyright M. D. Lamare)

Fig. 4 *L. nudifrons* in an aquarium (copyright T. Sandersfeld)

2. Manuscripts

This thesis includes four manuscripts. The manuscripts, their status as well as the candidate's contribution to them are shown in the manuscript outline. It is followed by reprints of the single manuscripts.

2.1. Manuscript outline

Manuscript I

Authors: Tina Sandersfeld, Felix C. Mark, Rainer Knust (2015)

Title: Temperature-dependent metabolism in Antarctic fish: Do habitat conditions affect acute thermal tolerance?

Status: Under review at *Polar Biology*

Contributions: RK and TS developed the idea and outline of the study. TS conducted the experiments. TS interpreted the data, wrote the manuscript and prepared the figures. RK supported data interpretation. RK, FCM and TS edited the manuscript.

Manuscript II

Authors: Tina Sandersfeld, William Davison, Miles D. Lamare, Rainer Knust, Claudio Richter (2015)

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

Status: Published in *The Journal of Experimental Biology* 218, 2373-2381, doi:10.1242/jeb.122804

Contributions: TS conceived the experiments with support of RK. MDL, WD and CR provided logistical support. MDL and TS collected experimental animals. TS designed and implemented the experiments. WD aided in experiment implementation. TS prepared the manuscript and figures. RK, MDL, WD, CR and TS edited the manuscript.

Manuscript III

Author: Tina Sandersfeld, Magnus Lucassen, Nils Koschnick, Claudio Richter, Rainer Knust (2015)

Title: Routine metabolism, growth and excretion in the Antarctic fish *Lepidonotothen nudifrons*: Does temperature affect the effective use of energy resources?

Status: Manuscript (Brief Communications)

Contributions: The idea of study was conceived by TS and RK. ML, NK and TS collected the model organisms. TS developed the experimental design and carried out the experiments. NK supported the sampling. TS prepared the manuscript and figures. The manuscript was reviewed by the co-authors.

Manuscript IV

Authors: Tina Sandersfeld, Kristina L. Kunz, Felix C. Mark, Claudio Richter, Holger Auel, Rainer Knust (2015)

Title: Energy allocation to growth as an indicator of sensitivity to climate change - an analysis of temperature-dependent growth of fish species from different latitudes

Status: Manuscript

Contributions: TS developed the outline of the study, wrote the manuscript and prepared the figures, based on former ideas of RK. The manuscript was reviewed by the co-authors.

2.2. Manuscript I

Temperature-dependent metabolism in Antarctic fish: Do habitat conditions affect acute thermal tolerance?

Tina Sandersfeld[1,2], Felix C. Mark[2], Rainer Knust[2]

Under review at *Polar Biology*

[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven and University of Bremen (FB 2), Germany

[2]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany

Abstract

Climatic warming is most pronounced in the polar regions. For marine ectotherms such as fish, temperature is a key abiotic factor, influencing metabolic processes. Species distribution and abundance are driven by reproduction and growth, which depend on available energy exceeding baseline maintenance costs. These routine metabolic costs make up a large part of the energy expenditure. Thermal stress can increase routine metabolism, affecting an organism's fitness.

Data of routine metabolic rates of Antarctic fish is scarce and comparability of existing data sets is often problematic due to ecological differences between species and in experimental protocols. Our objective was to compare routine metabolism and thermal sensitivity of species with similar ecotypes, but different thermal environments to assess possible implications of warming waters on energy expenditure in Antarctic fish, a fauna characterised by geographic isolation, endemism and putative thermal adaptation.

We measured routine metabolic rates of three benthic Antarctic fish species from sub-, low- and high-Antarctic regions at habitat temperatures and during acute temperature increase. Our analysis revealed differences in metabolic rates at the same temperature suggesting local adaptation to habitat temperature. Acute thermal stress induced a comparable response of metabolic rates to increasing temperature, suggesting that high-Antarctic species starting off from elevated metabolic rates might reach critical temperatures much quicker. We conclude that higher metabolic rates are associated with higher energetic costs and narrower thermal windows, a potential disadvantage to the endemic high-Antarctic fish fauna facing the challenge of climate change.

Key words: Routine metabolic rate, Polar fish, Notothenioids, Metabolic cold adaptation, Respiration

Introduction

The Polar Regions comprise some of the 'hot spots' of climatic warming. Around the Western Antarctic Peninsula, surface waters have risen in temperature about 1°C in the second half of the twentieth century and around South Georgia a temperature increase of 2.3°C has been recorded within the last 81 years (Meredith and King, 2005; Whitehouse et al., 2008). Although temperature changes have not yet been recorded for high-Antarctic regions such as the Weddell Sea, water temperature increases of up to 2°C have been projected by the year 2100 also for these areas (Hellmer et al., 2012; Turner et al., 2014).

Temperature is a key abiotic factor in the marine environment. In ectotherm organisms like fish, body temperature is determined by ambient temperature, affecting metabolic processes.

The thermal tolerance window of a species yields insight into physiological plasticity regarding changes in ambient temperature. According to the concept of oxygen- and capacity-limited thermal tolerance (OCLTT) (Pörtner, 2012), the temperature window of an organism is defined by the upper and lower critical and pejus temperatures. At a species' optimal temperature, low maintenance costs and maximised aerobic scope were found to come along with high growth rates (Koehn and Shumway, 1982; Wieser, 1994; Brodte et al., 2006). Even though supporting evidence for the OCLTT concept was found in various species (Mark et al., 2002; Lannig et al., 2004; Pörtner et al., 2004), its general applicability and how to measure it has still been discussed in the recent literature (Clark et al., 2013; Norin et al., 2014).

Fish play an important role in Antarctic food webs. Being predator and prey alike, they serve as an important link between lower and higher trophic levels (Hureau, 1994). Antarctica has been an oceanographically isolated and thermally very stable environment over geological time scales, leading to the evolution of an endemic Antarctic fish fauna with highly stenothermal species. Antarctic fish exhibit different adaptations to their constantly cold environment, such as a lack of heat shock response, expression of anti-freeze glycoproteins, a lack of haemoglobin and myoglobin, higher mitochondrial densities as well as other compensatory adaptation in the heart and circulatory system (e.g. DeVries and Eastman, 1981; Coppes Petricorena and Somero, 2007). Compared to temperate species that experience broader environmental temperature fluctuations, Antarctic fish have very narrow temperature windows (Somero and De Vries, 1967; Van Dijk et al., 1999; Brodte et al., 2006). However, habitat temperature does not only vary on global scales, but also within the Southern Ocean. In the lower, i.e. northern Antarctic region, shelf water temperatures are generally warmer

(Barnes et al., 2006; Clarke et al., 2009), compared to the very stable high Antarctic shelf region in the south with temperatures between -0.5 to -1.9°C (Hunt et al., 2003). Evolution in these thermally different regions is likely to have affected thermal tolerance within Antarctic notothenioids. Studies on critical thermal maxima (CT_{max}) showed thermal tolerance differences between high and low-Antarctic species of up to 4°C (Bilyk and DeVries, 2011). Moreover, organismal freeze avoidance (mostly anti-freeze glycoproteins) in Antarctic icefishes was shown to decrease with distribution in increasing latitudes (Bilyk and DeVries, 2010), indicating specific adaptation to the respective regional climate.

For a benthic marine fish species, Pörtner and Knust (2007) showed thermal limits determined in laboratory experiments to agree with ambient temperatures beyond which growth performance and abundance in the field declined. Growth and reproduction are the main driving forces for population dynamics and structure, thereby shaping a species' abundance and distribution. However, growth and reproduction of an organism depend upon aerobic energy available after baseline costs of maintenance have been met (Koehn and Shumway, 1982; Wieser, 1994). Thus, knowledge on the impact of temperature on energy budget factors from experimental trials, such as routine metabolic costs, helps to estimate possible impact of ocean warming on Antarctic fish.

Various studies are available focusing on differences in routine metabolism and thermal tolerance between temperate, tropical and polar species (Johnston et al., 1991; Clarke and Johnston, 1999; Vanella and Calvo, 2005; White et al., 2012). However, data for Antarctic species is limited (Robinson, 2008; Strobel et al., 2012; Enzor et al., 2013) and additionally comparability of single studies is complicated by differences in experimental setups, protocols and species' ecotypes, all of which have major effects on results (Chown et al., 2009; Bilyk and DeVries, 2011). The aim of this study is to compare routine metabolism and thermal sensitivity of different Antarctic fish species from different thermal environments i.e. sub-Antarctic, low northern and high southern Antarctic regions, to gain insight in the impact of environmental temperature variability within Antarctic waters on a species' thermal tolerance. While a population's thermal tolerance is an important factor to assess, as it has direct fitness consequences in a warming Southern Ocean, ambiguities of species and regional effects cannot be resolved with this approach. In this study, we compare three notothenioid species from different latitudes, namely *Lepidonotothen squamifrons*, *Lepidonotothen nudifrons* and *Trematomus hansoni*, which are all benthic, shelf inhabiting species. Distributions range from the sub-Antarctic Islands for *L. squamifrons*, the Scotia Arc and the Antarctic Peninsula (low-

Antarctic) for *L. nudifrons,* to high-Antarctic areas for *T. hansoni* (Gon and Heemstra, 1990). We present a data set of routine metabolic rates at habitat temperatures as well as in response to acute temperature increase of different Antarctic demersal fish species of similar ecotype measured with the same experimental setup and protocol. Thereby we want to approach the question how the geographically isolated and highly endemic Antarctic fish fauna will fare with progressing climate change and whether there will be differences in this putatively temperature sensitive species depending on habitat conditions.

Material and methods

Animals

In this study, the three Antarctic fish species, *Lepidonotothen squamifrons, Trematomus hansoni* and *Lepidonotothen nudifrons,* were investigated.

Lepidonotothen squamifrons were caught near South Georgia at a depth of ~310 m, water temperature of 2.1°C and salinity of 34.4‰ by bottom trawl in March/April 2011 (RV Polarstern, ANT-XXVII/3). Body weight of the fish ranged from 233.5 - 394.0 g.

Trematomus hansoni were collected in the Eastern Weddell Sea at a depth of ~225 m, water temperature between -1.5 to -1.9°C, and salinity of 34.4‰ by bottom trawl in April 2011 (RV Polarstern, ANT-XXVII/3). Body weight of the animals was between 213.2 and 300.8 g. Experiments with *L. squamifrons* and *T. hansoni* were carried out after a recovery period of a minimum of 14 days on board RV Polarstern. During this time *L. squamifrons* were kept at a temperature of 2°C (habitat temperature measured before trawling), while *T. hansoni* were kept at the lowest technically possible temperature on board the vessel of -0.5 to 0°C.

Lepidonotothen nudifrons were caught near Elephant Island at a depth of 70 to 322 m, water temperature of 0.0 to 0.8°C and salinity of 34.2 to 34.5‰ by bottom trawl in March/April 2012 (RV Polarstern, ANT-XXVIII/4). Fish weight was 32.2 to 41.0 g. Animals were transported to the Alfred Wegener Institute in Bremerhaven (Germany) and kept in aquaria at 0 to 1°C. Experiments were carried out in August 2012.

The temperature at which the animals were kept after being caught is here after referred to as habitat temperature and was used as the starting temperature for the respiration experiments. Experiments were stopped, when fish showed first signs of stress, indicated by a loss of

balance or irregular movements of opercula. Locations of catch for all species, as well as habitat temperature variations, are shown in Table 1.

Oxygen consumption measurement

Routine metabolic rates (RMR) in this study were measured by flow-through systems. Prior to each measurement, oxygen probes were calibrated at the starting temperature for 100% oxygen saturation with air equilibrated seawater and for 0% saturation with nitrogen bubbled seawater. Before and after each measurement period, a blank, i.e. without an animal inside the respiration chamber, was measured to estimate bacterial respiration and fluctuation of the flow. Blank data showed negligible oxygen consumptions and therefore were not considered in calculations of oxygen consumption. All animals were starved for about 10 days prior to experiments, to exclude effects of specific dynamic action on metabolic rates. The fish were placed in the respiration chamber at habitat temperature. After an acclimation period of at least 24 hours, oxygen consumption was measured for another 24 hours at habitat temperature for RMR determination. Subsequently, temperature was increased by 1°C per 24 hours. Temperature was continuously raised at the same time of day, usually in the morning. In this way, settings could be supervised while temperature was increased and data for analysis were recorded at stable temperature overnight, as disturbance levels by surroundings were lowest during this time. A dimmed light was turned on all day long in the experimental room to resemble summer light conditions. In the beginning of each measurement, the flow rate was set in a way that the out-flowing water displayed oxygen saturation between 95 and 90%.

The term ‘routine metabolic rates’ will be used in this study to describe oxygen consumption rates including all metabolic processes that contribute to keeping an organism alive (also often termed as basal or standard metabolism), plus spontaneous activity. Measured specimens were observed to adapt a tripod stance and showed very rare spontaneous activity.

For calculation of metabolic rates, usually mean oxygen consumption of 12 hour-periods was used (minimum 8 hours). For assessment of routine metabolic rates at habitat temperature, means were calculated over data of 24 hours.

Statistical analysis

Statistical analysis was performed using R statistical language (R Core Team, 2014; version 2.1.51). Data was checked for normality distribution (Shapiro-Wilk test, $p > 0.05$ for all groups, removing one data point of *L. squamifrons* (5°C) as an outlier (see Online Resource

1-3, 5)) and homogeneity of variances (Bartlett test, $p > 0.05$ across all species). The significance level was set to $\alpha = 0.05$ throughout the study. Logarithmic oxygen consumption data of single species in response to different temperatures was modelled linearly. Species-specific intercepts were tested for significant differences (ANOVA, common trend from linear model removed). Pairwise differences in intercepts were tested for significance by Tukey HSD (post-hoc, $p < 0.05$).

Results

Oxygen consumption of *T. hansoni* at 0°C was highest with a value of 34.63±4.2 mg O_2 kg^{-1} h^{-1}. This is followed by MO_2 of 34.44±3.5 mg O_2 kg^{-1} $h^{-1}$of *L. squamifrons* at 2°C. Lowest oxygen consumption of 21.12±1.8 mg O_2 kg^{-1} h^{-1} was measured for *L. nudifrons*. Detailed oxygen consumption values including standard errors (s.e.m.) of the single species at different temperatures are shown in Table 1.

Table 1 Routine metabolic rate in mg O_2 kg^{-1} h^{-1}± s.e.m. for different fish species measured at habitat temperature (first value for each species) and with increasing temperature of 1°C per 24 hours.

Temperature [°C]	*T. hansoni*	*L. squamifrons*	*L. nudifrons*
0	34.63 ±4.2 (n=5)	-	21.12 ±1.8 (n=6)
1	39.93 ±3.9 (n=5)	-	19.30 ±2.2 (n=6)
2	51.47 ±3.8 (n=5)	34.44±3.5 (n=8)	27.52 ±2.9 (n=5)
3	59.17 ±5.5 (n=5)	38.23±2.9 (n=8)	31.87 ±3.8 (n=6)
4	67.71 ±7.6 (n=5)	48.15 ±2.8 (n=8)	35.50 ±3.8 (n=6)
5	-	50.55 ±2.0 (n=7)	-
6	-	55.53 ±3.5 (n=6)	-

A linear fit revealed a significant effect of temperature on MO_2 ($p < 0.001$), i.e. a significant increase of oxygen consumption with increasing temperature (see Fig. 1).With no significant differences in slopes ($p > 0.05$), analysis supported model selection with a common slope for all species ($y = 0.150827 \times x + b$). Thus, temperature had a comparable effect on MO_2 of all analysed species. The full model with species specific slopes as well as the model with one common slope showed a significant difference in oxygen consumption between species ($p <$

0.05), which can be interpreted as differences in intercept between regression lines of the different species and thus significant differences in MO_2 at the same temperature between all species. Intercepts (b), y (x = habitat temperature) and RMR at habitat temperature are summarized in Table 2. Including the possible outlier in the data set of *L. squamifrons* (5°C) yielded in comparable results (not shown). Raw data and details of analysis are given in Online Resource 1 to 6.

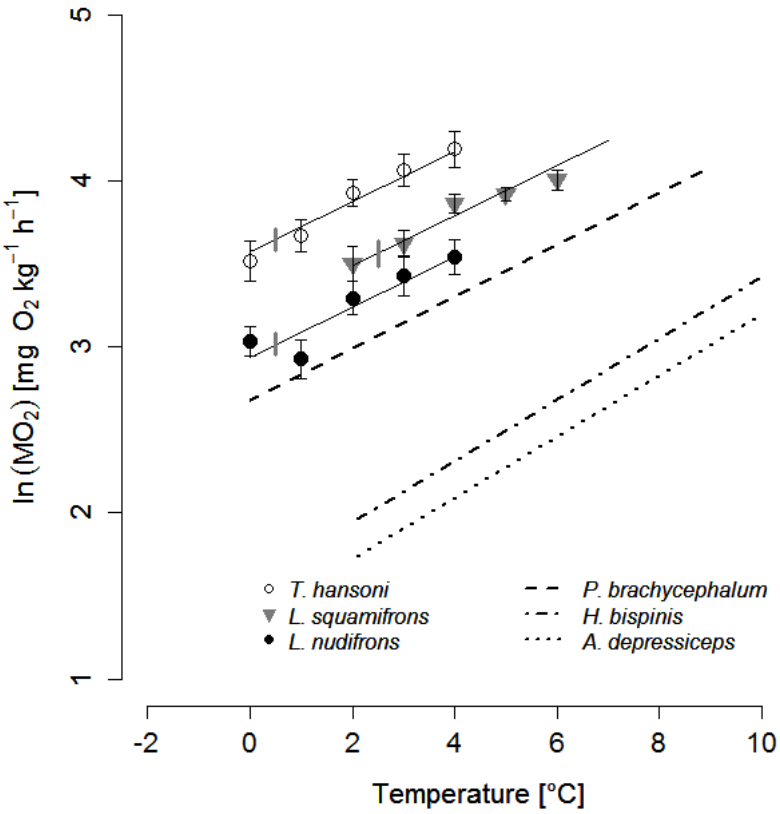

Fig. 1 Natural logarithm of routine metabolic rates in dependence of temperature of the notothenioid species *T. hansoni* (open circles), *L. squamifrons* (triangles) and *L. nudifrons* (filled circles). Black error bars indicate standard errors of logarithmic metabolic rates, while grey bars indicate standard errors of the linear model with common slope. Literature data of *P. brachycephalum* (dashed line) (Van Dijk et al., 1999), *Harpagifer bispinis* (dotted-dashed line) (Vanella and Calvo, 2005) and *Austrolycus depressiceps* (dotted line) (Vanella and Calvo, 2005) are indicated.

Table 2 Routine metabolic rate (RMR) in mg O_2 kg^{-1} $h^{-1}$for different fish species at habitat temperature according to the linear model with a common slope for all species y = 0.150827×x+b.

Species	Intercept	Habitat temperature [°C]	y (x = habitat temp.)	RMR at habitat temperature [mg O_2 kg^{-1} h^{-1}]
T. hansoni	3.57	0	3.57	35.58
L. squamifrons	3.18	2	3.49	32.68
L. nudifrons	2.93	0	2.94	18.93

Discussion

In this study, Antarctic fish species showed differences in metabolic rates at the same temperature, but no differences in respiratory response to warming. Generally, an increase of metabolic rate with increasing temperature complies well with our current understanding of temperature dependent metabolism (see Clarke and Fraser, 2004 and references therein) and is in line with the theory of oxygen-limited thermal tolerance (Pörtner, 2001). Comparing thermal tolerance of species from different thermal environments within the Southern Ocean, a common slope as response to increasing temperature could be a consequence of evolutionary adaptation to this cold environment with little temperature variation (cf. Table 2). Moreover, our results indicate significant differences in oxygen consumption at the same temperature. While highest oxygen consumption rates were found for high-Antarctic *T. hansoni*, intermediate metabolic rates were found for the sub-Antarctic *L. squamifrons*. Lowest rates were found for *L. nudifrons* from low-Antarctic regions (cf. Table 3).

Table 3 Location of catch (habitats) as well as minimum and maximum habitat temperatures of species used in this study. Temperatures are derived from Ocean Data View data sets SOA and WOA09 (Olbers et al., 1992; Locarnini et al., 2010; Schlitzer, 2011).

Species	Habitat	Latitude	Habitat temp. min. [°C]	Habitat temp. max. [°C]
T. hansoni	Southern Ocean/ Weddell Sea	70°S	-1.9	-1.5
L. squamifrons	Southern Ocean/ South Georgia	53°S	1.5	2.5
L. nudifrons	Southern Ocean/ Elephant Island	61°S	-1.5	1.0

Imagining there was a general upper limit of metabolic rate in benthic fish with an inactive lifestyle, a common thermal response would result in differences of thermal tolerance ranges (i.e. width of thermal windows), depending on the starting point (y-intercept, cf. Fig. 2).

A higher oxygen consumption of *T. hansoni* compared to *L. nudifrons* matches with the assumption of higher metabolic rates of Antarctic species from high latitudes and a colder environment. As shown in various studies, high-Antarctic species, such as *T. hansoni*, are considered to be highly temperature sensitive, displaying a very narrow thermal tolerance window (Pörtner and Farrell, 2008; Bilyk and DeVries, 2011). In contrast, data of *L. squamifrons* does not match with this hypothesis, as this species has a sub-Antarctic distribution, which would suggest a higher thermal tolerance. However, Beers & Sidell (2011) showed *L. squamifrons* to be especially sensitive towards high temperature and to have a

narrower thermal window compared to other red-blooded notothenioid species. They found the reason for this to be low haematocrit levels, which were negatively related to thermal tolerance (Beers and Sidell, 2011).

Literature data of temperature-dependent oxygen consumption measurements according to a comparable protocol is scarce and only available for the Antarctic eelpout *Pachycara brachycephalum* (Van Dijk et al., 1999). This species also shows a similar slope of oxygen consumption but a lower oxygen consumption at the same temperature compared to species analysed in this study (Fig. 1). The deep sea origin of the family Zoarcidae suggests lower metabolic rates for the eelpout species compared to species from shelf habitats (Hochachka, 1988). Moreover, it supports the finding of high metabolic rates in cold habitats, as shelf waters are usually colder than deep waters. For further comparisons, we added data of *Harpagifer bispinis*, a plunderfish species, and *Austrolycus depress*iceps, a zoarcid, from the Beagle Channel to Fig. 1. Even though experimental protocols of these species deviate and do include acclimation times, this data supports a similar picture (see Vanella and Calvo, 2005 for original data and details of expertimental protocol). Generally, oxygen consumption seems to be higher in species from cold habitats. The South American eelpout *A. depressiceps* displays lowest oxygen consumption, which is followed by the South American *H. bispinis*, the Antarctic eelpout *P. brachycephalum*, low-Antarctic *L. nudifrons* and high-Antarctic *T. hansoni*. Differences in oxygen consumption at the same temperature, but a similar response to an increase of temperature could be the basis of differences in thermal windows between species. Data gained in this study cannot be regarded as comprehensive enough to serve as a basis for a general theory on thermal windows. However, in the following we will explain our idea which will have to be tested and might serve as a starting point for further studies. Fig. 2 shows a schematic illustration of a small thermal window (light grey triangle) of a high Antarctic species that reaches metabolic limitations quicker, as it starts off with higher oxygen consumption (light grey line). In contrast, a northern or low-Antarctic species that starts off with a lower oxygen consumption (dark grey line) has more capacity to increase metabolism when temperature rises, shown by a larger thermal window (dark grey triangle). In consequence, this allows for and explains a relatively more eurythermal way of life for low-Antarctic species. But what sets metabolic limitations in these fish?

A limiting factor for an increase of metabolic rate might be set e.g. by the circulatory system as shown by Mark et al. (2002). (Sub-) cellular space requirements were suggested to constrain maximum scope for activity, as a higher mitochondrial volume is needed in the cold

for the same functional capacity as at warmer temperatures (O'Brien and Sidell, 2000; Pörtner, 2002). In Antarctic fish, metabolic rates are low, but metabolic increments e.g. due to increasing temperature are relatively high due to high amounts of enzymes to warrant functional capacities at low temperature, resulting in early capacity limitations (Pörtner et al., 1998; Pörtner et al., 2013).

This concept would comply well with findings of other studies. Declining width of thermal windows with decreasing habitat temperature variation has been observed for eelpout species from Antarctic and temperate regions (Van Dijk et al., 1999). Beers and Sidell (2011) suggested a positive relation between thermal tolerance and haematocrit levels, which matches well with the oxygen limited thermal tolerance model by Pörtner (2001). This also agrees well with our results of relatively high routine metabolism of *L. squamifrons* for a species with sub-Antarctic distribution, possibly indicating restricted scope for an increase of metabolism. A high performance sensitivity towards increasing temperature was suggested by various studies for species living at the warm-edge of their distributional range (Stillman, 2003; Deutsch et al., 2008; Neuheimer et al., 2011), which might also be true for *L. squamifrons*.

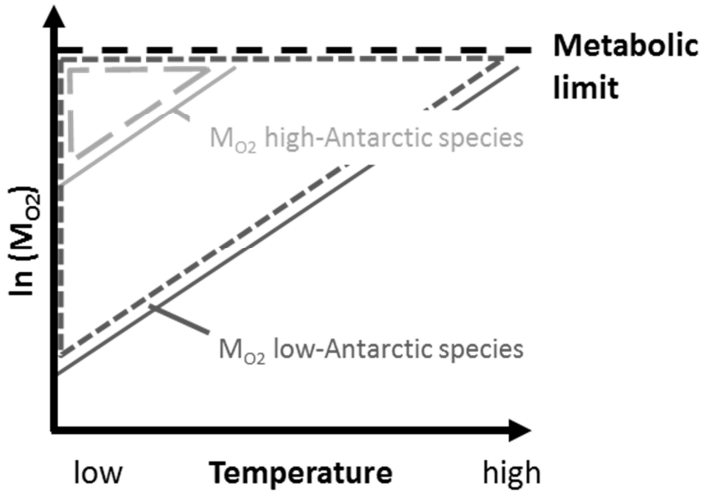

Fig. 2 Schematic illustration of differences in thermal windows of species with differences in oxygen consumption at the same temperature, but the same response to increasing temperature, given there was an upper limit to metabolic rate (black dashed line). Starting off at higher metabolic rates (light-grey line), high-Antarctic species display a smaller thermal window (long-dashed triangle), while low-Antarctic species start off with lower metabolic rates (dark-grey line), displaying a larger thermal window (short-dashed triangle).

A several-fold higher metabolic rate of polar compared to temperate or tropical ectotherms when extrapolated to the same temperature is hypothesised by the metabolic cold adaptation theory (MCA, Scholander et al., 1953; Wohlschlag, 1960). Since its introduction, the MCA concept has been vigorously discussed in the literature. While most studies support higher

activity rates for energy metabolism associated enzymes in species from polar regions (Hochachka, 1988; Crockett and Sidell, 1990; West et al., 1999; Kawall et al., 2002; but see also Magnoni et al., 2013), experimental results deviate widely at the whole-organism level (Holeton, 1974; Torres and Somero, 1988; Clarke and Johnston, 1999; Jordan et al., 2001; Steffensen, 2002; White et al., 2012). Our results agree well with a recent analysis by White et al. (2012), who found evidence for MCA at different levels of organisation in fish, with strongest support at the level of whole-animal metabolic rate. However, differences in metabolic rates in our, as well as in White's study, are not even close to a several-fold higher MO_2 for species from high latitudes as initially suggested by Scholander et al. (1953) and Wohlschlag (1960). About two-fold increased metabolic rates of species from higher latitudes were also considered in papers that actually disagree with the MCA hypothesis (cf. Holeton, 1974; Clarke, 1980).

Factors usually criticized in experiments supporting MCA, such as the lack of acclimation periods in the experimental protocol and comparison of species of different ecotypes can be excluded in this study. The use of flow-through respirometers was a major factor criticized by Steffensen (Steffensen, 1989; Steffensen, 2002), as this method was supposed to cause overestimation of metabolic rates. Steffensen and co-workers found differences in experimental protocols associated with flow-through respirometry, such as lack of acclimation times, short measuring periods and no consideration of diurnal rhythms, to result in high metabolic rate measurements. However, respiration rates obtained here comply well with values measured for similar species in other studies (Van Dijk et al., 1999; Mark et al., 2002; Steffensen, 2005; Brodte et al., 2006; Robinson, 2008; Robinson and Davison, 2008; Enzor et al., 2013). Moreover, methodological comparison of flow-through versus intermittent-flow respirometry showed no significant differences in oxygen consumption data of individual fish measured with both methods according to appropriate protocols (Sandersfeld and Knust unpublished). As in many other studies on high-Antarctic fish (Axelsson et al., 1992; Davison, 2001; Maffia et al., 2001), *T. hansoni* was not kept at its usual habitat temperature of below -1°C in this study. Although an elevation of metabolic rate due to higher acclimation temperature cannot be excluded, a comparison of literature data makes it seem unlikely (Robinson, 2008).

Yet, there are various factors possibly influencing metabolic rates, which cannot be completely controlled. While seasonal impacts on shelf water temperature expand only into the upper 100 to 200 m of the water column (Clarke, 1988; Barnes et al., 2006), seasonal

impacts on metabolic rates have been reported with respect to spawning as well as food availability in fish (Beamish, 1964; Karås, 1990). As experiments with notothenioid species were conducted after the spawning season (cf. Table 4), metabolic rates are likely to have been on a lower level. Moreover, various physiological and ecological as well as habitat-specific factors are known to possibly influence metabolic rates (Post, 1990; Campbell et al., 2009; Giesing et al., 2011; Ohlberger et al., 2012). However, only the parameters of lifestyle and activity could be considered and were regarded to be comparable for the analysed species.

Table 4 Time of experiment and time of spawning for species used in experiments. Spawning times are derived from Sil'yanova (1980) and Andriashev (1986).

Species	Time of experiment	Time of spawning
T. hansoni	April (autumn)	February/March
L. squamifrons	March/April (spring)	February
L. nudifrons	August (winter)	April/May

There are various attempts to explain high metabolic rates in Antarctic fish. The mitochondrial proton leak makes up a significant part of an organism's metabolic rate. Hardewig and co-workers (1999) estimated the proton leak to make up for about 10% of respiration in liver mitochondria of the Antarctic fish *L. nudifrons*. This makes a higher whole-animal metabolic rate in Antarctic fish with higher mitochondrial densities probable. While this is supported by results from studies on enzyme levels (e.g. Crockett and Sidell, 1990) and matches with observations of low growth in polar ectotherms (DeVries and Eastman, 1981; La Mesa and Vacchi, 2001; Pörtner et al., 2005), results of whole-organism metabolic rates are controversial. Studies at lower organisational levels are often less complex and yield clearer results. Nevertheless, when aiming to transfer results from lower levels to ecosystems, one can hardly get around taking the whole-organism level into account (cf. Barnes and Peck, 2008). It is questionable, whether measurements of metabolic rate via oxygen consumption are the optimal tools to estimate routine metabolic costs, as it includes a variety of complex processes (see also Clarke, 1991). However, alternatives are scarce. As routine metabolism makes up for about 50% of Antarctic fish energy expenditure (Brodte et al., 2006), differences in routine metabolic costs are likely to have fitness consequences (cf. Pörtner and Knust, 2007). High-Antarctic fish were shown to grow slower, compared to species from the seasonal pack-ice zone (La Mesa and Vacchi, 2001). While low growth performance is suggested to be linked to lifestyle and food resources (La Mesa and Vacchi,

2001), it could also suggest limitations in energy allocation of high Antarctic species. High routine metabolic costs could limit energy investment into growth (cf. Donelson and Munday, 2012), which is crucial for a species abundance and population structure. Regarding metabolic stressors, such as rising water temperatures and ocean acidification, which can additionally increase metabolic rates, generally elevated metabolic costs could be disadvantageous.

In conclusion, regarding regional thermal variability, habitat conditions did not affect acute thermal tolerance patterns in the studied species. Our results suggest that metabolic responses to rising temperature of species from Antarctic regions do not differ, but that species from high latitudes start off at higher metabolic rates and enzymatic capacities, which brings them to critical temperatures quicker than species from lower latitudes, resulting in narrower thermal windows. Increasing habitat temperature, even on small scales of some 0.1°C as suggested for the habitats of species studied here, are likely to increase routine metabolic costs. Recent models suggest that warm deep water entering the Filchner Trough in the Southern Weddell Sea can lead to temperature increases of up to 2°C (Hellmer et al., 2012). For high-latitude species with high routine metabolic rates and low growth performance, increasing habitat temperatures might further skew this imbalance affecting growth and reproduction and thereby population structures. In consequence, the discussion about higher or lower metabolic rates of polar fish species could be crucial for our understanding of thermal sensitivity of these animals in the current stage of climate change.

Acknowledgements

The authors would like to thank Nils Koschnick and Timo Hirse for technical assistance during experiments, Fredy Véliz Moraleda for animal care, the crew of RV Polarstern for valuable field support during ANT-XXVII/3 and ANT-XXVIII/4 as well as Christian Bock for helpful advice on experimental work and Stephan Frickenhaus for statistical support. This study was supported by Polarstern Grant No. AWI_PS77-03 and AWI_PS79-04. Tina Sandersfeld was partly supported by a Ph.D. scholarship of the Helmholtz Graduate School for Polar and Marine Research and by the PACES II programme. We disclose no conflict of interest in regard to this research.

Compliance with Ethical Standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This research was conducted in accordance with the ethics and guidelines of the German law. The experiments have been approved by the veterinary inspection office (Senatorin für Bildung, Wissenschaft und Gesundheit, Bremen, Germany) under the permit number AZ: 522-27-11/02-00 (93) issued on 15th January 2008 and valid until 14th January 2013.

References

Andriashev, A. (1986). Zoarcidae. In *Fishes of the north-eastern Atlantic and the Mediterranean*, vol. 3, (ed. E. A. Whitehead), pp. 1130-1150. Paris: UNESCO.
Axelsson, M., Davison, W., Forster, M. and Farrell, A. (1992). Cardiovascular responses of the red-blooded antarctic fishes *Pagothenia bernacchii* and *P. borchgrevinki*. *J. Exp. Biol.* **167**, 179-201.
Barnes, D. K. and Peck, L. S. (2008). Vulnerability of Antarctic shelf biodiversity to predicted regional warming. *Climate Res.* **37**, 149-163.
Barnes, D. K., Fuentes, V., Clarke, A., Schloss, I. R. and Wallace, M. I. (2006). Spatial and temporal variation in shallow seawater temperatures around Antarctica. *Deep-Sea Res. Pt II* **53**, 853-865.
Beamish, F. W. H. (1964). Seasonal changes in the standard rate of oxygen consumption of fishes. *Can. J. Zool.* **42**, 189-194.
Beers, J. M. and Sidell, B. D. (2011). Thermal tolerance of Antarctic notothenioid fishes correlates with level of circulating hemoglobin. *Physiol. Biochem. Zool.* **84**, 353-362.
Bilyk, K. T. and DeVries, A. L. (2010). Freezing avoidance of the Antarctic icefishes (Channichthyidae) across thermal gradients in the Southern Ocean. *Polar Biol.* **33**, 203-213.
Bilyk, K. T. and DeVries, A. L. (2011). Heat tolerance and its plasticity in Antarctic fishes. *Comp. Biochem. Physiol. A* **158**, 382-390.
Brodte, E., Knust, R. and Pörtner, H. O. (2006). Temperature-dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae). *Polar Biol.* **30**, 95-107.
Campbell, H., Davison, W., Fraser, K., Peck, L. and Egginton, S. (2009). Heart rate and ventilation in Antarctic fishes are largely determined by ecotype. *J. Fish Biol.* **74**, 535-552.
Chown, S. L., Jumbam, K. R., Sorensen, J. G. and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* **23**, 133-140.
Clark, T. D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771-2782.
Clarke, A. (1980). A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. *Biol. J. Linn. Soc.* **14**, 77-92.
Clarke, A. (1988). Seasonality in the Antarctic marine environment. *Comp. Biochem. Physiol. B* **90**, 461-473.
Clarke, A. (1991). What is cold adaptation and how should we measure It? *Am. Zool.* **31**, 81-92.
Clarke, A. and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *J. Anim. Ecol.* **68**, 893-905.

Clarke, A. and Fraser, K. (2004). Why does metabolism scale with temperature? *Funct. Ecol.* **18**, 243-251.
Clarke, A., Griffiths, H. J., Barnes, D. K., Meredith, M. P. and Grant, S. M. (2009). Spatial variation in seabed temperatures in the Southern Ocean: implications for benthic ecology and biogeography. *J. Geophys. Res.-Biogeo.* **114**, G03003.
Coppes Petricorena, Z. L. and Somero, G. N. (2007). Biochemical adaptations of notothenioid fishes: comparisons between cold temperate South American and New Zealand species and Antarctic species. *Comp. Biochem. Physiol. A* **147**, 799-807.
Crockett, E. L. and Sidell, B. D. (1990). Some pathways of energy metabolism are cold adapted in Antarctic fishes. *Physiol. Zool.*, 472-488.
Davison, W. (2001). Respiration of the emerald rockcod, a notothenioid fish from Antarctica. *New Zealand Natural Sciences* **26**, 13-20.
Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105**, 6668-6672.
DeVries, A. L. and Eastman, J. T. (1981). Physiology and ecology of notothenioid fishes of the Ross Sea. *J. Roy. Soc. New Zeal.* **11**, 329-340.
Donelson, J. M. and Munday, P. L. (2012). Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. *J. Anim. Ecol.* **81**, 1126-1131.
Enzor, L. A., Zippay, M. L. and Place, S. P. (2013). High latitude fish in a high CO_2 world: Synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids. *Comp. Biochem. Physiol. A* **164**, 154-161.
Giesing, E. R., Suski, C. D., Warner, R. E. and Bell, A. M. (2011). Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proc. R. Soc. Lond. B* **278**, 1753-1759.
Gon, O. and Heemstra, P. C. (eds) (1990). Fishes of the Southern Ocean. Grahamstown: JLB Smith Institute of Ichthyology.
Hardewig, I., Peck, L. and Pörtner, H. (1999). Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid *Lepidonotothen nudifrons*. *J. Comp. Physiol. B* **169**, 597-604.
Hellmer, H. H., Kauker, F., Timmermann, R., Determann, J. and Rae, J. (2012). Twenty-first-century warming of a large Antarctic ice-shelf cavity by a redirected coastal current. *Nature* **485**, 225-228.
Hochachka, P. (1988). Channels and pumps - determinants of metabolic cold adaptation strategies. *Comp. Biochem. Physiol. B* **90**, 515-519.
Holeton, G. F. (1974). Metabolic cold adaptation of polar fish: fact or artefact? *Physiol. Zool.*, 137-152.
Hunt, B. M., Hoefling, K. and Cheng, C.-H. C. (2003). Annual warming episodes in seawater temperatures in McMurdo Sound in relationship to endogenous ice in notothenioid fish. *Antarct. Sci.* **15**, 333-338.
Hureau, J. C. (1994). The significance of fish in the marine Antractic ecosystems. *Polar Biol.* **14**, 307-313.
Johnston, I. A., Clarke, A. and Ward, P. (1991). Temperature and metabolic rate in sedentary fish from the Antarctic, North Sea and Indo-West Pacific Ocean. *Mar. Biol.* **109**, 191-195.
Jordan, A. D., Jungersen, M. and Steffensen, J. (2001). Oxygen consumption of East Siberian cod: no support for the metabolic cold adaptation theory. *J. Fish Biol.* **59**, 818-823.
Karås, P. (1990). Seasonal changes in growth and standard metabolic rate of juvenile perch, *Perca fluviatilis* L. *J. Fish Biol.* **37**, 913-920.
Kawall, H., Torres, J., Sidell, B. and Somero, G. (2002). Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. *Mar. Biol.* **140**, 279-286.

Koehn, R. K. and Shumway, S. E. (1982). A genetic/physiological explanation for differential growth rate among individuals of the American oyster *Crassostrea virginica*. *Mar. Biol. Lett.* **3**, 35-42.
La Mesa, M. and Vacchi, M. (2001). Age and growth of high Antarctic notothenioid fish. *Antarct. Sci.* **13**, 227-235.
Lannig, G., Bock, C., Sartoris, F. J. and Pörtner, H. O. (2004). Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L., studied by magnetic resonance imaging and on-line venous oxygen monitoring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R902-R910.
Locarnini, R. A., Mishonov, A. V., Antonov, J. I., Boyer, T. P. and Garcia, H. E. (2010). World Ocean Atlas 2009, vol. 1 (ed. L. S), pp. 184 pp: NOAA Atlas NESDIS 69, U.S. Government Printing Office, Washington, D.C.
Maffia, M., Rizzello, A., Acierno, R., Rollo, M., Chiloiro, R. and Storelli, C. (2001). Carbonic anhydrase activity in tissues of the icefish *Chionodraco hamatus* and of the red-blooded teleosts *Trematomus bernacchii* and *Anguilla anguilla*. *J. Exp. Biol.* **204**, 3983-3992.
Magnoni, L. J., Scarlato, N. A., Ojeda, F. P. and Wohler, O. C. (2013). Gluconeogenic pathway does not display metabolic cold adaptation in liver of Antarctic notothenioid fish. *Polar Biol.* **36**, 661-671.
Mark, F. C., Bock, C. and Pörtner, H. O. (2002). Oxygen-limited thermal tolerance in Antarctic fish investigated by MRI and P-31-MRS. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R1254-R1262.
Meredith, M. P. and King, J. C. (2005). Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century. *Geophys. Res. Lett.* **32**.
Neuheimer, A., Thresher, R., Lyle, J. and Semmens, J. (2011). Tolerance limit for fish growth exceeded by warming waters. *Nature Clim. Change* **1**, 110-113.
Norin, T., Malte, H. and Clark, T. D. (2014). Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *J. Exp. Biol.* **217**, 244-251.
O'Brien, K. M. and Sidell, B. D. (2000). The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *J. Exp. Biol.* **203**, 1287-1297.
Ohlberger, J., Mehner, T., Staaks, G. and Hölker, F. (2012). Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. *Oikos* **121**, 245-251.
Olbers, D., Gouretski, V., Seiß, G. and Schröter, J. (1992). Hydrographic Atlas of the Southern Ocean. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany & Arctic and Antarctic Research Institute, St. Petersburg, Russia.
Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137-146.
Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol. A* **132**, 739-761.
Pörtner, H. O. (2012). Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* **470**, 273-290.
Pörtner, H. O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95-97.
Pörtner, H. O. and Farrell, A. P. (2008). Physiology and climate change. *Science* **322**, 690-692.

Pörtner, H. O., Mark, F. C. and Bock, C. (2004). Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. *Resp. Physiol. Neurobiol.* **141**, 243-260.
Pörtner, H. O., Storch, D. and Heilmayer, O. (2005). Constraints and trade-offs in climate-dependent adaptation: energy budgets and growth in a latitudinal cline. *Sci. Mar.* **69**, 271-285.
Pörtner, H. O., Walther, K. and Wittmann, A. (2013). Excess Oxygen in Polar Evolution: A Whole Organism Perspective. In *Adaptation and Evolution in Marine Environments, Volume 2*, (eds. C Verde, G. di Prisco), pp. 67-87. Berlin: Springer.
Pörtner, H. O., Hardewig, I., Sartoris, F. J. and Van Dijk, P. L. M. (1998). Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation. In *Cold Ocean Physiology*, (eds. H. O. Pörtner and R. C. Playle), pp. 88-120.Cambridge: Cambridge University Press.
Post, J. R. (1990). Metabolic allometry of larval and juvenile yellow perch (*Perca flavescens*): in situ estimates and bioenergetic models. *Can. J. Fish. Aqua. Sci.* **47**, 554-560.
R Core Team. (2014). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
Robinson, E. (2008). Antarctic Fish: Thermal Specialists or Adaptable Generalists?, PhD, Christchurch: University of Canterbury.
Robinson, E. and Davison, W. (2008). The Antarctic notothenioid fish *Pagothenia borchgrevinki* is thermally flexible: acclimation changes oxygen consumption. *Polar Biol.* **31**, 317-326.
Schlitzer, R. (2011). Ocean Data View, http://odv.awi.de.
Scholander, P., Flagg, W., Walters, V. and Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zool.* **26**, 67-92.
Sil'yanova, S. Z. (1981). Oogenesis and maturity stages of fishes of the family Nototheniidae. *J. Ichthyol.* **22**, 81-89.
Somero, G. N. and De Vries, A. L. (1967). Temperature tolerance of some Antarctic fishes. *Science* **156**, 257-258.
Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiol. Biochem.* **6**, 49-59.
Steffensen, J. F. (2002). Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artefact? Artefact! *Comp. Biochem. Physiol. A* **132**, 789-795.
Steffensen, J. F. (2005). Respiratory Systems and Metabolic Rates. In *The Physiology of Polar Fishes*, vol. 22, (eds. A. P. Farrell and J. F. Steffensen), pp. 203-238. San Diego: Academic Press.
Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65-65.
Strobel, A., Bennecke, S., Leo, E., Mintenbeck, K., Pörtner, H. O. and Mark, F. C. (2012). Metabolic shifts in the Antarctic fish *Notothenia rossii* in response to rising temperature and PCO_2. *Front. Zool.* **9**, 28-28.
Torres, J. J. and Somero, G. N. (1988). Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. *Mar. Biol.* **98**, 169-180.
Turner, J., Barrand, N. E., Bracegirdle, T. J., Convey, P., Hodgson, D. A., Jarvis, M., Jenkins, A., Marshall, G., Meredith, M. P., Roscoe, H. et al. (2014). Antarctic climate change and the environment: an update. *Polar Rec.* **50**, 237-259.
Van Dijk, P. L. M., Tesch, C., Hardewig, I. and Pörtner, H. O. (1999). Physiological disturbances at critically high temperatures: A comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). *J. Exp. Biol.* **202**, 3611-3621.
Vanella, F. A. and Calvo, J. (2005). Influence of temperature, habitat and body mass on routine metabolic rates of subantarctic teleosts. *Sci. Mar.* **69**, 317-323.

West, J. L., Bailey, J. R., Almeida-Val, V. M. F., Val, A. L., Sidell, B. D. and Driedzic, W. R. (1999). Activity levels of enzymes of energy metabolism in heart and red muscle are higher in north-temperate-zone than in Amazonian teleosts. *Can. J. Zool.* **77**, 690-696.
White, C. R., Alton, L. A. and Frappell, P. B. (2012). Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. *Proc. R. Soc. Lond. B* **279**, 1740-1747.
Whitehouse, M., Meredith, M., Rothery, P., Atkinson, A., Ward, P. and Korb, R. (2008). Rapid warming of the ocean around South Georgia, Southern Ocean, during the 20th century: forcings, characteristics and implications for lower trophic levels. *Deep-Sea Res. Pt.I* **55**, 1218-1228.
Wieser, W. (1994). Cost of growth in cells and organisms: general rules and comparative aspects. *Biol. Rev.* **69**, 1-33.
Wohlschlag, D. E. (1960). Metabolism of an Antarctic Fish and the Phenomenon of Cold Adaptation. *Ecology* **41**, 287-292.

2.3. Manuscript II

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

Tina Sandersfeld[1], William Davison[2], Miles D. Lamare[3], Rainer Knust[1], Claudio Richter[4]

Published in

The Journal of Experimental Biology 218, 2373-2381,

doi:10.1242/jeb.122804

[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Section Bentho-Pelagic Processes, Am Alten Hafen 26, 27568 Bremerhaven and University of Bremen (FB 2), Germany

[2]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

[3]Department of Marine Science, University of Otago, 30 Castle Street, Dunedin 9022, New Zealand.

[4]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Section Bentho-Pelagic Processes, Am Alten Hafen 26, 27568 Bremerhaven, Germany

© 2015. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2015) 218, 2373-2381 doi:10.1242/jeb.122804

RESEARCH ARTICLE

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

Tina Sandersfeld[1,*], William Davison[2], Miles D. Lamare[3], Rainer Knust[4] and Claudio Richter[1]

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).

[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Section Bentho-Pelagic Processes, Am Alten Hafen 26, 27568 Bremerhaven and University of Bremen, Germany. [2]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand. [3]Department of Marine Science, University of Otago, 30 Castle Street, Dunedin 9022, New Zealand. [4]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Section Bentho-Pelagic Processes, Am Alten Hafen 26, 27568 Bremerhaven, Germany.

*Author for correspondence (tina.sandersfeld@awi.de)

Received 26 March 2015; Accepted 18 May 2015

The Journal of Experimental Biology

RESEARCH ARTICLE 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

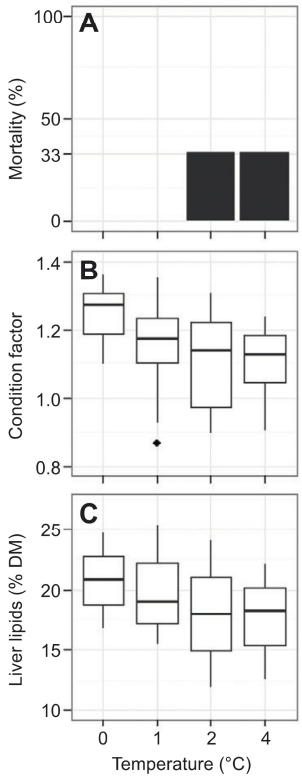

Fig. 1. Mortality and physiological condition of *Trematomus bernacchii* at different temperatures. The percentage mortality (A), condition factor (B) and liver lipid content (C) were measured in fish maintained in aquaria at 0, 1, 2 and 4°C (A and B, N=12 fish at 0 and 1°C; N=8 at 2 and 4°C; C, N=3 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

RESEARCH ARTICLE 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).

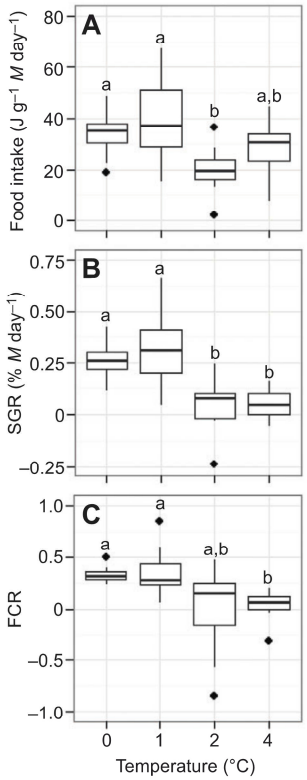

Fig. 2. Food consumption and growth of *T. bernacchii* at different temperatures. Food intake (A), specific growth rate (SGR, B) and food conversion ratio (FCR, C) were measured in fish maintained at 0, 1, 2 and 4°C (*N*=12 at 0 and 1°C, *N*=8 at 2 and 4°C). Different letters above boxes denote significant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

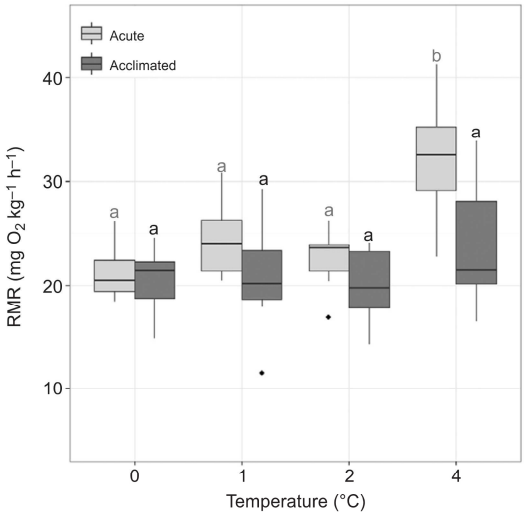

Fig. 3. Routine metabolism of *T. bernacchii* after acute temperature increase and temperature acclimation. Routine metabolic rate (RMR) was measured in fish following acute exposure to 0, 1, 2 and 4°C and after acclimation to these temperatures (acute: *N*=4 at 0°C, *N*=8 at 1 and 2°C, *N*=6 at 4°C;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

RESEARCH ARTICLE 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.

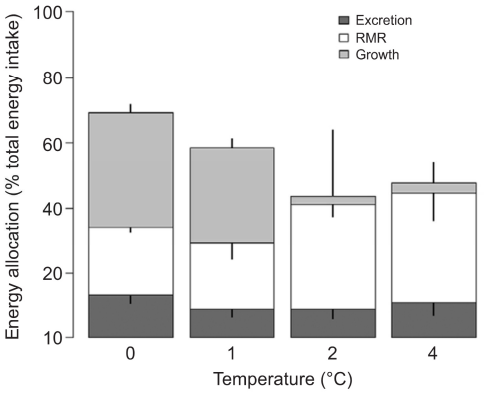

Fig. 4. Energy budget for *T. bernacchii* at different temperatures. Energy allocation to ammonia excretion, routine metabolism (RMR) and growth is given as a percentage of energy taken in as food (means±s.e.m.). Data are for fish maintained in aquaria at 0, 1, 2 and 4°C for which a complete energy budget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

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.

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.

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

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),

RESEARCH ARTICLE 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,

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

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).

Acknowledgements

The authors would like to thank the staff of Antarctic New Zealand for all their support in Antarctica and New Zealand. Moreover, we thank Rob Robins for his help collecting the animals, Rennie Bishop, Alan Woods and Jan McKenzie for their technical assistance during experiments as well as Christiane Lorenzen and Holger Auel for assistance with CN and lipid analysis. Moreover, we thank the two anonymous reviewers for their constructive comments that helped to improve the manuscript.

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.

Funding

This research was supported by a New Zealand Antarctic Research Institute grant to M.D.L. (NZARI 2103-4). T.S. was supported by a PhD scholarship as well as a travel grant of the Helmholtz Graduate School for Polar and Marine Research (POLMAR) and by the PACES II programme.

Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.122804/-/DC1

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

References

Bailey, D. M., Johnston, I. A. and Peck, L. S. (2005). Invertebrate muscle performance at high latitude: swimming activity in the Antarctic scallop, *Adamussium colbecki*. *Polar Biol.* **28**, 464-469.

Baudron, A. R., Needle, C. L. and Marshall, C. T. (2011). Implications of a warming North Sea for the growth of haddock *Melanogrammus aeglefinus*. *J. Fish Biol.* **78**, 1874-1889.

Bilyk, K. T. and DeVries, A. L. (2011). Heat tolerance and its plasticity in Antarctic fishes. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **158**, 382-390.

Björnsson, B. and Tryggvadóttir, S. V. (1996). Effects of size on optimal temperature for growth and growth efficiency of immature Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* **142**, 33-42.

Björnsson, B., Steinarsson, A. and Oddgeirsson, M. (2001). Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). *ICES J. Mar. Sci.* **58**, 29-38.

Boulenger, G. A. (1902). Pisces. Report of the collection of natural history made in the Antarctic regions during the voyage of the 'Southern Cross'. *Bull. Br. Mus. (Nat. Hist.)* **5**, 174-189.

Boyce, S. (1999). Nitrogenous excretion in the Antarctic plunderfish. *J. Fish Biol.* **54**, 72-81.

Boyce, S. J. and Clarke, A. (1997). Effect of body size and ration on specific dynamic action in the Antarctic plunderfish, *Harpagifer antarcticus* Nybelin 1947. *Physiol. Biochem. Zool.* **70**, 679-690.

Brauer, P. R., Sanmann, J. N. and Petzel, D. H. (2005). Effects of warm acclimation on Na^+, K^+-ATPase α-subunit expression in chloride cells of Antarctic fish. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **285A**, 600-609.

Brodte, E., Knust, R. and Pörtner, H. O. (2006). Temperature-dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae). *Polar Biol.* **30**, 95-107.

Bromwich, D. H., Nicolas, J. P., Monaghan, A. J., Lazzara, M. A., Keller, L. M., Weidner, G. A. and Wilson, A. B. (2013). Central West Antarctica among the most rapidly warming regions on Earth. *Nat. Geosci.* **6**, 139-145.

Buckley, B. A. and Somero, G. N. (2009). cDNA microarray analysis reveals the capacity of the cold-adapted Antarctic fish *Trematomus bernacchii* to alter gene expression in response to heat stress. *Polar Biol.* **32**, 403-415.

Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Frölicher, T. L., Lam, V. W. Y., Deng Palomares, M. L., Watson, R. and Pauly, D. (2013). Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nat. Clim. Change* **3**, 254-258.

Clarke, A. (1980). A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. *Biol. J. Linn. Soc.* **14**, 77-92.

Clarke, A. (1993). Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? *Funct. Ecol.* **7**, 139-149.

Cockcroft, A. and Du Preez, H. (1989). Nitrogen and energy loss via nonfaecal and faecal excretion in the marine teleost *Lithognathus lithognathus*. *Mar. Biol.* **101**, 419-425.

Coppes Petricorena, Z. L. and Somero, G. N. (2007). Biochemical adaptations of notothenioid fishes: comparisons between cold temperate South American and New Zealand species and Antarctic species. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **147**, 799-807.

Cussac, V. E., Fernández, D. A., Gómez, S. E. and López, H. L. (2009). Fishes of southern South America: a story driven by temperature. *Fish Physiol. Biochem.* **35**, 29-42.

Dockray, J. J., Reid, S. D. and Wood, C. M. (1996). Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration. *Can. J. Fish. Aquat. Sci.* **53**, 2752-2763.

Donnelly, J., Torres, J. J., Sutton, T. T. and Simoniello, C. (2004). Fishes of the eastern Ross Sea, Antarctica. *Polar Biol.* **27**, 637-650.

Dulvy, N. K., Rogers, S. I., Jennings, S., Stelzenmüller, V., Dye, S. R. and Skjoldal, H. R. (2008). Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. *J. App. Ecol.* **45**, 1029-1039.

Eastman, J. T. and Hubold, G. (1999). The fish fauna of the Ross Sea, Antarctica. *Antarct. Sci.* **11**, 293-304.

Elliott, J. M. and Davison, W. (1975). Energy equivalents of oxygen consumption in animal energetics. *Oecologia* **19**, 195-201.

Enzor, L. A. and Place, S. P. (2014). Is warmer better? Decreased oxidative damage in notothenioid fish after long-term acclimation to multiple stressors. *J. Exp. Biol.* **217**, 3301-3310.

Enzor, L. A., Zippay, M. L. and Place, S. P. (2013). High latitude fish in a high CO_2 world: synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **164**, 154-161.

Fang, J., Tian, X. and Dong, S. (2010). The influence of water temperature and ration on the growth, body composition and energy budget of tongue sole (*Cynoglossus semilaevis*). *Aquaculture* **299**, 106-114.

Fischer, T. (2003). The effects of climate induced temperature changes on cod (*Gadus morhua* L.): linking ecological and physiological investigations. [Die Effekte klimabedingter Temperaturveränderungen auf den Kabeljau (*Gadus morhua* L.): eine Verknüpfung von ökologischen und physiologischen Untersuchungen.] *Reports on Polar and Marine Research* [*Berichte zur Polarforschung*], Vol. **454** Germany: Alfred-Wegener-Institut für Polar- und Meeresforschung.

Folch, J., Lees, M. and Sloane-Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Foster, B. A. and Montgomery, J. C. (1993). Planktivory in benthic notothenid fish in McMurdo Sound, Antarctica. *Environ. Biol. Fish* **36**, 313-318.

Franklin, C. E., Davison, W. and Seebacher, F. (2007). Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *J. Exp. Biol.* **210**, 3068-3074.

Friedrich, C. and Hagen, W. (1994). Lipid contents of five species of notothenioid fish from high-Antarctic waters and ecological implications. *Polar Biol.* **14**, 359-369.

Gonzalez-Cabrera, P. J., Dowd, F., Pedibhotla, V. K., Rosario, R., Stanley-Samuelson, D. and Petzel, D. (1995). Enhanced hypo-osmoregulation induced by warm-acclimation in Antarctic fish is mediated by increased gill and kidney Na^+/K^+-ATPase activities. *J. Exp. Biol.* **198**, 2279-2291.

Handy, R. D. and Poxton, M. G. (1993). Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. *Rev. Fish Biol. Fish.* **3**, 205-241.

Herberich, E., Sikorski, J. and Hothorn, T. (2010). A robust procedure for comparing multiple means under heteroscedasticity in unbalanced designs. *PLoS ONE* **5**, e9788.

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A. and Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.* **56**, 1801-1808.

Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* **50**, 346-363.

Hubold, G. (1992). Ecology of Weddell sea fishes. [Zur Ökologie der Fische im Weddellmeer.] *Reports on Polar Research*. [*Berichte zur Polarforschung*], Vol. **103**. Germany: Alfred-Wegener-Institut für Polar- und Meeresforschung.

Hureau, J.-C. (1970). Biologie comparée de quelques poissons antarctiques (Nototheniidae). *Bull. Inst. Oceanogr. (Monaco)* **68**, 244.

Huth, T. J. and Place, S. P. (2013). De novo assembly and characterization of tissue specific transcriptomes in the emerald notothen, *Trematomus bernacchii*. *BMC Genomics* **14**, 805.

Kiest, K. A. (1993). A relationship of diet to prey abundance and the foraging behavior of *Trematomus bernacchii*. *Polar Biol.* **13**, 291-296.

Kock, K.-H. and Everson, I. (1998). Age, growth and maximum size of Antarctic notothenioid fish – revisited. In *Fishes of Antarctica: A Biological Overview* (ed. **G. di Prisco, E. Pisano and A. Clarke**), pp. 29-40. Milan: Springer.

Kock, K.-H. and Kellermann, A. (1991). Reproduction in Antarctic notothenioid fish. *Antarct. Sci.* **3**, 125-150.

Koehn, R. K. and Shumway, S. E. (1982). A genetic/physiological explanation for differential growth rate among individuals of the American oyster *Crassostrea virginica*. *Mar. Biol. Lett.* **3**, 35-42.

La Mesa, M. and Vacchi, M. (2001). Age and growth of high Antarctic notothenioid fish. *Antarct. Sci.* **13**, 227-235.

La Mesa, M., Vacchi, M., Arneri, E., Giannetti, G. and Greco, S. (1996). Age and growth of the notothenid fish *Trematomus bernacchii* Boulenger from Terra Nova Bay, Antarctica. *Polar Biol.* **16**, 139-145.

La Mesa, M., Dalú, M. and Vacchi, M. (2004). Trophic ecology of the emerald notothen *Trematomus bernacchii* (Pisces, Notothenidae) from Terra Nova Bay, Ross Sea, Antarctica. *Polar Biol.* **27**, 721-728.

Montgomery, J. C., Foster, B. A., Milton, R. C. and Carr, E. (1993). Spatial and temporal variations in the diet of notothenid fish in McMurdo Sound, Antarctica. *Polar Biol.* **13**, 429-431.

Peck, L. S., Souster, T. and Clark, M. S. (2013). Juveniles are more resistant to warming than adults in 4 species of Antarctic marine invertebrates. *PLoS ONE* **8**, e66033.

Peck, L. S., Morley, S. A., Richard, J. and Clark, M. S. (2014). Acclimation and thermal tolerance in Antarctic marine ectotherms. *J. Exp. Biol.* **217**, 16-22.

Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science* **308**, 1912-1915.

Podrabsky, J. E. and Somero, G. N. (2006). Inducible heat tolerance in Antarctic notothenioid fishes. *Polar Biol.* **30**, 39-43.

Pörtner, H. O. (2012). Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* **470**, 273-290.

Pörtner, H. O. and Farrell, A. P. (2008). Physiology and climate change. *Science* **322**, 690-692.

Pörtner, H. O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95-97.

Pörtner, H.-O. and Peck, M. A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J. Fish Biol.* **77**, 1745-1779.

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

The Journal of Experimental Biology

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

Ricker, W. (1975). Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Bd Can.* **191**, 1-382.
Robinson, E. (2008). Antarctic fish: thermal specialists or adaptable generalists? PhD thesis, University of Canterbury, Christchurch, New Zealand.
Seebacher, F., Davison, W., Lowe, C. J. and Franklin, C. E. (2005). A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fishes. *Biol. Lett.* **1**, 151-154.
Steffensen, J. F. (2005). Respiratory systems and metabolic rates. In *Fish Physiology*, Vol. 22 (ed. A. P. Farrell and J. F. Steffensen), pp. 203-238. Burlington, MA: Academic Press.
Strobel, A., Graeve, M., Pöertner, H. O. and Mark, F. C. (2013). Mitochondrial acclimation capacities to ocean warming and acidification are limited in the Antarctic nototheniid fish, *Notothenia rossii* and *Lepidonotothen squamifrons*. *PLoS ONE* **8**, e68865.
Suckling, C. C., Clark, M. S., Richard, J., Morley, S. A., Thorne, M. A. S., Harper, E. M. and Peck, L. S. (2015). Adult acclimation to combined temperature and pH stressors significantly enhances reproductive outcomes compared to short-term exposures. *J. Anim. Ecol.* **84**, 773-784.
Timmermann, R. and Hellmer, H. H. (2013). Southern Ocean warming and increased ice shelf basal melting in the twenty-first and twenty-second centuries based on coupled ice-ocean finite-element modelling. *Ocean Dyn.* **63**, 1011-1026.
Wieser, W. (1994). Cost of growth in cells and organisms: general rules and comparative aspects. *Biol. Rev.* **69**, 1-33.
Windisch, H. S., Kathöver, R., Pörtner, H.-O., Frickenhaus, S. and Lucassen, M. (2011). Thermal acclimation in Antarctic fish: transcriptomic profiling of metabolic pathways. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **301**, R1453-R1466.
Wood, C. M. (2001). Influence of feeding, exercise, and temperature on nitrogen metabolism and excretion. In *Fish Physiology*, Vol. 20 (ed. P. Wright and P. Anderson), pp. 201-238. London: Academic Press.
Xie, S., Zheng, K., Chen, J., Zhang, Z., Zhu, X. and Yang, Y. (2011). Effect of water temperature on energy budget of Nile tilapia, *Oreochromis niloticus*. *Aquacult. Nutr.* **17**, e683-e690.

2.4. Manuscript III

Routine metabolism, growth and excretion in the Antarctic fish *Lepidonotothen nudifrons*: Does temperature affect the effective use of energy resources?

Tina Sandersfeld[1], Magnus Lucassen[2], Nils Koschnick[2], Claudio Richter[1], Rainer Knust[2]

Manuscript (Brief Communications*)

[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Section Bentho-Pelagic Processes, Am Alten Hafen 26, 27568 Bremerhaven and University of Bremen (FB 2), Germany

[2]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany

*the formate 'Brief Communications' does not include any subdivision, but is one continuous text

Abstract

Measurements of temperature-dependent energy allocation of the low-Antarctic *Lepidonotothen nudifrons* were compared to published data of the high-Antarctic *Trematomus bernacchii* to reveal interspecies differences of thermal tolerance on the rarely-studied whole-organism level. In spite of a second stressor besides temperature causing ambiguous patterns in some parameters, energy budget studies proofed to be a useful tool to analyse thermal performance windows in Antarctic fish. Despite its limitations, our data revealed a higher thermal tolerance on the whole-organism level in the low-Antarctic *L. nudifrons* than in the high-Antarctic *T. bernacchii*.

Key words: Notothenioid, Energy allocation, Teleost, Energy budget, Ocean warming

Brief Communications

Climate change affects the ecophysiology of marine organisms with already visible consequences for production and population structures (Perry et al., 2005; Sundby and Nakken, 2008; Pörtner and Peck, 2010; Ohlberger, 2013). In the Antarctic region, marine organisms are highly adapted to the very cold and stable conditions of the Southern Ocean. However, some of the most rapidly warming places were found in this area (Meredith and King, 2005; Whitehouse et al., 2008; Schloss et al., 2012; Bromwich et al., 2013). Stenothermal Antarctic fish were shown to possess a very low thermal tolerance. Indeed, results are mostly derived from acute or short-term temperature exposure and often focused on lower organisational levels or metabolic rate (Bilyk and DeVries, 2011; Strobel et al., 2013; Enzor and Place, 2014; Sleadd et al., 2014). Especially for Antarctic fish, experimental studies at the whole-organism level are rare, but give most relevant information about potential population responses and possible associated consequences for ecosystems. Previously, we analysed the impact of elevated temperature on energy allocation of the high-Antarctic fish *Trematomus bernacchii*. In this species, a temperature increase of 2°C caused growth reductions of up to 84%, highlighting the low thermal tolerance of high-Antarctic fish (Sandersfeld et al., 2015). Differences in thermal tolerance between species from lower and higher Antarctic latitudes were shown in other studies (Brodte et al., 2006; Bilyk and DeVries, 2011). A comparison of ecologically similar species, is most interesting considering potential climate induced shifts in species distribution that might cause co-occurrence of low- and high-Antarctic species in some habitats, as observed in northern latitudes (Perry et al., 2005; Renaud et al., 2012).

Thus, we aimed to produce a completely comparable data set on energy allocation parameters of an Antarctic fish species with northern (low-)Antarctic distribution, for comparison with the high-Antarctic *T. bernacchii* (Sandersfeld et al., 2015). *Lepidonotothen nudifrons,* a benthic rather inactive species, mainly feeding on polychaetes, amphipods and isopods with a distribution ranging from the Antarctic Peninsula to South Georgia in the Scotia Arc was chosen as model organism (Gon and Heemstra, 1990). We hypothesised a higher thermal tolerance of the low-Antarctic *L. nudifrons* to be paralleled by lower trade-offs of elevated temperature for the whole-organism level, compared to the high-Antarctic *T. bernacchii*.

We measured the effects of acclimation to 0, 2, 4 and 6°C on routine metabolism, growth, food intake, ammonia as well as faecal excretion in this species to assess its acclimation capacity and potential consequences of warming for population structures and abundances.

The experiments have been approved by the veterinary inspection office (Senatorin für Bildung, Wissenschaft und Gesundheit, Bremen, Germany) under the permit references AZ: 522-27-11/02-00 (93) and ‚SYNKLIFisch'. *L. nudifrons* were caught near Elephant Island at a depth of 70 to 322 m, water temperature of 0.0 to 0.8°C and salinity of 34‰ by bottom trawl in March/April 2012 (RV Polarstern, ANT-XXVIII/4). Animals were transported to the Alfred Wegener Institute in Bremerhaven (Germany) and kept in aquaria at temperatures of 0 to 1°C until the start of the experiments in February 2013. Standard length of the fish varied from 8.8 to 16.5 cm, total length from 10.0 to 17.9 cm and body mass from 7.9 to 65.2 g, with no significant difference among treatment groups (ANOVA: $p > 0.05$). During the growth experiment, fish were held in four groups, separated in closed aquarium systems at 0, 2, 4 and 6°C (0°C n = 8, 2°C n = 7, 4°C n = 6, 6°C n = 7) for a period of about 60 days. During this time, individual food intake was recorded and energy allocation to growth (size/weight determination), routine metabolism (oxygen consumption measurements), ammonia excretion and faecal excretion were determined. The experimental set up and protocol was identical to that described in Sandersfeld et al. (2015) regarding growth, feeding intervals and routine metabolism measurements as well as tissue, stoichiometric and statistical analysis. Yet, krill (*Euphausia pacifica*) was used as food and food wet mass was used for food intake determination. Besides, standard length instead of total length was used for calculation of the condition factor. During the first respiration measurement, it was attempted to determine SDA. However, metabolic rate did not approach baseline levels ten days after feeding for the 2°C group. As long starvation periods were assumed to reduce growth, determinations of scope and duration of SDA were abandoned and routine metabolic rates including SDA were measured for all animals as described in Sandersfeld et al. (2015). Similarly, as in the study by Sandersfeld et al. (2015), ammonia and faecal excretion measurements were done in combination with oxygen consumption measurements before and after the acclimation period. Respiration measurements were carried out in a closed tank for every animal. After the respiration measurements, faeces produced by the fish were siphoned into a beaker and filtered onto pre-weight, organic-free glass fiber filters together with 100 ml of water from the sampling beaker. Additionally, a sample of 100 ml water was filtered onto a control filter to account for particulates in the water. Filters were stored at -20°C until analysis. Next, the water in the tank was mixed, a water sample was taken and frozen at -20°C until being analysed for ammonia concentration following Holmes et al. (1999). Filters were oven dried at 57°C. Faecal excretion was determined gravimetrically and by using Euro EA Elementar Analyser (Hekatech GmbH, Wegberg, Germany) for analysis of carbon and nitrogen.

Table 1 Condition, energy conversion and growth parameters of *L. nudifrons* at different temperatures (means ± s.e.m.). Number of replicates (n) is 6, 5, 4 and 1 for 0, 2, 4 and 6°C respectively, if not stated otherwise.
DM: dry mass; M: body mass

Temperature	0°C	2°C	4°C	6°C
Mortality [%]	25.0 (n=8)	28.6 (n=7)	33.3 (n=6)	85.7 (n=7)
Condition factor	1.32±0.04[a]	1.35±0.04[a]	1.10±0.05 [b]	1.38
Food intake [J g^{-1} M d^{-1}]	19.19±2.17[a]	30.63±3.49[ab]	34.73±5.92[b]	34.84
Specific growth rate [% M d^{-1}]	-0.09±0.08	0.01±0.02	-0.11±0.02	-0.05
Growth [% SL d^{-1}]	-0.006±0.011	0.024±0.013	0.005±0.006	-0.026
Feed conversion ratio	-0.14±0.11	0.00±0.02	-0.10±0.036	-0.03
Energy content white muscle [J g DM^{-1}]	22085±541 (n=3)	21118±1760 (n=3)	19094±688 (n=3)	24677 (n=1)
Water content white muscle [%]	81.77±0.63 (n=3)	81.80±0.30 (n=3)	82.28±0.41 (n=3)	81.63 (n=1)
Lipid content liver [% DM]	15.05±1.94 (n=3)	15.97±1.42 (n=3)	18.28±2.61 (n=3)	25.67 (n=1)
Faeces nitrogen [% N g^{-1} M g $food^{-1}$ d^{-1}]	0.37±0.16	0.90±0.39	1.45 ±0.50	0.41
NH_4 excretion [µmol g^{-1} M h^{-1}]	0.092±0.012 (n=6)	0.135±0.022 (n=4)	0.103±0.016 (n=2)	0.241 (n=1)

Faecal quantities were not sufficient for calorimetric analysis. Produced faeces were related to initial food intake at first feeding before the first respiration measurement for the first run. For the second run of faecal determination, faecal production was related to mean food intake per day during the experiment, as digestion times were unknown.

To enhance the comparison of data of high- and low-Antarctic species, as well as expected results and data recorded in this study, results are shown in parallel with and discussed in respect to data of *T. bernacchii* from a completely comparable experiment recently published by Sandersfeld et al. (2015) and highlighted in grey in Fig. 1 to 4.

In temperature experiments, elevated mortality is often an indicator of stress. For example the negative effect of temperature is shown by 33% mortality of *T. bernacchii* at 2 and 4°C compared to 0% in the control treatment (0°C) (Fig. 1A). However, in this experiment with *L. nudifrons*, elevated mortality of 25 to 29% in low temperature treatments at 0 and 2°C indicates the presence of an unknown stressor besides temperature and thereby questions the significance of results (Fig. 1A, Table 1). We will still analyse this data in comparison to

published data of *T. bernacchii*, to show that in spite of this limitation, a clear metabolic response to temperature can be identified. We show that even data of experiments with restricted explanatory power, as in this case, can contribute to our understanding of thermal tolerance in Antarctic fish.

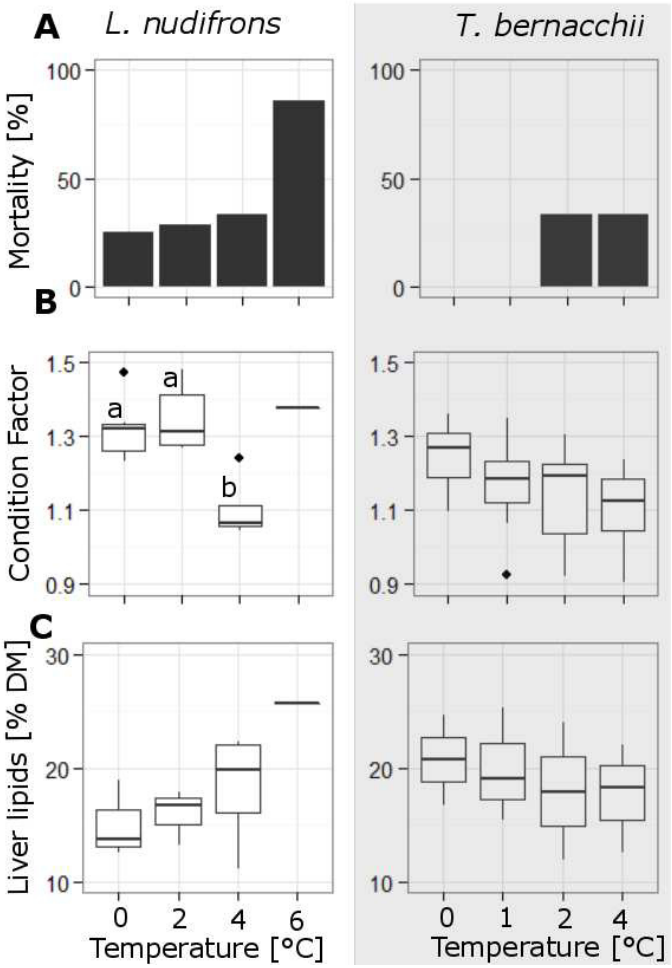

Fig. 1 Mortality (A), condition factor (B) and liver lipid content (C) of *L. nudifrons* and *T. bernacchii* at different temperatures (*L. nudifrons*: A: 0°C: n=8, 2°C: n=7, 4°C: n=6, 6°C: n=7; B: 0°C: n=6, 2°C: n=5, 4°C: n=4, 6°C: n=1; C: n=3 for all temperatures; data from this study; *T. bernacchii*: A & B: 0 & 1°C: n=12, 2 & 4°C: n=8; C: n=3 for all temperatures; *T. bernacchii*-data from Sandersfeld et al. 2015).

Despite a general high mortality, an increase of mortality from 33% at 4°C to 85% at 6°C indicates a significant impact of temperature on *L. nudifrons* (Fig. 1A, Table 1). Only one of seven fish at 6°C survived the complete acclimation period. Data of this individual is shown in Fig. 1 to 4 and Table 1. However, we will not further discuss this data point due to the limited sample size. A stress induced increase in mortality is usually paralleled with a decreasing condition, as seen for *T. bernacchii* (Fig. 1B). While the condition factor for *L. nudifrons* is comparable for fish at 0 and 1°C, a significant decrease at 4°C indicates a negative effect of temperature (ANOVA: $p = 0.003$; post hoc: 0 vs. 4°C: $p = 0.007$, 2 vs. 4°C:

p = 0.004, Fig. 1B). With decreasing condition at 4°C, a depletion of energy stores, e.g. in form of liver lipids, would be expected, as observed for *T. bernacchii* in Fig. 1C. In contrast, liver lipid content, showed a non-significant but increasing trend with increasing temperature in *L. nudifrons* (Fig. 1C), while the hepatosomatic index did not show any temperature correlation. Increasing energy storage in the liver could suggest surplus energy being available for deposition in storage sites, but it might also suggest lacking metabolic capacity for mobilisation of energy stores.

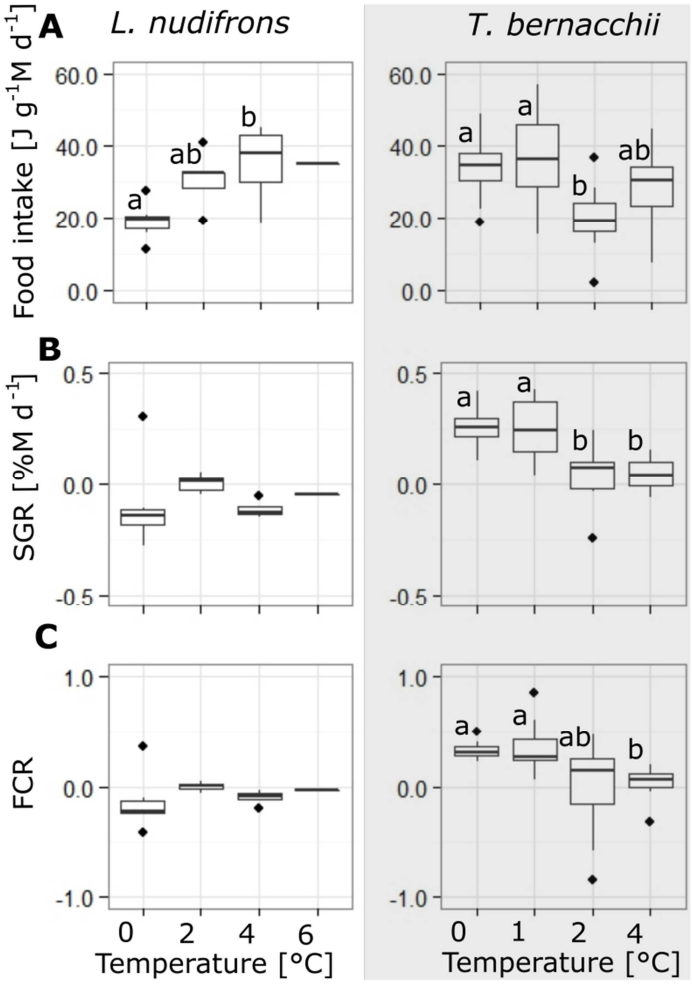

Fig. 2 Food intake (A), specific growth rate (SGR) (B) and feed conversion ratio (FCR) (C) of *L. nudifrons* and *T. bernacchii* at different temperatures. Different letters above boxes denote significant differences, similar letters denote lack of differences between temperatures (*L. nudifrons*: 0°C: n=6, 2°C n=5, 4°C: n=4, 6°C n=1; *T. bernacchii*: 0 & 1°C: n=12, 2 & 4°C: n=8; *T. bernacchii*-data from Sandersfeld et al. 2015).

Increased food intake of *L. nudifrons* with increasing temperature indicates a higher energy demand and thus a higher food intake of animals in warmer treatments, with a significant difference between 0 and 4°C (ANOVA: p = 0.026; Tukey: 0 vs. 4°C: p = 0.031, Fig. 2A). However, food conversion ratios (FCR) close to or below zero at all treatment temperatures

indicate an insufficient energy intake that did not allow energy assimilation (Fig. 2C). As a consequence, specific growth rate (SGR) of this low-Antarctic species showed a comparable development with a slightly positive value at 2°C and negative values at 0 and 4°C (Fig. 2B), while tissue energy and water content were comparable at all treatments (Table 1). Length growth was close to zero at all treatment temperatures, but 2°C. However, positive growth of *L. nudifrons* at 2°C was still low compared to growth rates (length) of the high-Antarctic *T. bernacchii* or the Antarctic eelpout *Pachycara brachycephalum* (Brodte et al., 2006; Sandersfeld et al., 2015).

Results of *L. nudifrons* shown in Fig. 2 suggest a general energy shortage in spite of *ad libitum* feeding. This is supported by a lower food intake compared to *T. bernacchii*, also at low temperatures (Fig. 2A). The krill food used for *L. nudifrons* closely resembled natural food sources of this species (Gon and Heemstra, 1990). However, the amount of physiologically useable net energy of krill might be lower due to higher costs of digestion for chitin containing crustaceans (Secor, 2009). Most *L. nudifrons* did not feed every second day, when food was offered. Thus, feeding intervals seemed to be sufficient.

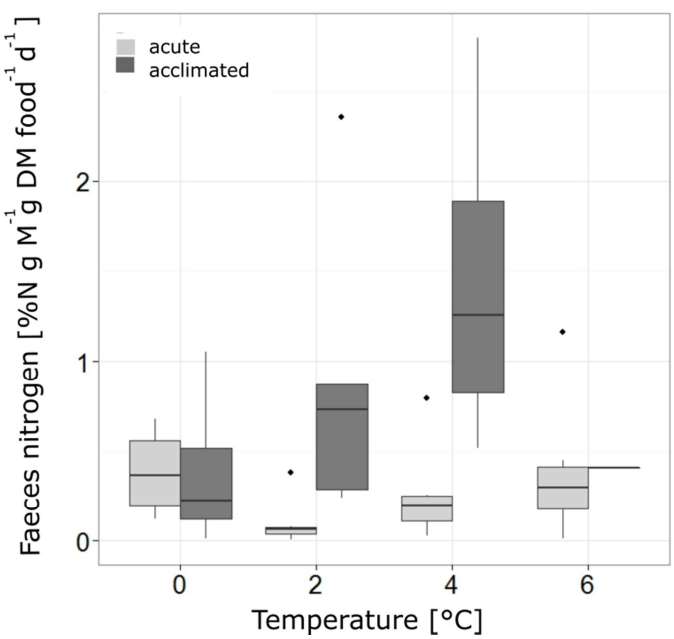

Fig. 3 Faeces nitrogen content of *L. nudifrons* after acute temperature increase (light grey boxes) and temperature acclimation (dark grey boxes) (acute 0°C: n=8, 2°C: n=7, 4°C: n=6, 6°C: n=7; acclimated 0°C: n=6, 2°C: n=5, 4°C: n=4, 6°C: n=1).

In the high-Antarctic *T. bernacchii*, SGR and FCR declined at 4°C, despite of similar food intake compared to the control group (Fig. 2B, C). This indicated less efficient energy assimilation in the high-Antarctic species and was supported by increasing trends of nitrogen

content in faeces with increasing temperature (data not shown, Sandersfeld et al., 2015). Similarly, an increasing trend of nitrogen content in faeces at higher temperatures was found in L. *nudifrons* (Fig. 3, Table 1). Ammonia excretion in *L. nudifrons* seemed to be low compared to that of *T. bernacchii* (Sandersfeld et al., 2015). Comparison to ammonia excretion data of other Antarctic fish species is difficult due to large deviations in experimental protocol (fish size, time of measurement, ration size) that likely affect excretion rates (Boyce and Clarke, 1997; Boyce, 1999). In combination with low growth rates, low ammonia excretion also hints to a potential energy shortage in *L. nudifrons*.

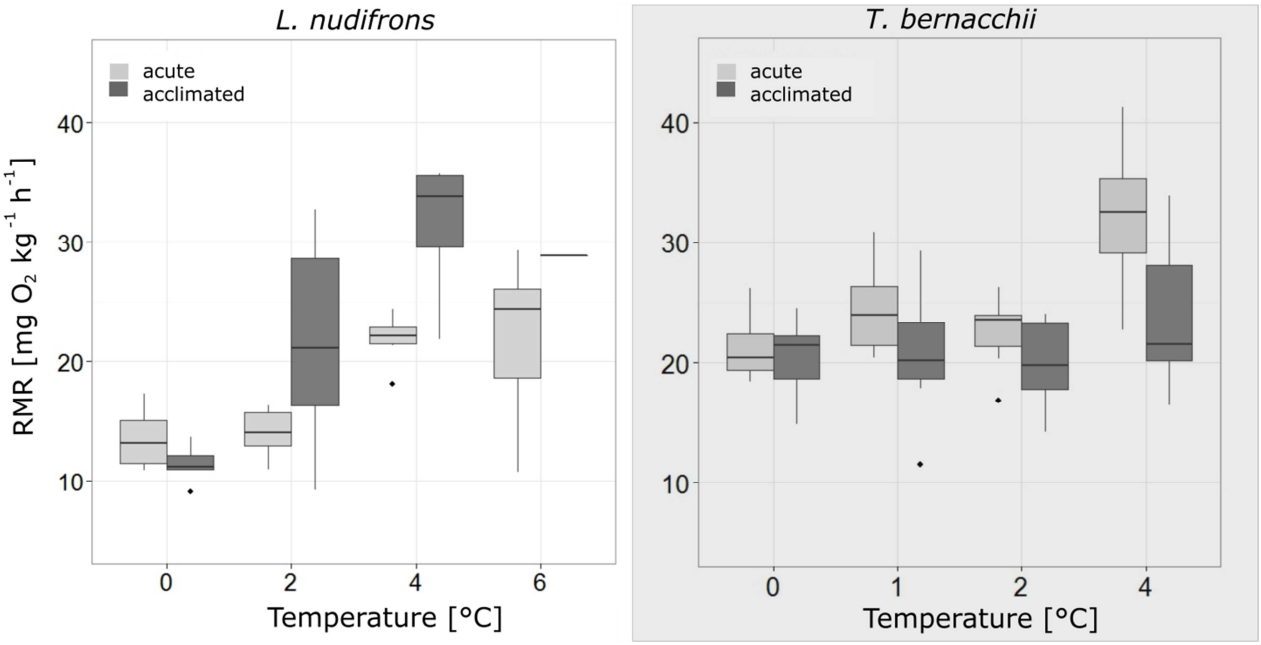

Fig. 4 Routine metabolic rate (RMR) of *L. nudifrons* and *T. bernacchii* after acute temperature increase (light grey boxes) and temperature acclimation (dark grey boxes). Different letters above boxes denote significant differences, similar letters denote lack of differences between measurements (*L. nudifrons*: acute 0°C: n=8, 2°C: n=7, 4°C: n=6, 6°C: n=7; acclimated 0°C: n=6, 2°C: n=5, 4°C: n=4, 6°C: n=1; *T. bernacchii*: acute 0°C: n=4, 1 & 2°C: n=8, 4°C: n=6; acclimated 0°C: n=7, 1, 2 & 4°C: n=8; ; *T. bernacchii*-data from Sandersfeld et al. 2015).

Routine metabolic rates after an acute temperature increase as well as after acclimation display an increase with temperature as expected from our current understanding of thermal tolerance (Fig. 4; Pörtner, 2010). Significantly elevated metabolic rates (RMR) at 4 and 6°C in *L. nudifrons* after acute temperature increase indicate elevated metabolic costs (ANOVA: $p < 0.001$; posthoc: 0 vs. 4°C: $p < 0.001$, 0 vs. 6°C: $p = 0.026$, 2 vs. 4°C: $p < 0.001$, 2 vs. 6°C: $p = 0.040$, Fig. 4). The comparison to data of *T. bernacchii* shows similar acute thermal tolerance of the high-Antarctic species (Fig. 4). After acclimation to treatment temperatures a decrease of metabolic rates would be expected, as metabolism is suggested to acclimate to stressful environmental conditions after a sufficient acclimation time (Robinson and Davison, 2008; Sandersfeld et al., 2015; Seebacher et al., 2015). For instance, acclimation of RMR at

4°C was observed for *T. bernacchii* (Fig. 4). In contrast, we observed an increasing trend of RMR after the acclimation period in *L. nudifrons*. After acclimation, fish at 4°C showed a significantly elevated metabolic rate compared to fish at 0°C (ANOVA: $p = 0.002$; posthoc: 0 vs. 4°C: $p < 0.001$). This observation implies that the stress level of the fish increased during the acclimation period, hinting to an additional stressor besides temperature, as suggested by various other parameters. Such a stressor could exist in the abiotic conditions of the aquarium system, e.g. regarding water quality, as well as in biotic conditions, such as the food source or the bacterial community in the water. However, artificial sea water was used in this experiment and water parameters were checked regularly to ensure water quality. As all fish were kept in individual cages, social stress is unlikely for this not in groups living notothenioid.

In summary, increasing food intake, liver lipid and faeces nitrogen content in parallel to low growth and FCR at warm temperatures in *L. nudifrons* are contradictory. While enough energy for the build-up of energy stores in the liver seems to be available, energy is not assimilated in form of body tissue but excreted via faeces. Based on this ambiguous data, we were not able to draw conclusions on the absolute thermal tolerance of *L. nudifrons*. A high mortality in the control treatment can generally question the results of an experiment. Though, it is noteworthy that a thermal response can still be distinguished in the data and is comparable to that of other Antarctic fish (Brodte et al., 2006; Sandersfeld et al., 2015). The combination of decreasing condition factor, SGR, FCR as well as increasing mortality and RMR at 4 and 6°C suggest a negative impact of temperature at 4°C. While condition and mortality of *L. nudifrons* are comparable at 0 and 1°C, food intake as well as growth is lower at 0°C, indicating lower performance around 0°C. In the Weddell Sea, *Lepidonotothen* species were found in a temperature range of -1 to 2°C, while abundances were highest at temperatures above 0°C (Meyer, 2012). This might suggest a lower thermal limit in this species around 0°C. Even though thermal sensitivity can be increased by additional stressors (Pörtner and Farrell, 2008), it is unlikely to be decreased. Thus, our results are rather underestimating the thermal tolerance of this species.

For the high-Antarctic *T. bernacchii*, significant trade-offs for the whole organism were found at 2°C (cf. Fig. 1-4; Sandersfeld et al., 2015), indicating a lower thermal tolerance of the high- compared to the low-Antarctic species. *L. nudifrons* might live close to its lower thermal limit at the western Antarctic Peninsula and our data suggest this species to have adaptation potential to warmer temperatures around 4°C. In contrast, the high-Antarctic *T. bernacchii* is

unlikely to have a wide scope for acclimation to increasing temperature in its natural habitat (cf. Sandersfeld et al., 2015). Projections suggest warming of sea water temperature in the Western Antarctic Peninsula region not to exceed 2°C within the next 100 years (Timmermann and Hellmer, 2013, R. Timmermann personal communication 2015). Disregarding associated ecological changes, our results suggest this to be a manageable scenario for *L. nudifrons*. Despite the limitations of data from this study due to the presence of an unknown second stressor, we were able to identify metabolic patterns in response to increased temperature. Thus, energy budget studies proofed to be a good tool to analyse the thermal tolerance window of Antarctic fish as well as temperature-dependent trade-offs to changing environmental conditions.

Acknowledgements

We would like to thank the master and crew of RV Polarstern for valuable field support during ANT-XXVIII/4, Fredy Véliz Moraleda for animal care, Ulrike Holtz and Richard Steinmetz for help with aquaria maintenance as well as Jessica Peterei for great support during experiments. This study was supported by Polarstern Grant No. AWI_PS79-04. Tina Sandersfeld was partly supported by a Ph.D. scholarship of the Helmholtz Graduate School for Polar and Marine Research and by the PACES II programme.

References

Bilyk, K. T. and DeVries, A. L. (2011). Heat tolerance and its plasticity in Antarctic fishes. *Comp. Biochem. Physiol. A* **158**, 382-390.
Boyce, S. (1999). Nitrogenous excretion in the Antarctic plunderfish. *J. Fish Biol.* **54**, 72-81.
Boyce, S. J. and Clarke, A. (1997). Effect of body size and ration on specific dynamic action in the Antarctic plunderfish, *Harpagifer antarcticus* Nybelin 1947. *Physiol. Biochem. Zool.* **70**, 679-690.
Brodte, E., Knust, R. and Pörtner, H. O. (2006). Temperature-dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae). *Polar Biol.* **30**, 95-107.
Bromwich, D. H., Nicolas, J. P., Monaghan, A. J., Lazzara, M. A., Keller, L. M., Weidner, G. A. and Wilson, A. B. (2013). Central West Antarctica among the most rapidly warming regions on Earth. *Nature Geosci.* **6**, 139-145.
Enzor, L. A. and Place, S. P. (2014). Is warmer better? Decreased oxidative damage in notothenioid fish after long-term acclimation to multiple stressors. *J. Exp. Biol.* **217**, 3301-3310.
Gon, O. and Heemstra, P. C. (eds) (1990). Fishes of the Southern Ocean. Grahamstown: JLB Smith Institute of Ichthyology.

Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A. and Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aqua. Sci.* **56**, 1801-1808.
Meredith, M. P. and King, J. C. (2005). Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century. *Geophys. Res. Lett.* **32**.
Meyer, S. (2012). The role of temperature on the distribution of fish fauna in Antarctic waters, B.Sc., Bremen: University of Bremen (FB2).
Ohlberger, J. (2013). Climate warming and ectotherm body size – from individual physiology to community ecology. *Funct. Ecol.* **27**, 991-1001.
Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science* **308**, 1912-1915.
Pörtner, H. O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **213**, 881-893.
Pörtner, H.- O. and Peck, M. (2010). Climate change effects on fishes and fisheries: towards a cause and effect understanding. *J. Fish Biol.* **77**, 1745-1779.
Pörtner, H. O. and Farrell, A. P. (2008). Physiology and climate change. *Science* **322**, 690-692.
Renaud, P. E., Berge, J., Varpe, Ø., Lønne, O. J., Nahrgang, J., Ottesen, C. and Hallanger, I. (2012). Is the poleward expansion by Atlantic cod and haddock threatening native polar cod, *Boreogadus saida*? *Polar Biol.* **35**, 401-412.
Robinson, E. and Davison, W. (2008). The Antarctic notothenioid fish *Pagothenia borchgrevinki* is thermally flexible: acclimation changes oxygen consumption. *Polar Biol.* **31**, 317-326.
Sandersfeld, T., Davison, W., Lamare, M., Knust, R. and Richter, C. (2015). Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. *J. Exp. Biol.* **218**, 2373-2381.
Schloss, I. R., Abele, D., Moreau, S., Demers, S., Bers, A. V., González, O. and Ferreyra, G. A. (2012). Response of phytoplankton dynamics to 19-year (1991–2009) climate trends in Potter Cove (Antarctica). *J. Marine Syst.* **92**, 53-66.
Secor, S. M. (2009). Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. B* **179**, 1-56.
Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Clim. Change* **5**, 61-66.
Sleadd, I. M., Lee, M., Hassumani, D. O., Stecyk, T. M., Zeitz, O. K. and Buckley, B. A. (2014). Sub-lethal heat stress causes apoptosis in an Antarctic fish that lacks an inducible heat shock response. *J. Therm. Biol.* **44**, 119-125.
Strobel, A., Graeve, M., Pörtner, H. O. and Mark, F. C. (2013). Mitochondrial acclimation capacities to ocean warming and acidification are limited in the Antarctic nototheniid fish, *Notothenia rossii* and *Lepidonotothen squamifrons*. *PloS One* **8**, e68865.
Sundby, S. and Nakken, O. (2008). Spatial shifts in spawning habitats of Arcto-Norwegian cod related to multidecadal climate oscillations and climate change. *ICES J. Mar. Sci.* **65**, 953-962.
Timmermann, R. and Hellmer, H. H. (2013). Southern Ocean warming and increased ice shelf basal melting in the twenty-first and twenty-second centuries based on coupled ice-ocean finite-element modelling. *Ocean Dynam.* **63**, 1011-1026.
Whitehouse, M., Meredith, M., Rothery, P., Atkinson, A., Ward, P. and Korb, R. (2008). Rapid warming of the ocean around South Georgia, Southern Ocean, during the 20th century: forcings, characteristics and implications for lower trophic levels. *Deep-Sea Res. Pt.I* **55**, 1218-1228.

2.5. Manuscript IV

Energy allocation to growth as an indicator of sensitivity to climate change - an analysis of temperature-dependent growth of fish species from different latitudes

Tina Sandersfeld[1], Kristina L. Kunz[1], Felix C. Mark[2], Claudio Richter[1], Holger Auel[3], Rainer Knust[2]

Manuscript

[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven and University of Bremen (FB 2), Germany

[2]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany

[3]University of Bremen (FB 2), BreMarE - Bremen Marine Ecology, Leobener Str. NW2, 28359 Bremen, Germany

Abstract

Climate change affects the physiology of marine ectotherm organisms, such as fish. In recent years, impacts of rising temperatures on fish distribution have been reported from several marine regions. Growth and reproduction are among the driving forces for fish population structures and abundances. In the framework of this study, we analysed temperature-dependent growth performance from experimental studies of fish species with different latitudinal distribution. We found a decreasing width of the thermal tolerance window for growth performance with increasing latitude. Moreover, lower growth rates at higher latitudes imply differences in energy allocation to growth. These results are discussed with regard to recent physiological models and energy allocation patterns. We hypothesise that high-latitude fish species are particularly susceptible to climate change due to the combination of slow growth and high thermal sensitivity of growth performance. Narrow thermal windows might make polar species more likely to encounter temperatures unfavourable for growth compared to temperate species with a broader thermal tolerance. Given the low growth rates at high latitudes, additional temperature induced reductions with progressive ocean warming may have significant consequences for population structures and species distribution of polar fish.

Key words: Thermal window, Ocean warming, Teleost, Thermal tolerance, Polar fish

Introduction

The efficiency of energy utilisation determines the fitness of an organism. For heterotrophic organisms, food intake represents the energy input, which is used to cover all vital functions. While routine metabolism covers the essential processes to keep an organism alive, including costs of digestion and spontaneous activity, the energy allocated to growth and reproduction is the driving factor for population structure and abundance (Jobling, 1994; Pörtner et al., 2001; Pörtner and Knust, 2007). Energy allocation is influenced by biotic factors, such as prey availability, predation pressure and lifestyle, depending on habitat characteristics. Moreover, abiotic factors, such as temperature, affect energy allocation. At optimal temperature conditions, an organism allocates a large part of its energy input into growth and reproduction as well as in the build-up of energy stores. In contrast, under stress, i.e. at sub-optimal temperature conditions, energy is often limited and can only cover costs of essential functions, such as standard metabolism (Pörtner, 2010; Sokolova et al., 2012). Thus, the width of an organism's thermal window describes the temperature-dependent performance and thereby the efficient use of energy resources of single individuals of a population. In the course of climate change, temperature in the ocean changes at an exceptional pace, making an organism's thermal tolerance an essential trait (Pörtner and Knust, 2007).

Published models and literature describe a shift of fish abundances as well as decreases in production as consequences of ocean warming (Murawski, 1993; Perry et al., 2005; Dulvy et al., 2008; Cheung et al., 2010). While migration to colder areas cannot always fully compensate for the effects of progressive warming (Pörtner et al., 2014), decreasing production is likely to be connected to impacts of temperature on energy budgets, becoming evident in limited energy allocation to growth (Sokolova et al., 2012).

Variations of growth rates between species as well as populations across latitudes have often been discussed in literature. In general, high-latitude species seem to show slower growth rates compared to temperate species (DeVries and Eastman, 1981; Jobling, 1997; Kock and Everson, 1998; Pörtner et al., 2005). Low temperature at high-latitudes limits catabolic as well as anabolic biochemical reactions including growth. In addition, low food availability can limit growth in polar regions. Moreover, factors such as density effects, predation pressure, genetic differences, activity and seasonality can account for differences in growth between species (Boehlert and Kappenman, 1980; Kock and Everson, 1998; Björnsson and Steinarsson, 2002; Lorenzen and Enberg, 2002; Pörtner et al., 2005; Yamahira et al., 2007).

Here, we present a comparison of temperature-dependent growth rates of fish species with different latitudinal distributions under comparable laboratory conditions from Arctic, Antarctic and temperate regions. We test the hypothesis that in spite of comparable maintenance and feeding conditions, absolute growth rates and thermal tolerance of growth performance decline with increasing latitude from temperate to polar fish. The results will be discussed in an energy allocation framework as well as regarding potential implications of ocean warming on growth performance.

Methods

We performed a meta-analysis of published data on temperature-dependent growth rates of species with different latitudinal distribution, as summarised in Table 1. This new combination of data from fish covering a latitudinal range from 77°N to 77°S allows a comparison of species from both polar regions to the temperate counterparts.

All studies included, used a comparable experimental design (Table 1). Fish species were acclimated to different temperatures, while being fed *ad libitum*. Feeding intervals were depending on the feeding activity of the fish, associated to acclimation temperature. Fish kept at warm temperatures were more active and fed daily (Fonds, 1989; Fonds et al., 1992), while fish at lower temperatures were fed only every second or third day (Brodte et al., 2006a; Sandersfeld et al., 2015). Supplied food was usually similar to natural food sources of the respective species. Fish, cockle and mussel meat were used as food in most studies. Only during experiments with the two gadoid species, industrial fish feed was used and fish were fed only every fourth day. With the exceptions of *Gadus morhua* and *Boreogadus saida*, which were relatively active, all other species had a rather inactive lifestyle. All studies were conducted in the respective summer season, except for that of *Pachycara brachycephalum*, which was carried out throughout the year. However, it is questionable whether seasonal factors affect growth of this species with deep-sea origin, caught from relatively deep depth (200-800 m) (Brodte et al., 2006b). Studies on *Pleuronectes platessa* and *Platichthys flesus* at high temperatures were conducted in summer, while low temperature experiments took place in winter. Periods of growth measurements (i.e. acclimation times) varied from 11 to 120 days. A shorter acclimation time likely increases stress levels of animals and potentially reduces growth. However, shortest acclimation times were used for warm-temperate species with highest growth rates (cf. Table 1) and are unlikely to bias data interpretation. For a comparison of growth rates, size differences among species have to be considered.

Table 1 Summary of data used in this study, including distributional range, habitat temperature range and lifestyle of the species as well as experimental temperature range, acclimation time, experimental feeding interval, season in which experiments were conducted, size range of fish and references of data.

Species	Distribution range	Habitat temp. range [°C]	Lifestyle	Experimental temp. range [°C]	Acclimation time [d]	Exp. Feeding interval [d]/ food	Season	Fish size range [cm]	Reference
Platichthys flesus	temperate	5 - 25	inactive	2 - 22	11 - 28	1-3*/ mussel meat	high temp. summer, low temp. winter	4 - 30	Fonds et al. 1992
Pleuronectes platessa	temperate	2 - 15	inactive	2 - 22	11 - 28	1-3*/ mussel meat	high temp. summer, low temp. winter	3 - 31	Fonds et al. 1992
Zoarces viviparus	temperate	3 - 24	inactive	2 - 22	28 - 36	1/ shrimp & mussel meat	summer	10 - 24	Fonds et al. 1989
Gadus morhua	temperate	0 - 8	active	3 - 16	133	4/ industrial feed	summer	11 - 25	Kunz et al. unpublished
Myxoscephalus scorpius	temperate/ Arctic	-1.5 - 16	inactive	5 - 20	28 - 36	1/ shrimp & mussel meat	summer	8 - 18	Fonds et al. 1989
Boreogadus saida	Arctic	-1.5 - 3.2	intermediate active	0 - 8	130	4/ industrial feed	summer	11 - 16	Kunz et al. unpublished
Pachycara brachycephalum	Antarctic	0 - 0.6	inactive	0 - 6	120	2/ cockle meat	summer/ winter**	15 - 27	Brodte et al. 2006
Trematomus bernacchii	Antarctic	-1.9 - -0.3	inactive	0 - 4	59 - 70	2/ fish fillet	summer	8 - 17	Sandersfeld et al. 2015

Additional information **Table 1**:
*depending on experimental temperature; **experiments were conducted throughout the year
Habitat temperature ranges derived from fishbase.org (Froese and Pauly, 2015) for *P. flesus*, *P. platessa*, *M. scorpius*; from Brodte et al. (2006a) for *Z. viviparus*; from Ottersen et al. (1998) for *G. morhua*; from Falk-Petersen et al. (1986) for *B. saida*; from Brodte et al. (2006b) for *P. brachycephalum*; from Hunt et al. (2003) for *T. bernacchii*.

Yet, for data used in this analysis, fish sizes were similar in all studies (cf. Table 1). For data analysis, 2nd order polynomial fits were used to relate growth data to temperature (cf. Fig. 1).

Results

Fig. 1 displays temperature-dependent growth rates of different fish species. Highest maximum growth rates were found for the temperate species *P. flesus* and *P. platessa*, exceeding maximum growth rates of the temperate *Z. viviparus* and *G. morhua* by a factor of two. The Arctic *M. scorpius* and *B. saida* showed intermediate growth rates, followed by the Antarctic *P. brachycephalum* and finally *T. bernacchii* with the lowest absolute growth rates recorded. A similar pattern is apparent for the width of the growth performance window. While the warm-temperate species *P. flesus* and *P. platessa* display a broad thermal window regarding growth performance, the width of the thermal window decreases towards polar species, with the high-Antarctic *T. bernacchii* displaying the narrowest thermal tolerance window. The Antarctic species *T. bernacchii* and *P. brachycephalum* start with low growth rates at low temperatures, which increase only little when temperature rises. Subsequently, growth rates decline quickly when experimental temperatures exceed the habitat temperature range, resulting in short and shallow curves. In contrast, for temperate species, such as *Z. viviparus* and *P. platessa*, growth rates increase with increasing temperature over a range of several degrees, before maximum growth rate is reached and growth declines again with further increasing temperature, showing large bell-shaped curves. The Arctic species, *B. saida* and *M. scorpius* show steeper curves compared to Antarctic species, which are, however, still smaller and shallower than curves of temperate species.

Discussion

In spite of comparable experimental conditions, data presented here show clear differences in absolute growth rates as well as in temperature-dependent growth performance between fish species from different latitudinal ranges. We will first focus on possible reasons and

implications of differences in general growth performance and subsequently discuss the role of thermal sensitivity for growth performance and potential implications of these findings.

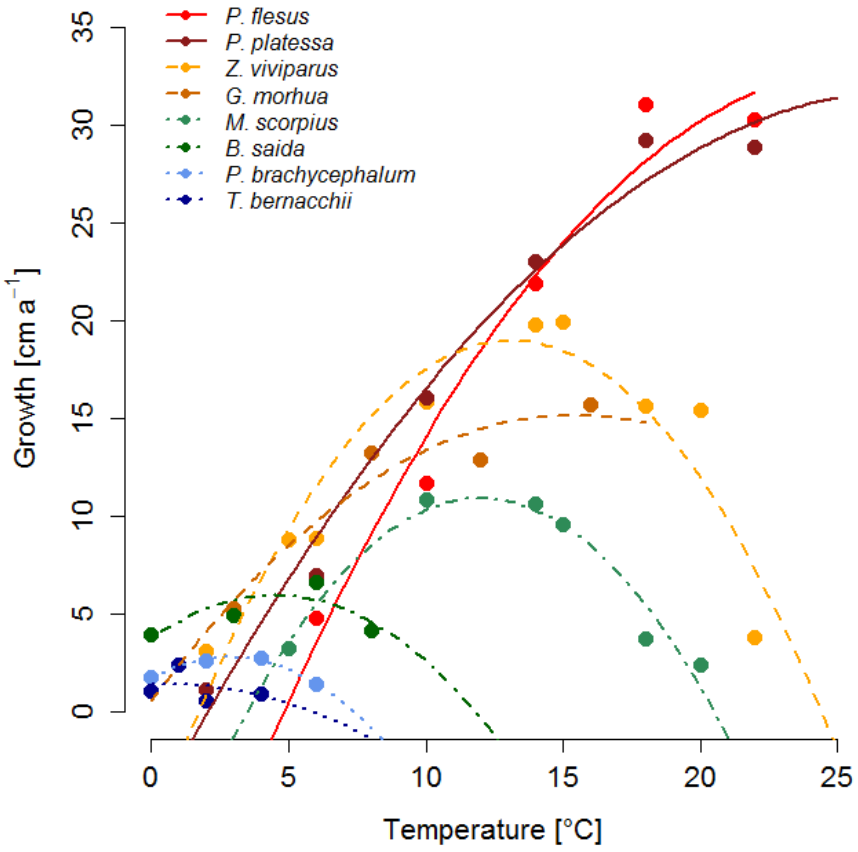

Fig. 1 Temperature-dependent growth rates in cm a^{-1} of fish species with different latitudinal distribution, *P. flesus* (Fonds et al., 1992), *P. platessa* (Fonds et al., 1992), *Z. viviparus* (Fonds, 1989), *G. morhua* (Kunz et al., unpublished), *M. scorpius* (Fonds, 1989), *B. saida* (Kunz et al., unpublished), *P. brachycephalum* (Brodte et al., 2006a) and *T. bernacchii* (Sandersfeld et al., 2015).

Differences in growth rates

Fig. 1 shows generally higher growth rates for temperate *P. flesus* and *P. platessa* than for polar *P. brachycephalum* and *T. bernacchii* under *ad libitum* feeding conditions. This implies an effect of temperature on growth performance, with higher growth at warmer temperatures. In fact, studies observed higher growth rates in populations of the same species living in the warmer distributional ranges compared to populations from colder areas, supporting similar findings from latitudinal comparisons between different species (Clarke, 1983; Jobling, 1997; Kock and Everson, 1998; La Mesa and Vacchi, 2001; Pörtner et al., 2001; Hildebrandt et al., 2011; Trip et al., 2014). Some polar ectotherms display seasonal variation in growth patterns, while others show constant growth throughout the year (Barnes, 1995; Peck et al., 1997; Peck

et al., 2000). In pelagic habitats, growth can be limited by highly seasonal food supply at high latitudes (cf. Pörtner et al., 2005). However, in most benthic habitats, food is constantly available throughout the year (DeVries and Eastman, 1981; Kock, 1992). As shown in this study, for benthic fish species, food availability is unlikely to limit growth. Seasonal rhythms, possibly influenced by light regime, temperatures as well as hormonal triggers have been suggested to impact feeding activity in Antarctic fish, irrespective of food availability (Clarke and North, 1991; Johnston and Battram, 1993; Campbell et al., 2008). For instance, reduced feeding activity in winter was indicated by experiments with *Notothenia* species despite sufficient food supply (Targett, 1990; Coggan, 1997). Generally, differences in food composition, such as different lipid and protein content, influence the growth efficiency (e.g. García et al., 2015). However, the food used in growth experiments closely resembled natural food sources of the respective species, except for the gadoids. Studies on different fish populations indicate population-specific differences in food conversion efficiency and food consumption, thereby indicating differences in energy utilisation (Reinitz et al., 1978; Present and Conover, 1992; Jonassen et al., 2000). Such differences in energy allocation patterns could also influence interspecies variation in growth.

Additionally, differences in the lifestyle of a species can impact growth performance. The rather inactive eelpouts species, *Z. viviparus* and *P. brachycephalum*, show a comparable lifestyle to *T. bernacchii* and *M. scorpius*, as well as the flatfish species *P. flesus* and *P. platessa*. In contrast, the gadoid species, *G. morhua* and *B. saida*, are more active, showing demersal as well as pelagic presence. In general, a pelagic lifestyle limits energy allocation to growth due to elevated costs for activity and potentially lower or more seasonal prey availability. Benthic species spend less energy for activity and benefit from a relatively constant food supply. Besides, growth rates of *G. morhua* from the study by Kunz et al. (unpublished) are low, compared to values reported e.g. by Fischer (2003) or Björnsson et al. (2001). These differences can be related to feeding conditions (Fischer, 2003), as well as population-specific differences (Björnsson et al., 2001). For this study, we chose the data set of Kunz et al., as these data were produced using an identical protocol as for *B. saida*, thus enhancing comparability between the two more active species. In spite of slight differences in feeding regime, data of *G. morhua* and *B. saida* serve as a valuable addition, demonstrating comparable growth patterns, independent of lifestyle.

Regarding differences in energy allocation of species from different latitudes, a higher metabolic rate of species from high latitudes has been vigorously discussed in recent years

(Scholander et al., 1953; Wohlschlag, 1960; Holeton, 1974; Clarke, 1980; Clarke, 1991; Steffensen, 2002; White et al., 2012). Though the existence of elevated metabolism in polar species is still under debate, it could serve as an explanation for differences in growth. A higher energy allocation to routine metabolism could cause trade-offs in energy allocation to growth at high latitudes, as illustrated in Fig. 2a (cf. Koehn and Shumway, 1982; Wieser, 1994). Pörtner et al. (2005) showed a negative relationship between routine metabolism and growth for low- and high-Antarctic fish, with high-Antarctic species having highest metabolic rates, but lowest growth. Jobling (1997) argued that a higher metabolic rate might be essential for high-latitude fish to be able to fully utilise available food sources during the shorter growing seasons. However, this is unlikely to play a role in benthic habitats.

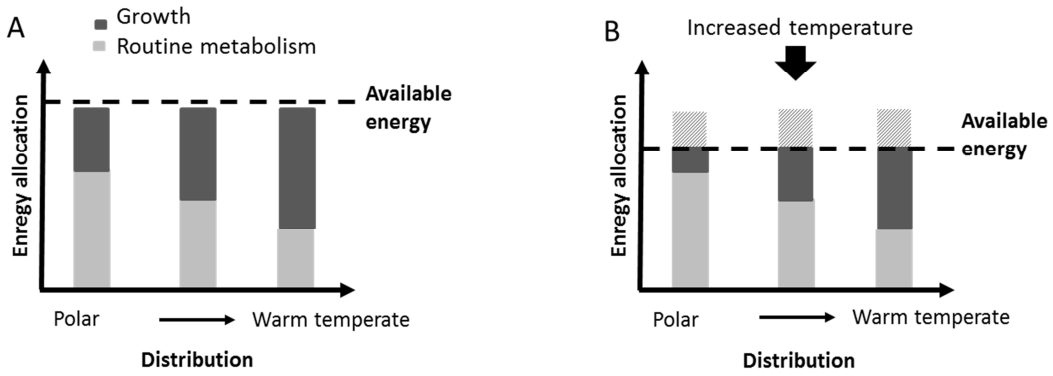

Fig. 2 Schematic illustration of energy allocation patterns of fish species with different latitudinal distributions. As polar species show the lowest growth rates (A), the effect of temperature is most severe by reducing these low growth rates even further (B).

Besides the obvious differences between temperate and polar species, growth rates of *B. saida* and *M. scorpius* display an intermediate level between the temperate and Antarctic species. Even though the Arctic and Antarctic seem to be comparable regions in terms of average temperature, environmental conditions differ distinctly in terms of variability. The geographically isolated Southern Ocean displays extremely stable and cold temperature conditions. Thus, Antarctic fish are highly stenotherm organisms. In contrast, the Arctic Ocean is a cold, but more variable thermal habitat. Therefore, Arctic fish are adapted to larger temperature fluctuations and warmer temperatures than Antarctic fish, potentially coming along with intermediate growth rates.

Thermal tolerance of growth performance

In addition to differences in growth rates, temperate species display a broad thermal window of growth performance, which decreases towards the Arctic species and is narrowest for the

high-Antarctic species. A narrow thermal performance window makes a species more sensitive towards temperature changes. For the high-Antarctic *T. bernacchii*, mass growth reductions of up to 84% were recorded in response to a temperature increase of 2°C, while growth of the temperate *Z. viviparus* was similar between 4 and 12°C, with highest growth performance at 12°C (Brodte et al., 2006a; Sandersfeld et al., 2015). Energy can only be allocated to growth after all vital metabolic costs have been covered (Koehn and Shumway, 1982; Wieser, 1994). Stress, such as increased temperature, can elevate energetic costs for cell protection and repair, decrease food conversion efficiency and impact aerobic ATP production. Thus, stress-induced reductions of aerobic metabolic scope and increased routine metabolic costs can limit energy available for other functions (Sokolova et al., 2012; Sokolova, 2013, cf. Fig. 2). However, the shift in temperature necessary to cause these consequences depends on the width of a species' thermal tolerance window (Fig. 1). While warming is likely to have negative impacts on growth for very stenothermal species, such as *T. bernacchii*, small increases of temperature might influence growth rates of temperate, more thermal tolerant species, such as *P. platessa*, even positively. For example, increasing stock size of capelin in the Barents Sea (Arctic Ocean) from the 1990's onwards has been linked with increasing temperatures enhancing growth and reproduction (Hop and Gjøsæter, 2013).

Ecological implications

Different life strategies are associated with differences in energy allocation. Garvey and Marshall (2003) showed different energy allocation strategies in largemouth bass (*Micropterus salmoides*) to be connected to low- and high-latitude distribution. They found low-latitude fish to mainly invest energy in body growth and reproduction to enhance length growth and reproductive output. High-latitude fish allocated energy to body growth in summer, to energy reserves during fall (to increase survival during winter) and to reproduction early before the spawning season. Similarly, gender and region specific differences in energy allocation to growth and reproduction were found in polar cod and were suggested to be caused by differences in diet and predation risk in different habitats (Nahrgang et al., 2014).

Antarctic fish usually grow to high age and reproduce late in their life cycle, indicating that energy is first allocated to somatic growth (Kock and Kellermann, 1991; Kock, 1992). Some high-Antarctic species only spawn after having reached about 70 to 80% of their maximal size (Kock and Kellermann, 1991). In contrast, the Arctic *B. saida* reproduces at the age of 2 to 3 years (males/females; Craig et al., 1982). While attaining a maximum age of 7 years,

individuals older than 5 years are rarely encountered (Hop and Gjøsæter, 2013). *G. morhua*, reaching a maximum age of about 10 years, matures comparatively late at an age of 6 to 8 years (Ottersen and Sundby, 1994; Brander, 1995). Thus, different life strategies affect the allocation of energy between growth and reproduction. Usually, somatic growth can be retained in a broader range of environmental conditions than reproductive output. Thus, when impacts on growth performance can be measured, reproduction is likely to be affected to a higher degree already (Jobling, 1997).

In conclusion, we suggest that the impact of ocean warming on a species depends on the width of the thermal tolerance window and the general growth performance. Data presented here indicate that polar fish are affected first and most severely by climate change. Disregarding temperature compensation, the combination of a low thermal tolerance and slow growth results in a high sensitivity towards ocean warming.

While Arctic fish species display a higher thermal tolerance and higher growth rates compared to Antarctic species, the Arctic region will face the largest temperature increase globally. Although predicted temperature increases for Antarctic waters are comparatively low (Hellmer et al., 2012; Timmermann and Hellmer, 2013), the lower thermal tolerance of the highly adapted Southern Ocean fish fauna might lead to similarly severe ecological consequences.

References

Barnes, D. K. (1995). Seasonal and annual growth in erect species of Antarctic bryozoans. *J. Exp. Mar. Biol. Ecol.* **188**, 181-198.

Björnsson, B. and Steinarsson, A. (2002). The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aqua. Sci.* **59**, 494-502.

Björnsson, B., Steinarsson, A. and Oddgeirsson, M. (2001). Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). *ICES J. Mar. Sci.* **58**, 29-38.

Boehlert, G. and Kappenman, R. (1980). Variation of growth with latitude in two species of rockfish (*Sebastes pinniger* and *S. diploproa*) from the northeast Pacific Ocean. *Mar. Ecol. Prog. Ser* **3**, 1-10.

Brander, K. (1995). The effect of temperature on growth of Atlantic cod (*Gadus morhua* L.). *ICES J. Mar. Sci.* **52**, 1-10.

Brodte, E., Knust, R. and Pörtner, H. O. (2006a). Temperature-dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae). *Polar Biol.* **30**, 95-107.

Brodte, E., Knust, R., Pörtner, H. O. and Arntz, W. (2006b). Biology of the Antarctic eelpout *Pachycara brachycephalum*. *Deep-Sea Res. Pt II* **53**, 1131-1140.

Campbell, H. A., Fraser, K. P., Bishop, C. M., Peck, L. S. and Egginton, S. (2008). Hibernation in an Antarctic fish: on ice for winter. *PLoS One* **3**, e1743.

Cheung, W. W., Lam, V. W., Sarmiento, J. L., Kearney, K., Watson, R., Zeller, D. and Pauly, D. (2010). Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. *Global Change Biol.* **16**, 24-35.
Clarke, A. (1980). A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. *Biol. J. Linn. Soc.* **14**, 77-92.
Clarke, A. (1983). Life in cold water: the physiological ecology of polar marine ectotherms. *Oceanogr. Mar. Biol.* **21**, 341-453.
Clarke, A. (1991). What is cold adaptation and how should we measure It? *Am. Zool.* **31**, 81-92.
Clarke, A. and North, A. (1991). Is the growth of polar fish limited by temperature? In *Biology of Antarctic fish*, (eds. G. Di Prisco, B. maresca and B. Tota), pp. 54-69. Berlin: Springer.
Coggan, R. (1997). Growth: Ration relationships in the antarctic fish *Notothenia coriiceps* Richardson maintained under different conditions of temperature and photoperiod. *J. Exp. Mar. Biol. Ecol.* **210**, 23-35.
Craig, P., Griffiths, W., Haldorson, L. and McElderry, H. (1982). Ecological studies of Arctic cod (*Boreogadus saida*) in Beaufort Sea coastal waters, Alaska. *Can. J. Fish. Aqua. Sci.* **39**, 395-406.
DeVries, A. L. and Eastman, J. T. (1981). Physiology and ecology of notothenioid fishes of the Ross Sea. *J. Roy. Soc. New Zeal.* **11**, 329-340.
Dulvy, N. K., Rogers, S. I., Jennings, S., Stelzenmüller, V., Dye, S. R. and Skjoldal, H. R. (2008). Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. *J. App. Ecol.* **45**, 1029-1039.
Falk-Petersen, I.-B., Frivoll, V., Gulliksen, B. and Haug, T. (1986). Occurrence and size/age relations of polar cod, *Boreogadus Saida* (Lepechin), in Spitsbergen coastal waters. *Sarsia* **71**, 235-245.
Fischer, T. (2003). The effects of climate induced temperature changes on cod (*Gadus morhua* L.): linking ecological and physiological investigations. *Reports on Polar and Marine Research* **454**.
Fonds, M. (1989). Metabolism, food consumption, growth and food conversion of shorthorn sculpin (*Myoxocephalus scorpius*) and eelpout (*Zoarces viviparus*). In *ICES Council Meeting 1989 (collected papaers)*, G31, pp. 19. Copenhagen: ICES.
Fonds, M., Cronie, R., Vethaak, A. and Van der Puyl, P. (1992). Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. *Neth. J. Sea Res.* **29**, 127-143.
Froese, R. and Pauly, D. (2015). FishBase. *World Wide Web electronic publication, www.fishbase.org (04/2015)*.
García, V., Celada, J. D., González, R., Carral, J. M., Sáez□Royuela, M. and González, Á. (2015). Response of juvenile tench (*Tinca tinca* L.) fed practical diets with different protein contents and substitution levels of fish meal by soybean meal. *Aquac. Res.* **46**, 28-38.
Garvey, J. E. and Marschall, E. A. (2003). Understanding latitudinal trends in fish body size through models of optimal seasonal energy allocation. *Can. J. Fish. Aqua. Sci.* **60**, 938-948.
Hellmer, H. H., Kauker, F., Timmermann, R., Determann, J. and Rae, J. (2012). Twenty-first-century warming of a large Antarctic ice-shelf cavity by a redirected coastal current. *Nature* **485**, 225-228.
Hildebrandt, N., Bergmann, M. and Knust, R. (2011). Longevity and growth efficiency of two deep-dwelling Arctic zoarcids and comparison with eight other zoarcid species from different climatic regions. *Polar Biol.* **34**, 1523-1533.
Holeton, G. F. (1974). Metabolic cold adaptation of polar fish: fact or artefact? *Physiol. Zool.*, 137-152.

Hop, H. and Gjøsæter, H. (2013). Polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) as key species in marine food webs of the Arctic and the Barents Sea. *Mar. Biol. Res.* **9**, 878-894.
Hunt, B. M., Hoefling, K. and Cheng, C.-H. C. (2003). Annual warming episodes in seawater temperatures in McMurdo Sound in relationship to endogenous ice in notothenioid fish. *Antarct. Sci.* **15**, 333-338.
Jobling, M. (1994). Fish bioenergetics. London: Chapman & Hall.
Jobling, M. (1997). Temperature and growth: modulation of growth rate via temperature change. In *Global Warming : Implications for freshwater and marine fish*, vol. 61, (eds C. M. Wood and D. G. McDonald), pp. 225-254. Cambridge: Cambridge University Press.
Johnston, I. and Battram, J. (1993). Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. *Mar. Biol.* **115**, 7-14.
Jonassen, T., Imsland, A., Fitzgerald, R., Bonga, S., Ham, E., Naevdal, G., Stefánsson, M. O. and Stefansson, S. (2000). Geographic variation in growth and food conversion efficiency of juvenile Atlantic halibut related to latitude. *J. Fish Biol.* **56**, 279-294.
Kock, K.-H. (1992). Antarctic fish and fisheries. Cambridge: Cambridge University Press.
Kock, K.-H. and Kellermann, A. (1991). Reproduction in Antarctic notothenioid fish. *Antarct. Sci.* **3**, 125-150.
Kock, K.-H. and Everson, I. (1998). Age, growth and maximum size of Antarctic notothenioid fish—revisited. In *Fishes of Antarctica*, (eds. G. di Prisco, E. Pisano and A. Clarke), pp. 29-40. Milan: Springer.
Koehn, R. K. and Shumway, S. E. (1982). A genetic/physiological explanation for differential growth rate among individuals of the American oyster *Crassostrea virginica*. *Mar. Biol. Lett.* **3**, 35-42.
La Mesa, M. and Vacchi, M. (2001). Age and growth of high Antarctic notothenioid fish. *Antarct. Sci.* **13**, 227-235.
Lorenzen, K. and Enberg, K. (2002). Density-dependent growth as a key mechanism in the regulation of fish populations: evidence from among-population comparisons. *Proc. R. Soc. Lond. B* **269**, 49-54.
Murawski, S. (1993). Climate change and marine fish distributions: forecasting from historical analogy. *Trans. Am. Fish. Soc.* **122**, 647-658.
Nahrgang, J., Varpe, Ø., Korshunova, E., Murzina, S., Hallanger, I. G., Vieweg, I. and Berge, J. (2014). Gender specific reproductive strategies of an Arctic key species (*Boreogadus saida*) and implications of climate change. *PloS ONE* **9**, e98452.
Ottersen, G. and Sundby, S. (1994). Arcto-Norwegian cod. In *Spawning and life history information for North Atlantic cod stocks*, (ed. K. Brander), pp. 152. Copenhagen: ICES.
Ottersen, G., Michalsen, K. and Nakken, O. (1998). Ambient temperature and distribution of north-east arctic cod. *ICES J. Mar. Sci.* **55**, 67-85.
Peck, L., Colman, J. and Murray, A. (2000). Growth and tissue mass cycles in the infaunal bivalve *Yoldia eightsi* at Signy Island, Antarctica. *Polar Biol.* **23**, 420-428.
Peck, L. S., Brockington, S. and Brey, T. (1997). Growth and metabolism in the Antarctic brachiopod *Liothyrella uva*. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **352**, 851-858.
Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science* **308**, 1912-1915.
Pörtner, H. O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **213**, 881-893.
Pörtner, H. O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T. and Knust, R. (2001). Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod *Gadus morhua* and common eelpout *Zoarces viviparus*. *Cont. Shelf Res.* **21**, 1975-1997.

Pörtner, H. O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95-97.
Pörtner, H. O., Storch, D. and Heilmayer, O. (2005). Constraints and trade-offs in climate-dependent adaptation: energy budgets and growth in a latitudinal cline. *Sci. Mar.* **69**, 271-285.
Pörtner, H. O., Karl, D., Boyd, P. W., Cheung, W., Lluch-Cota, S. E., Nojiri, Y., Schmidt, D. N. and Zavialov, P. (2014). Ocean systems. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change*, (eds. C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova et al.), pp. 411-484. Cambridge/New York: Cambridge University Press.
Present, T. and Conover, D. (1992). Physiological basis of latitudinal growth differences in *Menidia menidia*: variation in consumption or efficiency? *Funct. Ecol.* ***6***, 23-31.
Reinitz, G. L., Orme, L. E., Lemm, C. A. and Hitzel, F. N. (1978). Differential performance of four strains of rainbow trout reared under standardized conditions. *The Progressive Fish-Culturist* **40**, 21-23.
Sandersfeld, T., Davison, W., Lamare, M. D., Knust, R. and Richter, C. (2015). Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. *J. Exp. Biol.* **218**, 2373-2381.
Scholander, P., Flagg, W., Walters, V. and Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zool.* **26**, 67-92.
Sokolova, I. M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr. Comp. Biol.* **53**, 597-608.
Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G. and Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar. Environ. Res.* **79**, 1-15.
Steffensen, J. F. (2002). Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artefact? Artefact! *Comp. Biochem. Physiol. A* **132**, 789-795.
Targett, T. (1990). Feeding, digestion and growth in Antarctic fishes: ecological factors affecting rates and efficiencies. In *Second international symposium on the biology of Antarctic fishes. Naples: IIGB Press*, pp. 37-39.
Timmermann, R. and Hellmer, H. H. (2013). Southern Ocean warming and increased ice shelf basal melting in the twenty-first and twenty-second centuries based on coupled ice-ocean finite-element modelling. *Ocean Dynam.* **63**, 1011-1026.
Trip, L., Elizabeth, D., Clements, K. D., Raubenheimer, D. and Choat, J. H. (2014). Temperature- related variation in growth rate, size, maturation and life span in a marine herbivorous fish over a latitudinal gradient. *J. Anim. Ecol.* **83**, 866-875.
White, C. R., Alton, L. A. and Frappell, P. B. (2012). Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. *Proc. R. Soc. Lond. B* **279**, 1740-1747.
Wieser, W. (1994). Cost of growth in cells and organisms: general rules and comparative aspects. *Biol. Rev.* **69**, 1-33.
Wohlschlag, D. E. (1960). Metabolism of an Antarctic Fish and the Phenomenon of Cold Adaptation. *Ecology* **41**, 287-292.
Yamahira, K., Kawajiri, M., Takeshi, K. and Irie, T. (2007). Inter- and intrapopulation variation in thermal reaction norms for growth rate: evolution of latitudinal compensation in ectotherms with a genetic constraint. *Evolution* **61**, 1577-1589.

3. Discussion

In the studies of this thesis, the impact of increasing temperature on Antarctic fish was analysed from different perspectives. The collected data allow to gain a glimpse into acclimation capacities of Antarctic fish with regard to ocean warming and it can contribute to improve the knowledge of thermal responses in these unique organisms. In the following, the data of the second manuscript will be used to show the relevance of the assessment of the whole-organism level for the interpretation of data at various organisational levels. This is followed by a methodological discussion on critical aspects of thermal tolerance measurements at the whole-organism level. Subsequently, the objectives and results of this thesis will be discussed in a wider ecological framework.

3.1. Assembling pieces - the thermal response of *Trematomus bernacchii*

Regarding Antarctic fish, *T. bernacchii* is a very well studied model organism. This chapter will review knowledge on the metabolic response of this species to temperature changes, trying to establish a broader picture of its acclimation capacity and underlying physiological mechanisms.

The common and nearly universal response to stress, the heat shock response, is absent in *T. bernacchii*. A continuous up-regulation of heat shock proteins has been observed in this species (Hofmann et al., 2000) and it is suggested to be a requirement to mitigate the effects of constant sub-zero temperatures (Place et al., 2004; Place and Hofmann, 2005). However, this is thought to make these fish incapable of any further up-regulations of heat shock proteins (Hofmann et al., 2000; Place et al., 2004). Nevertheless, various responses to temperature stress at different organisational levels of this species have been revealed in the second study of this thesis (manuscript II), as well as various other studies, that will be discussed in the following (Table 1). A metabolic response depends on the magnitude of a temperature increase, as well as on the exposure time. This paragraph will focus on studies conducted at an elevated water temperature of 4°C (cf. Table 1).

Due to its ecological relevance, the studies of this thesis focused on the whole-organism level. However, measurements on the whole-organism level display only the sum of various processes on lower organisational levels. An organism is made up of cells, thus changes in cellular processes affect cellular energy demand and become evident at the whole-organism level. For instance, low hepatocyte respiration was shown to comply with low whole-

organism metabolic rate and maximum growth in the Antarctic eelpout *Pachycara brachycephalum* at 4°C (Mark et al., 2005; Brodte et al., 2006a). Thus, the integration of knowledge from the whole organism, cellular and molecular levels can yield insight into the underlying mechanisms of thermal tolerance and can help to improve the understanding of thermal response patterns and thus thermal limits of organisms (Pörtner, 2012; Somero, 2012).

Temperature accelerates biochemical reaction times, which generally becomes evident in an elevated whole-organism metabolic rate after an acute temperature increase (Jensen et al., 1993). Such an elevation of metabolic rate was also measured in *T. bernacchii* in experiments of this thesis (manuscript II), as well as in other studies, after an acute increase of temperature. Increased metabolism was observed for up to 28 days after a temperature increase (Robinson, 2008; Enzor et al., 2013). This increase of metabolism likely displays an increased energy demand (Wells, 1978; Buckley and Somero, 2009). In aerobic metabolism, oxygen is needed for energy conversion. Thus, increased oxygen transport capacity (haematocrit) measured in the blood of *T. bernacchii* is suggested to be a (temporary) measure to support aerobic energy supply (Davison et al., 1994; Hudson et al., 2008; cf. also Sleadd et al., 2014). When energy supply can no longer match the increasing demand, a shift to anaerobic metabolism assures energy supply of the tissue (Pörtner, 2010). Results of different studies indicate a shift to anaerobic metabolism in *T. bernacchii* after a temperature increase (Wells, 1978; Jayasundara et al., 2013). A shift to anaerobic metabolism is suggested to mark temperatures which affect the metabolic scope of an organism, potentially influencing e.g. activity and growth. Beyond these temperature threshold, it its suggested that conditions can only be tolerated for limited time (Pörtner, 2010).

Changes in metabolic enzyme activities, as well as an up-regulation of genes involved in reorganisation of metabolic pathways, indicate a shift in metabolic fuel preferences from lipids to carbohydrates after an acute increase of temperature in *T. bernacchii* (Buckley and Somero, 2009; Jayasundara et al., 2013). Moreover, indicators of inflammation, apoptosis, cell-cycle arrest (Sleadd and Buckley, 2013; Sleadd et al., 2014) and increased oxidative tissue damage (Enzor and Place, 2014) imply elevated cellular stress levels. In addition, up-regulation of genes involved in intracellular signalling, as well as cytoskeletal organisation, cell cycle arrest and proteolysis (Buckley and Somero, 2009) also suggest increased cellular stress after exposure to increased temperature. While mitigation of damage and cell repair seem to be important processes happening parallel to metabolic reorganisation, activity seems

to be unimpaired by acute increases of temperature, indicating no impact on crucial activities, such as escape or hunting capabilities (Wilson et al., 2001).

Generally, temperature changes are thought to trigger a two-stage stress response (Kültz, 2005; Buckley and Somero, 2009). The first phase, the 'cellular stress response', generally assures the short-term survival of the cell independent of the stressor. It comprises growth control, the protection of macromolecular integrity, the modulation of major energetic pathways and apoptosis (Kültz, 2005). The aforementioned thermal metabolic responses in *T. bernacchii* seem to comply well with this first phase of the stress response. According to Kültz (2005), the second phase, the 'cellular homeostatic response', aims at the restoration of cellular homeostasis depending on the stressors effect. Declining cellular oxidative damage and compensated whole-animal metabolic rate after an acclimation time of about 56 days could suggest a return to organismal homeostasis in *T. bernacchii*. However, transcriptomic changes indicate reduced overall transcription, reduced protein turnover capacity, as well as a mobilisation of energy stores (Huth and Place, 2013), which is supported by decreasing trends in condition factors and energy storage, as well as decreasing growth rates at the whole-organism level after long-term acclimation to elevated temperature (manuscript II). An increasing trend in nitrogen content of faeces found in manuscript II indicates less efficient food assimilation. These results could suggest a lower efficiency of energy metabolism after acclimation to increased temperature. However, whether e.g. a reduced protein turnover is a cause for reduced food conversion efficiency or whether inefficient food assimilation contributes to reduced protein turnover rates, will have to be assessed in further studies.

In the short-term, increasing temperature seems to trigger various molecular, cellular and organismal compensation mechanisms in *T. bernacchii*, which seem to make these fish able to cope with changing temperatures. Nevertheless, it is questionable whether the energetic costs for these 'mitigation' measures can be met without cut-offs for other traits and whether a complete return to an organismal homeostasis is possible in the long-term. In the face of rapid temperature changes, resistance mechanisms and short-term responses to assure physiological functioning, such as oxygen and energy supply of the tissue, are most crucial processes (Peck et al., 2009; Pörtner, 2010). However, in an ecological context of slow environmental change, physiological and ecological processes interact. Then, the combination of physiological factors, such as the efficient utilisation of energy reserves and the degree of acclimation of various physiological processes, as well as ecological factors, such as food availability and predator/prey interactions, can be pivotal for survival (Barnes and Peck, 2008; Peck et al.,

2009). Knowledge on physiological ranges of thermal tolerance is most important, but these ranges are likely to be additionally confined by ecological limitations in a species' natural environment, such as limited food supply or suitable habitat (Peck et al., 2009). Thus, physiological optima and the ecological niche that is actually realised by a species do not always comply. For example, this is again demonstrated by the Antarctic eelpout *Pachycara brachycephalum*, which was found to show best growth performance at 4°C in laboratory studies, but lives at water temperatures around 0 to 0.6°C (Brodte et al., 2006b; Brodte et al., 2006a).

In spite of the described response patterns that maintain all vital functions, the energetic trade-offs for growth indicated by long-term experiments are significant. Neglecting potentially mitigating effects of transgenerational plasticity and cross-generational shifts of adaptation capacities, the results collected here make the acclimation ability to increased temperatures around 2 to 4°C on population-relevant time scales in *T. bernacchii* highly questionable.

Table 1 Schematic summary of studies on thermal tolerance of *T. bernacchii*, focusing on acute (up to 36 hours) as well as acclimatory responses of different organisational levels to 4°C (if not stated otherwise).

Influenced factor	Acute	≥14 d	≥28 d	≥56 d	References
Whole-organism level					
Growth				↓	Manuscript II
Food conversion ratio				↓	Manuscript II
Routine metabolism	↑	↑	↑	●	Enzor et al. 2013; Morrison et al. 2006; Robinson 2008; Manuscript II
Drinking rates			↑		Petzel 2005
Burst swimming	●				Wilson et al. 2001
Tissue & cellular level					
Gill tissue respiration		●			Somero et al. 1968
Brain tissue respiration		●			Somero et al. 1968
Apoptotic hepatocytes (2°C, 6°C)	↑				Sleadd et al. 2014
Oxidative damage (gill)		7d ↑	●	↓	Enzor and Place 2014
Oxidative damage (liver)		7d ↑	↑	●	Enzor and Place 2014
Hematocrit (acute 10°C)	↑	●	●		Davison et al. 1994; Hudson et al. 2008
Serum osmolality	↓	↓	↓		Brauer et al. 2005; Gonzalez-Cabrera et al. 1995; Guynn et al. 2002; Hudson et al. 2008; Morrison et al. 2006
Blood ATP concentration (5°C)	↓				Wells 1978
Blood lactate concentration (5°C)	↑				Wells 1978
Serum cortisol		●	●		Hudson et al. 2008
Hemoglobin (10°C)	↑				Davison et al. 1994
Molecular level					
Na^+/K^+-ATPase (gill)			↑		Gonzalez-Cabrera et al. 1995; Guynn et al. 2002
Na^+/K^+-ATPase (muscle)			↓		Gonzalez-Cabrera et al. 1995
Na^+/K^+-ATPase (liver)			●		Gonzalez-Cabrera et al. 1995
Na^+/K^+-ATPase (kidney)			↑		Gonzalez-Cabrera et al. 1995
HOAD (ventricular tissue)		↓			Jayasundara et al. 2013
CS (ventricular tissue)		↓			Jayasundara et al. 2013
LDH (ventricular tissue)		↓			Jayasundara et al. 2013
HOAD (skeletal muscle)		●			Jayasundara et al. 2013
CS (skeletal muscle)		↓			Jayasundara et al. 2013
LDH (skeletal muscle)		↑			Jayasundara et al. 2013
Gene regulation					
Transcription/Translation (general)			↓		Huth and Place 2013
Cytochrome C Oxidase (gill)			↑		Huth and Place 2013
Apolipoproteins (liver)			↑		Huth and Place 2013
C/EBP δ (white muscle)	6h ↑		↑		Sleadd and Buckley 2013

HOAD: 3-Hydroxyacyl CoA dehydrogenase, CS: Citrate synthase LDH: Lactate dehydrogenase; C/EBP δ: CCAAT/enhancer-binding protein δ (mediating inflammatory and pro-apoptotic processes)

3.2. Thermal tolerance at the whole-organism level - a methodological discussion

In this section, some major aspects of the methodology for thermal tolerance assessment will be discussed, regarding their relevance for this study. There are several approaches to assess the thermal tolerance of an organism. Estimates of thermal tolerance can vary widely, depending on the condition of the animal, its sex, its life stage and its feeding status, but also on the type of experiment, such as acute or acclimation experiments, or the rate of temperature increase or temperature intervals (Post, 1990; O'Connor et al., 2000; Chown et al., 2009; Peck et al., 2009; Pörtner and Peck, 2010; Terblanche et al., 2011; cf. Fig. 5).

Typically, slow heating rates are thought to result in lower estimates of thermal tolerance and closer resemble conditions experienced by an organism in the field (Peck et al., 2009). In contrast, the application of acute temperature changes often focuses on comparative or mechanistic approaches that are not feasible to realise in the framework of acclimation experiments. Acute temperature experiments are often used for multi-species comparisons, such as the thermal response patterns of *Lepidonotothen nudifrons*, *Lepidonotothen squamifrons* and *Trematomus hansoni* that were compared in manuscript I using oxygen consumption measurements. The discussion of this first manuscript highlights that the comparability of studies using acute measures is often confined by differences in experimental protocols, such as rates of temperature increase or prior acclimation.

This does not only apply to oxygen consumption measurements. For example, the application of the critical thermal maximum (CT_{max}) focuses on the determination of the temperature at which locomotory ability and the escape response from lethal conditions is impaired (Cowles and Bogert, 1944). The use of e.g. different heating rates, for the determination of $CT_{max,}$ often makes comparison of data difficult (Terblanche et al., 2007). Additionally, acute measures (as well as long-term measures) can vary depending on prior exposure or acclimation to elevated temperatures that can cause shifts of tolerance limits (Pörtner and Peck, 2010; Bilyk et al., 2012). For instance, when CT_{max} of *T. bernacchii* was determined twice for the same individual within 24 hours, the second measurement resulted in a higher CT_{max} than the first measurement (Bilyk et al., 2012). On the other hand, Prodrabsky and Somero (2006) analysed thermal tolerance in a group of *T. bernacchii* directly transferred to 4°C, as well as in a group slowly heated to 4°C over 24 hours. They found comparable warm acclimation patterns resulting in an increased thermal tolerance (comparable lethal temperature) of both groups after 24 hours.

In contrast to these acute thermal challenges, experiments with longer acclimation times are suggested to reveal more ecologically relevant capacities of thermal tolerance (Peck et al., 2009). These long-term acclimation capacities were aimed to be determine in energy budget studies of this thesis. The energy budget proofed to be a good tool to assess acclimation patterns, as shown in manuscript II and III using temperature dependent energy allocation of *T. bernacchii* and *L. nudifrons* as an example. However, various factors have to be taken into account that might possibly impact results, by affecting metabolism or thermal tolerance of the experimental organism, and could limit data interpretation.

An important factor influencing metabolism and thereby possibly thermal response capacities is food quality and quantity (Peck et al., 2003; Secor, 2009; Norin and Malte, 2011). O'Connor (2000) found food deprivation to cause changes in the rank order of Atlantic salmon metabolic rate, indicating that fish were able to adjust metabolic expenses according to food availability. As Antarctic fish display generally low growth rates, food was supplied *ad libitum* in the energy budget studies of manuscript II and III. By maximising energy input and thereby energy availability for growth, it was aimed to maximize the growth potential of fish. One reason for this was the standardisation of measured growth rates for comparison with other studies. However, as suggested in manuscript II, the food source, nutrient composition, costs of digestion and physiologically usable energy have to be considered and can affect the net energy gain from food. Nutritional composition of food is known to impact food conversion ratios and physiologically usable energy (García et al., 2015). In nature, food composition varies, possibly facilitating a more or less balanced and comprehensive nutritional source. In the energy budget experiment with *T. bernacchii,* the discrepancy between energy input in form of food and the amount of energy allocated to vital functions demonstrated potential trade-offs in energy estimations, when physiological usable or digestive energy cannot be determined. In the experiment with *L. nudifrons* (manuscript III), higher energetic costs for the digestion of chitin containing crustacean food (Secor, 2009) in combination with temperature and an unknown second stressor might have contributed to declining condition and negative growth. Though, increased liver lipid content at elevated temperature did not indicate energy shortage in these fish (manuscript III). Moreover, an unlimited food supply in experiments has to be taken into account regarding any ecological interpretation of the results from the laboratory studies of this thesis, since they do not necessarily reflect environmental conditions. In nature, food availability might be limited and foraging costs decrease net energy gain from food (Flore and Keckeis, 1998; Giacomini et al., 2013).

In general, captivity cannot be avoided in fish energy budget studies. The separation of individuals, as done by cages in experiments of this thesis, could either pose additional stress or even reduce social stress, since this avoids hierarchical pressure (Pottinger and Pickering, 1992; Morgan and Tromborg, 2007). The Antarctic species used in experiments of this thesis are known to be inactive, display low activity ranges, territorial behaviour and are likely to be loners (Miyamoto and Tanimura, 1999). Thus, separation was unlikely to cause but rather reduce social stress for these fish. Specimens from experiments of this thesis did not show any behavioural changes after separation into cages, except from some smaller *T. bernacchii*, which seemed less shy and fed more after separation (T. Sandersfeld, personal observation). Lower stress levels during captivity were also reported by Davison et al. (1995). They compared heart rate and cardiovascular parameters in groups of *T. bernacchii* measured in Antarctica directly with that of a group transported to and kept in captivity at Christchurch/NZ. Fish kept in aquaria in Christchurch for three weeks displayed lower heart rates compared to those measured in Antarctica (Davison et al., 1995). In combination with even lower heart rates measured after a minimum of six weeks in captivity in Christchurch by Axelsson et al. (1992), these results could indicate lower stress levels in captivity (Davison et al., 1995).

Furthermore, the behavioural aspects on thermal tolerance in Antarctic fish are hardly studied and often neglected due to feasibility reasons, as also in this study. Thermal effects on behaviour can influence boldness and shyness in fish, with potential impacts on feeding activity. In damselfish, increased aggression, boldness and activity were found at elevated temperature (Biro et al., 2010). Moreover, behavioural responses to environmental temperature changes have been observed for various species (Claireaux et al., 1995; Perry et al., 2005; Morita et al., 2010). For instance, thermoregulating behaviour, such as migration to cooler/warmer water layers or higher latitudes, has been observed for cod (*Gadus morhua*) in laboratory trials as well as in their natural habitat (Perry and Neilson, 1988; Claireaux et al., 1995). However, regarding Antarctic and especially high-Antarctic fish species, evasive manoeuvers such as migration to deeper water layers or higher latitudes are limited and will be discussed in section 3.3.

Additionally, the social status was shown to be positively correlated with *in situ* metabolic rate and growth in Atlantic salmon (Metcalfe et al., 1995). However, due to unknown hierarchical structures and social behaviour in most species as well as potential impacts of captivity, this is hardly controllable in laboratory experiments. Moreover, energy allocation

patterns change within the life cycle of a fish (Post, 1990; Jonsson and Jonsson, 2003). Besides differences between juvenile and adults, energy allocation can vary depending on reproductive cycles and strategies (Post, 1990; Post and Parkinson, 2001; Garvey and Marschall, 2003). However, access to model organisms is often limited in studies on Antarctic fish. In the study on *L. nudifrons* (manuscript III), selection of animals was limited, as animals were caught by trawling. Thus, the inclusion of animals of different sizes and possibly different reproductive status could not be avoided. As *T. bernacchii* were caught by hook and line fishing, selection of juvenile animals allowed to at least partly control impacts of reproductive cycles (manuscript II). Yet, the impact of different sexes could not be prohibited, as visual sex determination was not possible in the used species.

As described above, the whole-organism level can be influenced by a variety of factors, making it hard to establish a completely controlled experimental setup. This is demonstrated by the contradictory data set of *L. nudifrons* (manuscript III). Acute oxygen consumption measurements display an increase of metabolic rates with increasing temperature, similar to data of *T. bernacchii* (manuscript III, Fig. 3). However, after the acclimation period, higher metabolic rates were recorded compared to those measured after an acute temperature increase. Under unstressed conditions, one could expect metabolic rates after temperature acclimation to be comparable to or lower than measurements after an acute temperature challenge. During metabolic rate measurements of *L. nudifrons*, an unknown additional stressor was suspected to have elevated metabolism after the acclimation period, illustrating the sensitivity of the experimental work at the whole-organism level.

In spite of influencing factors and potential limitations of experiments at the whole-organism level, the organism is the pivotal factor that needs to be understood. Clear and detailed experimental protocols, e.g. regarding heating rates, acclimation times, feeding regime and handling, can help to enhance comparability between studies and support the identification of potential influencing factors. Combinations of studies, at lower as well as at higher organisational levels, if possible taking into account behavioural and ecosystem aspects, are of great value, as discussed above in section 3.1. Moreover, experimental results at the whole-organism level enable us to gain insight into potential consequences of elevated temperature at ecosystem-relevant levels, which will be further elaborated in the next section.

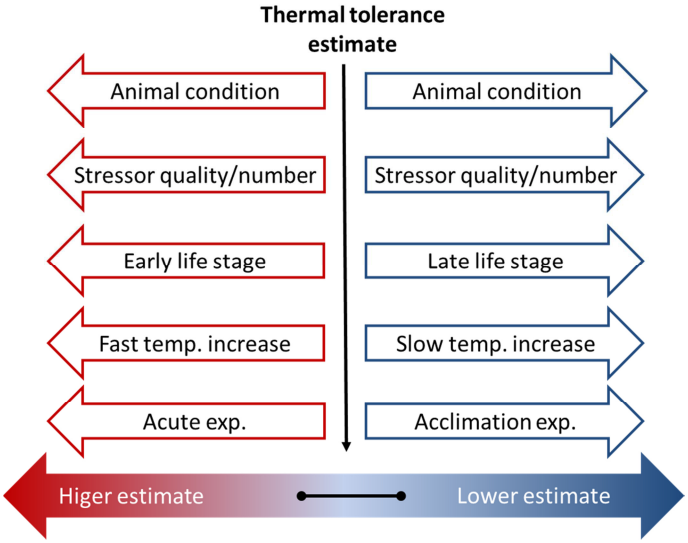

Fig. 5 Thermal tolerance estimates are influenced by animal as well as experimental condition. While some factors (red) can contribute to a higher estimated thermal tolerance, other factors (blue) can contribute to a lower estimate of thermal tolerance.

3.3. Sensitivity of Antarctic fish to ocean warming

Regarding vulnerability to climate change, organisms living at the upper limit of their thermal window are thought to be especially sensitive towards temperature changes (Stillman, 2003; Neuheimer et al., 2011). Moreover, the temperature range experienced in an environment is suggested to be a driving factor for the capacity to cope with rising temperatures (Tewksbury et al., 2008; Peck et al., 2014). Thus, various studies suggest a relatively low thermal tolerance of Antarctic fish compared to temperate counterparts, due to their evolutionary adaptation to lower temperature fluctuations in Antarctic waters (Somero, 2010; Peck et al., 2014). In turn, differences in thermal regimes in low- and high-Antarctic areas suggest differences of thermal tolerance within Antarctic fish species (cf. Fig. 1; Stillman 2003; Tewksbury et al. 2008, Bilyk and DeVries 2011). **Accordingly, a lower thermal tolerance of high-Antarctic compared to low-Antarctic fish species was hypothesised.** In the first study of this thesis (manuscript I), routine metabolic rates of different Antarctic fish species at their habitat temperature as well as after acute temperature increase were compared. A comparable effect of increased temperature on routine metabolism suggests no difference in the thermal response of the analysed species. Though, routine metabolic rates at habitat temperature suggested higher metabolic rates of species from higher latitudes. This partly supports the hypothesis of metabolic cold adaptation (MCA), however, in a much weaker sense. The hypothesis of MCA suggests polar fish to display several-fold elevated metabolic rates than temperate or tropical fish when projected to the same temperatures (Scholander et al., 1953;

Wohlschlag, 1960). While MCA has been found at lower organisational levels in fish (Hochachka, 1988; Kawall et al., 2002; but also see Magnoni et al., 2013), it is still vigorously discussed at the whole-organism level (Holeton, 1974; Clarke, 1991; Jordan et al., 2001; White et al., 2012). The size of the data set of manuscript I does not allow identification of broad scale patterns and conclusions on this topic, but it can serve as further evidence enhancing this discourse. Higher routine metabolic costs could have far reaching consequences for energy budgets by reducing energy availability for other vital functions, such as growth and reproduction. Moreover, according to the hypothesis of manuscript I, it could limit the scope for further elevations of metabolism, e.g. caused by increasing temperature, thereby decreasing thermal tolerance due to energetic constraints. Differences between high- and low-Antarctic species could be crucial in competition scenarios that will be discussed later in this paragraph.

Assuming that temperature affects energy allocation, it was hypothesised that increasing temperature causes metabolic trade-offs at the whole-organism level in high-Antarctic fish. In this context, the temperature-dependent energy allocation in the high-Antarctic *T. bernacchii* (manuscript II) was analysed. Results of the second study show that temperature does cause trade-offs at the whole-organism level. These trade-offs were most evident in form of growth reductions. In *T. bernacchii*, a temperature increase of only 2°C caused mass growth reductions of up to 84%, which was likely due to inefficient food conversion. In agreement with the first study (manuscript I), *L. nudifrons* showed a broader thermal tolerance, displayed by shifts in energy allocation after temperature increases to 4°C in contrast to 2°C for *T. bernacchii* (manuscript III). Absolute comparisons of shifts in energy allocation between the two species were prohibited by high mortality in control treatments of *L. nudifrons*. However, in spite of an additional unknown stressor in this experiment, a clear thermal response could be identified, emphasising the use of energy budget experiments for the identification of ecologically relevant thermal tolerance windows.

Starting from the assumption that elevated temperature significantly impacts growth in high-Antarctic fish, it was hypothesised that differences in growth rates as well as temperature dependent growth performance influence a species' production and thereby its sensitivity to ocean warming. To address this hypothesis, temperature-dependent growth rates of fish species with different latitudinal distributions were analysed. Polar and especially Antarctic fish species were found to show low growth rates and low thermal tolerance of growth performance compared to temperate species (manuscript IV). As

hypothesised in manuscript I, higher routine metabolic costs could contribute or account for low growth rates at high-latitudes. Moreover, a very low thermal tolerance of growth performance, as shown in manuscript II for *T. bernacchii*, can cause further reductions in already low growth rates of Antarctic species. Changes in temperature can potentially disrupt the balance between energy allocation to growth and reproduction as well as metabolic costs. While consequences of increasing temperature might be smaller for temperate species with relatively higher growth rates and a broad thermal tolerance, consequences might be more severe for Antarctic fish species with low growth rates and a high thermal sensitivity, making these fish particularly sensitive to ocean warming.

Shifts in species distribution, to colder water layers or higher latitudes, have already been observed in response to increasing temperature (Perry et al., 2005; Dulvy et al., 2008; Sundby and Nakken, 2008). For example in the North Sea, especially species with fast life cycles and small body size, such as dragonet (*Callionymus lyra*), were observed to shift their distribution, while species with slower life cycles, such as spurdog (*Squalus acanthias*), seemed not to be able to adapt to changing conditions (Perry et al., 2005). Antarctic fish show much slower life cycles compared to temperate species (Kock, 1992; La Mesa and Vacchi, 2001), calling their response capacities into question.

However, avoidance strategies, such as migration to colder waters in deeper depth or higher latitudes can only be partially exploited by Antarctic fish. In the high-latitudes, deep waters are warmer than upper water layers (Mantyla and Reid, 1983), making migration to deeper depth an unsuitable avoidance manoeuver from rising temperatures. In contrast, warming of shelf waters might facilitate the introduction or increasing abundance of deep-sea, bathy- or mesopelagic fish species, as well as non-Antarctic invaders (Mintenbeck et al., 2012). For instance in the Palmer deep, a large population of king crabs was found and is suspected to be restricted in upward migration by cold temperatures (Smith et al., 2011). Besides, migration to colder waters in higher latitudes is only possible for low-Antarctic fish. For these species, north-south aligned shelves, such as at the Antarctic Peninsula, the Kerguelen Plateau or Victoria Landon might offer potential habitats (Barnes et al., 2009). Though, for high-Antarctic species pole-ward migration is limited by the borders of the Antarctic continent.

In such a migration scenario, low-Antarctic species could intrude high-Antarctic waters and compete for niches and other resources with high-Antarctic species. Analysis of temperature dependent abundance structures of fish species in the Atlantic sector of the Weddell Sea showed typical low-Antarctic species, such as of the genus *Lepidonotothen*, to occur in a

temperature range of -1 to 2°C, while abundances were highest at temperatures above 0°C (Meyer, 2012). Typically high-Antarctic species, such as of the genus *Trematomus*, were mostly found below 0°C, with peak abundances between -1 and -2°C (Meyer, 2012). However, temperature-induced overlaps in abundances of species could enhance competition. Here, a higher thermal sensitivity of high-Antarctic species might be a disadvantage with progressing climate change. As presumed in manuscript I, a higher metabolic rate of high-Antarctic fish could restrict thermal tolerance and cause these species to reach physiological critical temperatures earlier compared to low-Antarctic species. Low growth rates in addition to a higher thermal sensitivity of growth performance, as shown in manuscripts II, III and IV, could reduce production rates of high-Antarctic fish at elevated temperatures by a higher degree than those of low-Antarctic species. Besides the thermal effects on physiology, temperature associated changes in intra- and interspecies interaction, food web as well as habitat structures may have advantages and disadvantages for high- and low-Antarctic fish alike.

Regarding food availability, some species, such as *T. bernacchii* and *L. nudifrons*, show a quite broad prey spectrum, feeding on various benthic and epifaunal organisms as well as planktonic copepods, amphipods and fish (Gon and Heemstra, 1990; Montgomery et al., 1993; Fanta, 1999; La Mesa et al., 2004). Feeding strategies that target sessile, crawling as well as swimming organisms alike, suggest sufficient flexibility to cope with fluctuations and possible changes in prey spectrums (cf. Montgomery et al., 1993). However, even flexible predators can be impacted by changes in the food web that significantly limit available energy sources. For example, decreasing sea ice cover coming along with decreasing krill abundance and increasing salp abundance was reported to impact food webs at the Western Antarctic Peninsula (Atkinson et al., 2004; Turner et al., 2014). Such changes in food webs may change the distribution of food sources for pelagic life stages of marine organisms, as well as top predators, such as chinstrap penguins, and disrupt established recruitment cycles (Trivelpiece et al., 2011; Flores et al., 2012). Moreover, the substitution of a high-energy food source by a low-energy prey can decrease energy input and thus reduce energy available for higher trophic levels (Mintenbeck et al., 2012). Ruck et al. (2014) found regional differences in energy content of common prey species of predators, such as large fish, seals and penguins. For example, lipid content of Antarctic krill (*Euphausia superba*) increased with latitude and was suggested to vary between in- and offshore areas (Ruck et al., 2014). In case of distribution shifts, such regional differences in energy content of important prey species, can affect energy availability for predators. Increased temperature also influences predator-prey

interaction among fish species (Grigaltchik et al., 2012). For instance, in reef fish, elevated temperature increased predator performance positively, but negative effects on prey escape responses increased predation rates (Allan et al., 2015). However, fish are predator and prey alike. Impacts on fish as predators are likely to be followed by impacts on organisms preying on fish, such as seabirds, seals and penguins (Casaux and Barrera-Oro, 2006; Mintenbeck et al., 2012). After all, limited knowledge on complex food webs and interspecies interactions make predictions of temperature impacts on Antarctic ecosystems difficult.

In an optimistic future scenario, the situation of Antarctic fish regarding distribution overlaps could be similar to that of fish species in the Norwegian Sea, Barents Sea and waters around Svalbard (Norway). Here, shifts in species distribution due to warming waters have led to co-occurrence of Atlantic cod, haddock and polar cod in some regions (Sundby and Nakken, 2008; Drinkwater, 2009; Renaud et al., 2012). Even though, inference of niches was suspected in this case, no evidence for strong competition has yet been found (Renaud et al., 2012). Foraging in different depth and targeting of different prey species or life stages of prey seem to be mechanisms to prevent competition for food sources among Antarctic fish today (Moreira et al., 2014). Additionally, Brenner et al. (2001) found small-scale horizontal gradients in areas disturbed and undisturbed by iceberg scouring to affect niche separation in *Trematomus* species in the Weddell Sea. However, climate change induced food web changes could facilitate resource competition, e.g. regarding food and habitat availability, and might affect mechanisms that reduce competition today. Potential competition scenarios might point out winners and losers in the challenge of climate change.

Considering climate impacts on fish, complex life cycles and differences in thermal sensitivity of different life stages have to be considered (Rijnsdorp et al., 2009; Petitgas et al., 2013). Increasing temperatures might affect recruitment of Antarctic fish, for example with regard to timing or location (Van der Veer et al., 2000; Wilderbuer et al., 2002). Such changes could result in mismatches between hatching and larval food supply (Edwards and Richardson, 2004; Voss et al., 2006). Additionally, around the Antarctic Peninsula warming is paralleled by decreasing sea ice, reducing spawning habitat of ice associated species, such as the Antarctic silverfish (*Pleuragramma antarctica*) (La Mesa et al., 2015a). Various larvae of Antarctic fish hatch in the short Antarctic summer (Kock and Kellermann, 1991; Kock, 1992), when food is abundant. Thus, impacts on timing of hatching could be severe regarding short production peaks in the pelagic zone of the Southern Ocean (Clarke, 1988). Generally, early life stages are suggested to be more temperature sensitive than adult fish (Pörtner and Farrell,

2008; Pörtner and Peck, 2010). Experimental studies on early life stages of Antarctic fish are scarce, but available data indicates theses to be most vulnerable to environmental change (Mintenbeck et al., 2012). To date the only available study on thermal tolerance of Antarctic fish early life stages by Flynn et al. (2015) found increased metabolic and developmental rate as well as increased mortality of developing Antarctic dragon fish embryos at 2°C.

Most notothenioid larvae are pelagic and thus transported by ocean currents (Kock, 1992; Damerau et al., 2012; Mintenbeck et al., 2012; La Mesa et al., 2015b). Increased temperatures are suggested to reduce larval-stage duration, thereby limiting dispersal distance (O'Connor et al., 2007). Additionally, potential climate induced changes of ocean currents might affect larval dispersal patterns with potential consequences on availability of food and other resources (Rijnsdorp et al., 2009; Vestfals et al., 2014). Due to reduced or lacking swimming ability, migration from warming areas is often restricted for larval stages (Rijnsdorp et al., 2009). Considering the high sensitivity of early life stages to abiotic as well as biotic factors, they might be critical for response patterns of Antarctic fish in a warming ocean (Rijnsdorp et al., 2009; Mintenbeck et al., 2012).

From a genetic perspective, a limited connectivity between habitats in the Antarctic region is assumed to restrict genetic mixing (Patarnello et al., 2011; Agostini et al., 2015). A reduction of polymorphisms can facilitate locally adapted genotypes, but might also limit capacities to react to environmental change (Patarnello et al., 2011; Agostini et al., 2015). Indicators of developmental plasticity and cross generational shifts in stress tolerance have been observed for fish as well as for invertebrates and suggest mitigating effects (Schaefer and Ryan, 2006; Parker et al., 2012; Donelson et al., 2014). For example, transgenerational plasticity and environment-genotype interactions were reported to mitigate impacts of increased temperature on metabolic capacity and growth of sticklebacks (Shama et al., 2014).

In a worst case scenario, ocean warming could reduce population sizes and thereby genetic variability, or cause local extinctions, by reducing available habitat (Patarnello et al., 2011; La Mesa et al., 2015a). On the other hand, adaptation over life cycles as well as tolerance shifts over generations could mediate effects of increasing temperatures (Schaefer and Ryan, 2006; Shama et al., 2014). Besides, changing ocean currents and temperatures might influence larval drift and developmental patterns (O'Connor et al., 2007) with possible advantageous or disadvantageous impacts on habitat connectivity. Warming temperatures might facilitate invasion of non-indigenous species and possibly destabilise Antarctic ecosystems (Aronson et

al., 2007). Besides, the exploitation of living resources, such as krill and fish, puts additional stress on Antarctic ecosystems (Kock et al., 2007; Ainley and Pauly, 2013).

Projections suggest a maximum water temperature increase of up to 2°C within the next 100 years for the southern Weddell Sea as well as the Antarctic Peninsula (Hellmer et al., 2012; Timmermann and Hellmer, 2013, R. Timmermann personal communication 2015), the southernmost areas of *T. bernacchii*'s and *L. nudifrons*' distributional range respectively. The results of this thesis suggest that progressing climate change is likely to affect population structures and abundances of Antarctic fish species, with high-Antarctic species, such as *T. bernacchii*, being most vulnerable to ocean warming.

4. Conclusion & Future Perspectives

In the framework of this thesis, different aspects of the thermal tolerance of Antarctic fish were analysed. In the first study (manuscript I), a comparable acute thermal response of routine metabolism of different Antarctic fish species from low- and high-Antarctic regions was found. Moreover, results of the first study indicate differences in routine metabolic rates between low- and high-Antarctic species. This could contribute to a limited thermal tolerance of these fish. In the second study (manuscript II), impacts of increased temperature at the whole-organism level in *T. bernacchii* were analysed. As a significant trade-off of elevated temperature in this species, growth rates were reduced by up to 84% at 2°C. The third study on temperature dependent energy allocation in *L. nudifrons* (manuscript III) indicated a higher thermal tolerance of energy allocation in this low-Antarctic species. In the fourth study (manuscript IV), a metadata-analysis showed growth rates of polar and especially Antarctic fish to be lower, compared to those of temperate fish. Besides, polar fish displayed a narrower thermal window compared to temperate species.

In summary, these results suggest that a low thermal tolerance of Antarctic fish could be caused by differences in energy allocation. Elevated temperature affects this energy allocation, leading to significant reductions in growth, likely affecting production and making Antarctic fish most sensitive towards ocean warming. Additionally, a high thermal sensitivity of growth performance could also reduce the competitive force of high-Antarctic fish, when climate change induced distribution shifts might lead to co-occurrence of low- and high-Antarctic species in some regions.

While a wealth of short-term studies on thermal tolerance of Antarctic fish is available, the low number of long-term experiments is surprising and more studies on acclimated animals are urgently needed. This is particularly important since the second study of this thesis and various other studies on thermal tolerance of Antarctic fish indicate acclimation to take 21 to 69 days (Robinson and Davison, 2008; Bilyk and DeVries, 2011; Strobel et al., 2012; Peck et al., 2014). At the whole-organism level, energy budget studies (manuscript II & III) proofed to be a good mean to assess temperature impacts. Further, transgenerational plasticity, such as effects of maternal temperature acclimation on thermal tolerance of offspring, would be most interesting. However, cross-generation experiments with Antarctic fish are mostly hindered by problems of larval rearing and breeding.

Additionally, the combination of physiological and genetic studies can yield valuable insights and enhance the effective use of animal and work resources. In this context, a further processing of tissue samples collected in the energy budget experiment with *T. bernacchii* is planned to follow-up findings from this thesis (manuscript II). An analysis of transcriptomic changes among temperature treatments could be a great chance to track down changes identified at the whole-organism to genetic levels. Such an analysis could include e.g. transcriptomic profiles. Real time quantitative polymerase chain reaction (qPCR) assays on single genes could yield insight into anabolic and catabolic pathways and heat shock protein responses to increased temperature. Later, epigenetics may give some insight into factors influencing thermal tolerance, e.g. by analysis of DNA methylation and histone modification patterns.

Considering differences in thermal tolerance within Antarctic species, ambiguities of species and regional effects are a very interesting aspect. Studies on the thermal sensitivity of different populations of the same species from different Antarctic habitats could help to assess e.g. genetic contributions to thermal response capacities, local adaptations to biotic and abiotic factors as well as assumptions of countergradient variation (Conover and Present, 1990). In this context, a comparable energy budget study to the one from this thesis with a northern population of *T. bernacchii*, e.g. from the Antarctic Peninsula, would be of great value to assess the phenotypic plasticity and acclimation capacity of the species. The combination of such a study with the above mentioned genetic analysis could potentially help to identify genetic patterns associated with a higher thermal tolerance across populations. Knowledge on genetic patterns involved in thermal tolerance and warm acclimation capacities might allow a scan of different populations of *T. bernacchii* for their thermal response capacities to gain insight in the overall temperature tolerance of this circum-Antarctic species.

From an ecological perspective, the assessment of potential consequences of climate change based on knowledge of a species' thermal tolerance is most crucial. Regarding the example of the model species *T. bernacchii*, the identification of thermal limits for the whole animal could be followed by the assessment of mitigation scenarios. The identification of temperature-dependent abundance structures in combination with knowledge on thermal tolerance can allow the identification of potentially suitable habitats for a species (c.f. Galparsoro et al., 2009). Climate and ecosystem models could then be used to analyse possible suitable habitats and potential shifts in abundances based on projected future ocean conditions.

Furthermore, data from energy budget experiments, food web as well as abundance studies could be combined with temperature predictions and estimations of available habitat to serve as a framework for an ecological model (cf. Albouy et al., 2014). Such a model could allow the estimation of energy fluxes within ecosystems, as well as potential changes due to elevated temperature, thereby supporting to assess potential consequences of progressing climate change. Linking data on thermal tolerance of single fish species from physiological studies to ecological data sets is an important step to gain a better understanding of what might happen in Antarctic areas in the future.

The Southern Ocean is a unique ecosystem inhabited by a variety of organisms with diverse adaptations to this extreme environment. Antarctic fish display various features adjusting their physiology and ecology to the cold, making them most sensitive to temperature changes (O'Brien and Crockett, 2013; Peck et al., 2014). The assessment of potential consequences of ocean warming for Antarctic fish is particularly important regarding their significant role for Antarctic ecosystems and food webs. Antarctic fish are an important link between higher and lower trophic levels in Antarctic food webs, as they serve as food source for predatory species such as seals, penguins and sea birds (Hureau, 1994; Kock et al., 2012). Thus, impacts on fish could have far reaching consequences for food webs and whole Antarctic ecosystems.

Climate change has the potential to irrevocably change the Southern Ocean. The studies of this thesis demonstrate the low thermal tolerance of Antarctic fish species and indicate a high vulnerability towards increasing temperatures. Ocean warming is also paralleled by e.g. ocean acidification and changes in salinity (Mintenbeck et al., 2012). The Interaction of different stressors can have antagonistic or synergistic effects, with potential implications for physiology and ecology of fish species (Perry et al., 2005; Pörtner and Peck, 2010; Mintenbeck et al., 2012; Gräns et al., 2014). While warming waters have been recorded to affect fish especially on the warm end of their distributional range (Perry et al., 2005), a recent study found ocean acidification to negatively affect species at the cold edge of their distributional range (Gräns et al., 2014). The combination of different stressors influencing marine environments thus raises the need for multi-stressor experiments. A clear understanding of already visible and potential future consequences is urgently needed to assess options to mitigate and stop anthropogenic climate change.

5. References

Abele, D. (2002). Toxic oxygen: the radical life-giver. *Nature* **420**, 27-27.
Abele, D. and Puntarulo, S. (2004). Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comp. Biochem. Physiol. A* **138**, 405-415.
Agostini, C., Patarnello, T., Ashford, J. R., Torres, J. J., Zane, L. and Papetti, C. (2015). Genetic differentiation in the ice-dependent fish *Pleuragramma antarctica* along the Antarctic Peninsula. *J. Biogeogr.* **42**, 1103-1113.
Ainley, D. G. and Pauly, D. (2013). Fishing down the food web of the Antarctic continental shelf and slope. *Polar Rec.* **50**, 92-107.
Albouy, C., Velez, L., Coll, M., Colloca, F., Loc'h, F., Mouillot, D. and Gravel, D. (2014). From projected species distribution to food-web structure under climate change. *Global Change Biol.* **20**, 730-741.
Allan, B. J., Domenici, P., Munday, P. L. and McCormick, M. I. (2015). Feeling the heat: the effect of acute temperature changes on predator–prey interactions in coral reef fish. *Conserv. Physiol.* **3**, cov011.
Anderson, J. B. (1991). The Antarctic continental shelf: results from marine geological and geophysical investigations. In *The Geology of Antarctica*, (ed. R. Tingey), pp. 285-334. Oxford: Clarendon Press.
Archer, S. D. and Johnston, I. A. (1991). Density of cristae and distribution of mitochondria in the slow muscle fibers of Antarctic fish. *Physiol. Zool.* **64**, 242-258.
Arntz, W. E., Gutt, J. and Klages, M. (1997). Antarctic marine biodiversity: an overview. In *Antarctic communities: species, structure and survival*, (ed. B. Battaglia), pp. 3-14. Cambridge: Cambridge University Press.
Aronson, R. B., Thatje, S., Clarke, A., Peck, L. S., Blake, D. B., Wilga, C. D. and Seibel, B. A. (2007). Climate change and invasibility of the Antarctic benthos. *Annu. Rev. Ecol. Evol. S.* **38**, 129-154.
Atkinson, A., Siegel, V., Pakhomov, E. and Rothery, P. (2004). Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature* **432**, 100-103.
Axelsson, M., Davison, W., Forster, M. and Farrell, A. (1992). Cardiovascular responses of the red-blooded antarctic fishes *Pagothenia bernacchii* and *P. borchgrevinki*. *J. Exp. Biol.* **167**, 179-201.
Barnes, D. K. and Peck, L. S. (2008). Vulnerability of Antarctic shelf biodiversity to predicted regional warming. *Climate Res.* **37**, 149-163.
Barnes, D. K., Griffiths, H. J. and Kaiser, S. (2009). Geographic range shift responses to climate change by Antarctic benthos: where we should look. *Mar. Ecol. Prog. Ser.* **393**, 13-26.
Barnes, D. K., Fuentes, V., Clarke, A., Schloss, I. R. and Wallace, M. I. (2006). Spatial and temporal variation in shallow seawater temperatures around Antarctica. *Deep-Sea Res. Pt II* **53**, 853-865.
Battram, J. C. and Johnston, I. A. (1991). Muscle growth in the Antarctic teleost, *Notothenia neglecta* (Nybelin). *Antarct. Sci.* **3**, 29-33.
Bilyk, K. T. and DeVries, A. L. (2011). Heat tolerance and its plasticity in Antarctic fishes. *Comp. Biochem. Physiol. A* **158**, 382-390.
Bilyk, K. T., Evans, C. W. and DeVries, A. L. (2012). Heat hardening in Antarctic notothenioid fishes. *Polar Biol.* **35**, 1447-1451.
Biro, P. A., Beckmann, C. and Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proc. R. Soc. Lond. B* **277**, 71-77.

Brauer, P. R., Sanmann, J. N. and Petzel, D. H. (2005). Effects of warm acclimation on Na^+, K^+-ATPase α-subunit expression in chloride cells of Antarctic fish. *Anat. Rec.* **285**, 600-609.
Brenner, M., Buck, B., Cordes, S. and Dietrich, L. (2001). The role of iceberg scours in niche separation within the antarctic fish genus *Trematomus*. *Polar Biol* **24**, 502-507.
Brodte, E., Knust, R. and Pörtner, H. O. (2006a). Temperature-dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae). *Polar Biol.* **30**, 95-107.
Brodte, E., Knust, R., Pörtner, H.-O. and Arntz, W. (2006b). Biology of the Antarctic eelpout *Pachycara brachycephalum*. *Deep-Sea Res. Pt II* **53**, 1131-1140.
Buckley, B. A. and Somero, G. N. (2009). cDNA microarray analysis reveals the capacity of the cold-adapted Antarctic fish *Trematomus bernacchii* to alter gene expression in response to heat stress. *Polar Biol.* **32**, 403-415.
Casaux, R. and Barrera-Oro, E. (2006). Shags in Antarctica: their feeding behaviour and ecological role in the marine food web. *Antarct. Sci.* **18**, 3-14.
Celik, Y., Drori, R., Pertaya-Braun, N., Altan, A., Barton, T., Bar-Dolev, M., Groisman, A., Davies, P. L. and Braslavsky, I. (2013). Microfluidic experiments reveal that antifreeze proteins bound to ice crystals suffice to prevent their growth. *Proc. Natl. Acad. Sci. USA* **110**, 1309-1314.
Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Frolicher, T. L., Lam, V. W. Y., Deng Palomares, M. L., Watson, R. and Pauly, D. (2013). Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nature Clim. Change* **3**, 254-258.
Chown, S. L., Jumbam, K. R., Sorensen, J. G. and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* **23**, 133-140.
Claireaux, G., Webber, D., Kerr, S. and Boutilier, R. (1995). Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. *J. Exp. Biol.* **198**, 49-60.
Clark, T. D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771-2782.
Clarke, A. (1988). Seasonality in the Antarctic marine environment. *Comp. Biochem. Physiol. B* **90**, 461-473.
Clarke, A. (1991). What is cold adaptation and how should we measure it? *Am. Zool.* **31**, 81-92.
Clarke, A. and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *J. Anim. Ecol.* **68**, 893-905.
Clarke, A., Barnes, D. K. and Hodgson, D. A. (2005). How isolated is Antarctica? *Trends Ecol. Evol.* **20**, 1-3.
Conover, D. O. and Present, T. M. (1990). Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* **83**, 316-324.
Costa, D. P., Huckstadt, L. A., Crocker, D. E., McDonald, B. I., Goebel, M. E. and Fedak, M. A. (2010). Approaches to studying climatic change and its role on the habitat selection of Antarctic pinnipeds. *Integr. Comp. Biol.*, icq054.
Cowles, R. B. and Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist* **83**, 261-296.
Crockett, E. L. and Sidell, B. D. (1990). Some pathways of energy metabolism are cold adapted in Antarctic fishes. *Physiol. Zool.*, 472-488.
Damerau, M., Matschiner, M., Salzburger, W. and Hanel, R. (2012). Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. *Polar Biol.* **35**, 1073-1086.

Daniels, R. A. (1982). Feeding ecology of some fishes of the Antarctic Peninsula. *Fish. Bull.* **80**, 575-588.
Davison, W., Franklin, C. E. and McKenzie, J. C. (1994). Haematological changes in an Antarctic teleost, *Trematomus bernacchii*, following stress. *Polar Biol.* **14**, 463-466.
Davison, W., Axelsson, M., Forster, M. and Nilsson, S. (1995). Cardiovascular responses to acute handling stress in the Antarctic fish *Trematomus bernacchii* are not mediated by circulatory catecholamines. *Fish Physiol. Biochem.* **14**, 253-257.
Detrich III, H. (1991). Polymerization of microtubule proteins from Antarctic fish. In *Biology of Antarctic fish*, (eds. G. di Prisco, B. Maresca and B. Tota), pp. 248-262. Berlin: Springer.
DeVries, A. L. (1971). Glycoproteins as biological antifreeze agents in Antarctic fishes. *Science* **172**, 1152-1155.
DeVries, A. L. and Eastman, J. T. (1981). Physiology and ecology of notothenioid fishes of the Ross Sea. *J. Roy. Soc. New Zeal.* **11**, 329-340.
Donelson, J. M., McCormick, M. I., Booth, D. J. and Munday, P. L. (2014). Reproductive acclimation to increased water temperature in a tropical reef fish. *PLoS ONE* **9**, e97223.
Drinkwater, K. (2009). Comparison of the response of Atlantic cod (*Gadus morhua*) in the high-latitude regions of the North Atlantic during the warm periods of the 1920s–1960s and the 1990s–2000s. *Deep-Sea Res. Pt II* **56**, 2087-2096.
Dulvy, N. K., Rogers, S. I., Jennings, S., Stelzenmüller, V., Dye, S. R. and Skjoldal, H. R. (2008). Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. *J. App. Ecol.* **45**, 1029-1039.
Eastman, J. T. (1993). Antarctic fish biology: evolution in a unique environment. San Diego: Academic Press.
Eastman, J. T. and DeVries, A. L. (1981). Buoyancy adaptations in a swim-bladderless Antarctic fish. *J. Morphol.* **167**, 91-102.
Eastman, J. T. and Eakin, R. R. (2000). An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments on Antarctic species. *Arch. Fish. Mar. Res.* **48**, 11-20.
Edwards, M. and Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* **430**, 881-884.
Egginton, S. (1996). Blood rheology of Antarctic fishes: viscosity adaptations at very low temperatures. *J. Fish Biol.* **48**, 513-521.
Enzor, L. A. and Place, S. P. (2014). Is warmer better? Decreased oxidative damage in notothenioid fish after long-term acclimation to multiple stressors. *J. Exp. Biol.* **217**, 3301-3310.
Enzor, L. A., Zippay, M. L. and Place, S. P. (2013). High latitude fish in a high CO_2 world: Synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids. *Comp. Biochem. Physiol. A* **164**, 154-161.
Fan, T., Deser, C. and Schneider, D. P. (2014). Recent Antarctic sea ice trends in the context of Southern Ocean surface climate variations since 1950. *Geophys. Res. Lett.* **41**, 2419-2426.
Fanta, E. (1999). Laboratory tests on feeding interactions and food preferences of some Antarctic fish from Admiralty Bay, King George Island, South Shetland Islands. *Pol. Polar. Res.* **20**, 335-346.
Fitch, N., Johnson, I. and Wood, R. (1984). Skeletal muscle capillary supply in a fish that lacks respiratory pigments. *Respir. Physiol.* **57**, 201-211.
Flore, L. and Keckeis, H. (1998). The effect of water current on foraging behaviour of the rheophilic cyprinid *Chondrostoma nasus* (L.) during ontogeny: evidence of a trade-off between energetic gain and swimming costs. *Regul. River* **14**, 141-154.

Flores, H., van Franeker, J., Siegel, V., Haraldsson, M., Strass, V., Meesters, E. H., Bathmann, U. and Wolff, J. W. (2012). The Association of Antarctic Krill *Euphausia superba* with the Under-Ice. *PLoS ONE* **7**, e31775.
Flynn, E. E., Bjelde, B. E., Miller, N. A. and Todgham, A. E. (2015). Ocean acidification exerts negative effects during warming conditions in a developing Antarctic fish. *Conserv. Physiol.* **3**, 1-16.
Fonds, M., Cronie, R., Vethaak, A. and Van der Puyl, P. (1992). Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. *Neth. J. Sea Res.* **29**, 127-143.
Forcada, J., Trathan, P., Reid, K., Murphy, E. and Croxall, J. (2006). Contrasting population changes in sympatric penguin species in association with climate warming. *Global Change Biol.* **12**, 411-423.
Franklin, C. E., Davison, W. and Seebacher, F. (2007). Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *J. Exp. Biol.* **210**, 3068-3074.
Gagné, M. È., Gillett, N. and Fyfe, J. (2015). Observed and simulated changes in Antarctic sea ice extent over the past 50 years. *Geophys. Res. Lett.* **42**, 90-95.
Galparsoro, I., Borja, Á., Bald, J., Liria, P. and Chust, G. (2009). Predicting suitable habitat for the European lobster (*Homarus gammarus*), on the Basque continental shelf (Bay of Biscay), using Ecological-Niche Factor Analysis. *Ecol. Model.* **220**, 556-567.
García, V., Celada, J. D., González, R., Carral, J. M., Sáez-Royuela, M. and González, Á. (2015). Response of juvenile tench (*Tinca tinca* L.) fed practical diets with different protein contents and substitution levels of fish meal by soybean meal. *Aquac. Res.* **46**, 28-38.
Garvey, J. E. and Marschall, E. A. (2003). Understanding latitudinal trends in fish body size through models of optimal seasonal energy allocation. *Can. J. Fish. Aqua. Sci.* **60**, 938-948.
Giacomini, H. C., Shuter, B. J. and Lester, N. P. (2013). Predator bioenergetics and the prey size spectrum: Do foraging costs determine fish production? *J. Theor. Biol.* **332**, 249-260.
Gon, O. and Heemstra, P. C. (1990). Fishes of the Southern Ocean. Grahamstown: JLB Smith Institute of Ichthyology.
Gonzalez-Cabrera, P. J., Dowd, F., Pedibhotla, V. K., Rosario, R., Stanley-Samuelson, D. and Petzel, D. (1995). Enhanced hypo-osmoregulation induced by warm-acclimation in Antarctic fish is mediated by increased gill and kidney Na^+/K^+-ATPase activities. *J. Exp. Biol.* **198**, 2279-2291.
Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O. and Einarsdottir, I. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. *J. Exp. Biol.* **217**, 711-717.
Grebmeier, J. M., Overland, J. E., Moore, S. E., Farley, E. V., Carmack, E. C., Cooper, L. W., Frey, K. E., Helle, J. H., McLaughlin, F. A. and McNutt, S. L. (2006). A major ecosystem shift in the northern Bering Sea. *Science* **311**, 1461-1464.
Grigaltchik, V. S., Ward, A. J. and Seebacher, F. (2012). Thermal acclimation of interactions: differential responses to temperature change alter predator-prey relationship. *Proc. R. Soc. Lond. B* **279**, 4058-4064.
Guynn, S., Dowd, F. and Petzel, D. (2002). Characterization of gill Na/K-ATPase activity and ouabain binding in Antarctic and New Zealand nototheniid fishes. *Comp. Biochem. Physiol. A* **131**, 363-374.
Hellmer, H. H., Kauker, F., Timmermann, R., Determann, J. and Rae, J. (2012). Twenty-first-century warming of a large Antarctic ice-shelf cavity by a redirected coastal current. *Nature* **485**, 225-228.

Hemmingsen, E. A., Douglas, E. L., Johansen, K. and Millard, R. W. (1972). Aortic blood flow and cardiac output in the hemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol. A* **43**, 1045-1051.
Hildebrandt, N., Bergmann, M. and Knust, R. (2011). Longevity and growth efficiency of two deep-dwelling Arctic zoarcids and comparison with eight other zoarcid species from different climatic regions. *Polar Biol.* **34**, 1523-1533.
Hochachka, P. (1988). Channels and pumps-determinants of metabolic cold adaptation strategies. *Comp. Biochem. Physiol. B* **90**, 515-519.
Hoegh-Guldberg, O., Cai, R., Poloczanska, E. S., Brewer, P. G., Sundby, S., Hilmi, K., Fabry, V. J. and Jung, S. (2014). The Ocean. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change*, (eds. V. R. Barros, C. B. Field, D. J. Dokken, M. D. Mastrandrea, K. J. Mach, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova et al.), pp. 1655-1731. Cambridge/New York: Cambridge University Press.
Hofmann, G. E., Buckley, B. A., Airaksinen, S., Keen, J. E. and Somero, G. N. (2000). Heat-shock protein expression is absent in the Antarctic fish *Trematomus bernacchii* (family Nototheniidae). *J. Exp. Biol.* **203**, 2331-2339.
Holeton, G. F. (1970). Oxygen uptake and circulation by a hemoglobinless Antarctic fish (*Chaenocephalus aceratus* Lonnberg) compared with three red-blooded Antarctic fish. *Comp. Biochem. Physiol.* **34**, 457-&.
Holeton, G. F. (1974). Metabolic cold adaptation of polar fish: fact or artefact? *Physiol. Zool.*, 137-152.
Hubold, G. (1992). Zur Ökologie der Fische im Weddellmeer. *Berichte zur Polarforschung* **103**.
Hudson, H., Brauer, P., Scofield, M. and Petzel, D. (2008). Effects of warm acclimation on serum osmolality, cortisol and hematocrit levels in the Antarctic fish, *Trematomus bernacchii*. *Polar Biol.* **31**, 991-997.
Hunt, B. M., Hoefling, K. and Cheng, C.-H. C. (2003). Annual warming episodes in seawater temperatures in McMurdo Sound in relationship to endogenous ice in notothenioid fish. *Antarct. Sci.* **15**, 333-338.
Hureau, J. C. (1994). The significance of fish in the marine Antractic ecosystems. *Polar Biol.* **14**, 307-313.
Huth, T. J. and Place, S. P. (2013). De novo assembly and characterization of tissue specific transcriptomes in the emerald notothen, *Trematomus bernacchii*. *BMC Genomics* **14**, 805.
Jayasundara, N., Healy, T. M. and Somero, G. N. (2013). Effects of temperature acclimation on cardiorespiratory performance of the Antarctic notothenioid *Trematomus bernacchii*. *Polar Biol.* **36**, 1047-1057.
Jensen, F. B., Nikinmaa, M. and Weber, R. E. (1993). Environmental perturbations of oxygen transport in teleost fishes: causes, consequences and compensations. In *Fish ecophysiology*, (eds. J. C. Rankin and F. B. Jensen), pp. 161-179. London: Chapman & Hall.
Jobling, M. (1994). Fish bioenergetics. London: Chapman & Hall.
Johnston, I., Calvo, J., Guderley, H., Fernandez, D. and Palmer, L. (1998). Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *J. Exp. Biol.* **201**, 1-12.
Johnston, I. A., Fernández, D. A., Calvo, J., Vieira, V. L., North, A. W., Abercromby, M. and Garland, T. (2003). Reduction in muscle fibre number during the adaptive radiation of notothenioid fishes: a phylogenetic perspective. *J. Exp. Biol.* **206**, 2595-2609.
Jonsson, N. and Jonsson, B. (2003). Energy allocation among developmental stages, age groups, and types of Atlantic salmon (*Salmo salar*) spawners. *Can. J. Fish. Aqua. Sci.* **60**, 506-516.

Jordan, A. D., Jungersen, M. and Steffensen, J. (2001). Oxygen consumption of East Siberian cod: no support for the metabolic cold adaptation theory. *J. Fish Biol.* **59**, 818-823.
Kawall, H., Torres, J., Sidell, B. and Somero, G. (2002). Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. *Mar. Biol.* **140**, 279-286.
Kock, K.-H. (1992). Antarctic fish and fisheries. Cambridge: Cambridge University Press.
Kock, K.-H. and Kellermann, A. (1991). Reproduction in Antarctic notothenioid fish. *Antarct. Sci.* **3**, 125-150.
Kock, K.-H. and Everson, I. (1998). Age, growth and maximum size of Antarctic notothenioid fish - revisited. In *Fishes of Antarctica*, (eds. G. di Prisco, E. Pisano and A. Clarke), pp. 29-40. Milan: Springer.
Kock, K.-H., Reid, K., Croxall, J. and Nicol, S. (2007). Fisheries in the Southern Ocean: an ecosystem approach. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **362**, 2333-2349.
Kock, K.-H., Barrera-Oro, E., Belchier, M., Collins, M., Duhamel, G., Hanchet, S., Pshenichnov, L., Welsford, D. and Williams, R. (2012). The role of fish as predators of krill (*Euphausia superba*) and other pelagic resources in the Southern Ocean. *CCAMLR Sci.* **19**, 115-169.
Kültz, D. (2005). Molecular and evolutionary basis of the cellular stress response. *Annu. Rev. Physiol.* **67**, 225-257.
La Mesa, M. and Vacchi, M. (2001). Age and growth of high Antarctic notothenioid fish. *Antarct. Sci.* **13**, 227-235.
La Mesa, M., Dalú, M. and Vacchi, M. (2004). Trophic ecology of the emerald notothen *Trematomus bernacchii* (Pisces, Nototheniidae) from Terra Nova Bay, Ross Sea, Antarctica. *Polar Biol.* **27**, 721-728.
La Mesa, M., Riginella, E., Mazzoldi, C. and Ashford, J. (2015a). Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the Western Antarctic Peninsula. *Marine Ecology* **36**, 235-245.
La Mesa, M., Piñones, A., Catalano, B. and Ashford, J. (2015b). Predicting early life connectivity of Antarctic silverfish, an important forage species along the Antarctic Peninsula. *Fisheries Oceanography* **24**, 150-161.
Lindquist, S. (1986). The heat-shock response. *Annu. Rev. Biochem.* **55**, 1151-1191.
Macdonald, J. and Wells, R. (1991). Viscosity of body fluids from Antarctic notothenioid fish. In *Biology of Antarctic fish*, (eds. G. di Prisco, B. Maresca and B. Tota), pp. 163-178. Berlin: Springer.
Magnoni, L. J., Scarlato, N. A., Ojeda, F. P. and Wohler, O. C. (2013). Gluconeogenic pathway does not display metabolic cold adaptation in liver of Antarctic notothenioid fish. *Polar Biol.* **36**, 661-671.
Mantyla, A. W. and Reid, J. L. (1983). Abyssal characteristics of the World Ocean waters. *Deep-Sea Res. Pt I* **30**, 805-833.
Mark, F. C., Hirse, T. and Pörtner, H. O. (2005). Thermal sensitivity of cellular energy budgets in Antarctic fish hepatocytes. *Comp. Biochem. Physiol. A* **141**, S368-S369.
Meredith, M. P. and King, J. C. (2005). Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century. *Geophys. Res. Lett.* **32**.
Metcalfe, N. B., Taylor, A. C. and Thorpe, J. E. (1995). Metabolic rate, social status and life-history strategies in Atlantic salmon. *Anim. Behav.* **49**, 431-436.
Meyer, S. (2012). The role of temperature on the distribution of fish fauna in Antarctic waters, B.Sc., Bremen: University of Bremen (FB 2).
Mintenbeck, K., Barrera-Oro, E., Brey, T., Jacob, U., Knust, R., Mark, F. C., Moreira, E., Strobel, A. and Arntz, W. (2012). Impact of climate change on fishes in complex Antarctic ecosystems. *Adv. Ecol. Res.* **46**, 351-426.

Miyamoto, Y. and Tanimura, A. (1999). Behavior of the Antarctic fish *Trematomus bernacchii* (Pisces, Nototheniidae) beneath the sea ice near the Antarctic station Syowa using acoustic biotelemetry. *Fisheries Sci.* **65**, 315-316.
Moline, M. A., Claustre, H., Frazer, T. K., Schofield, O. and Vernet, M. (2004). Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. *Global Change Biol.* **10**, 1973-1980.
Montgomery, J. C., Foster, B. A., Milton, R. C. and Carr, E. (1993). Spatial and temporal variations in the diet of nototheniid fish in McMurdo Sound, Antarctica. *Polar Biol.* **13**, 429-431.
Moreira, E., Juáres, M. and Barrera-Oro, E. (2014). Dietary overlap among early juvenile stages in an Antarctic notothenioid fish assemblage at Potter Cove, South Shetland Islands. *Polar Biol.* **37**, 1507-1515.
Morgan, K. N. and Tromborg, C. T. (2007). Sources of stress in captivity. *Appl. Anim. Behav. Sci.* **102**, 262-302.
Morita, K., Fukuwaka, M. a., Tanimata, N. and Yamamura, O. (2010). Size-dependent thermal preferences in a pelagic fish. *Oikos* **119**, 1265-1272.
Morris, R. W. and Schneider, M. J. (1969). Brain fatty acids of an antarctic fish, *Trematomus bernacchii*. *Comp. Biochem. Physiol.* **28**, 1461-1465.
Morrison, J. F., Guynn, S. R., Scofield, M. A., Dowd, F. J. and Petzel, D. H. (2006). Warm acclimation changes the expression of the Na^+, K^+-ATPase α subunit isoforms in Antarctic fish gills. *J. Exp. Mar. Biol. Ecol.* **333**, 129-139.
Murawski, S. (1993). Climate change and marine fish distributions: forecasting from historical analogy. *Trans. Am. Fish. Soc.* **122**, 647-658.
Nahrgang, J., Varpe, Ø., Korshunova, E., Murzina, S., Hallanger, I. G., Vieweg, I. and Berge, J. (2014). Gender specific reproductive strategies of an Arctic key species (*Boreogadus saida*) and implications of climate change. *PloS ONE* **9**, e98452.
Neuheimer, A., Thresher, R., Lyle, J. and Semmens, J. (2011). Tolerance limit for fish growth exceeded by warming waters. *Nature Clim. Change* **1**, 110-113.
Norin, T. and Malte, H. (2011). Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *J. Exp. Biol.* **214**, 1668-1675.
O'Brien, K., Skilbeck, C., Sidell, B. and Egginton, S. (2003). Muscle fine structure may maintain the function of oxidative fibres in haemoglobinless Antarctic fishes. *J. Exp. Biol.* **206**, 411-421.
O'Brien, K. M. and Crockett, E. L. (2013). The promise and perils of Antarctic fishes. *EMBO reports* **14**, 17-24.
O'Connor, K., Taylor, A. and Metcalfe, N. (2000). The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. *J. Fish Biol.* **57**, 41-51.
O'Connor, M. I., Bruno, J. F., Gaines, S. D., Halpern, B. S., Lester, S. E., Kinlan, B. P. and Weiss, J. M. (2007). Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proc. Natl. Acad. Sci. USA* **104**, 1266-1271.
Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A. and Pörtner, H. O. (2012). Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biol.* **18**, 82-92.
Parkinson, C. and Cavalieri, D. (2012). Antarctic sea ice variability and trends, 1979–2010. *The Cryosphere* **6**, 871-880.
Parsell, D. and Lindquist, S. (1993). The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genet.* **27**, 437-496.
Patarnello, T., Verde, C., Di Prisco, G., Bargelloni, L. and Zane, L. (2011). How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study. *BioEssays* **33**, 260-268.

Peck, L. S., Morley, S. A., Richard, J. and Clark, M. S. (2014). Acclimation and thermal tolerance in Antarctic marine ectotherms. *J. Exp. Biol.* **217**, 16-22.
Peck, L. S., Clark, M. S., Morley, S. A., Massey, A. and Rossetti, H. (2009). Animal temperature limits and ecological relevance: effects of size, activity and rates of change. *Funct. Ecol.* **23**, 248-256.
Peck, M. A., Buckley, L. J., Caldarone, E. M. and Bengtson, D. A. (2003). Effects of food consumption and temperature on growth rate and biochemical-based indicators of growth in early juvenile Atlantic cod *Gadus morhua* and haddock *Melanogrammus aeglefinus*. *Mar. Ecol. Prog. Ser.* **251**, 233-243.
Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science* **308**, 1912-1915.
Perry, R. I. and Neilson, J. D. (1988). Vertical distributions and trophic interactions of age-0 Atlantic cod and haddock in mixed and stratified waters of Georges Bank. *Mar. Ecol. Prog. Ser.* **49**, 199-214.
Petitgas, P., Rijnsdorp, A. D., Dickey-Collas, M., Engelhard, G. H., Peck, M. A., Pinnegar, J. K., Drinkwater, K., Huret, M. and Nash, R. D. (2013). Impacts of climate change on the complex life cycles of fish. *Fish. Oceanogr.* **22**, 121-139.
Petzel, D. (2005). Drinking in Antarctic fishes. *Polar Biol.* **28**, 763-768.
Place, S. P. and Hofmann, G. E. (2005). Constitutive expression of a stress-inducible heat shock protein gene, hsp70, in phylogenetically distant Antarctic fish. *Polar Biol.* **28**, 261-267.
Place, S. P., Zippay, M. L. and Hofmann, G. E. (2004). Constitutive roles for inducible genes: evidence for the alteration in expression of the inducible hsp70 gene in Antarctic notothenioid fishes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R429-R436.
Podrabsky, J. E. and Somero, G. N. (2006). Inducible heat tolerance in Antarctic notothenioid fishes. *Polar Biol.* **30**, 39-43.
Pörtner, H. O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **213**, 881-893.
Pörtner, H. O. and Peck, M. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J. Fish Biol.* **77**, 1745-1779.
Pörtner, H. O. (2012). Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* **470**, 273-290.
Pörtner, H. O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95-97.
Pörtner, H. O. and Farrell, A. P. (2008). Physiology and climate change. *Science* **322**, 690-692.
Pörtner, H. O., Peck, L. and Somero, G. (2007). Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **362**, 2233-2258.
Post, J. R. (1990). Metabolic allometry of larval and juvenile yellow perch (*Perca flavescens*): in situ estimates and bioenergetic models. *Can. J. Fish. Aqua. Sci.* **47**, 554-560.
Post, J. R. and Parkinson, E. (2001). Energy allocation strategy in young fish: allometry and survival. *Ecology* **82**, 1040-1051.
Pottinger, T. and Pickering, A. (1992). The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress. *J. Fish Biol.* **41**, 435-447.
Renaud, P. E., Berge, J., Varpe, Ø., Lønne, O. J., Nahrgang, J., Ottesen, C. and Hallanger, I. (2012). Is the poleward expansion by Atlantic cod and haddock threatening native polar cod, *Boreogadus saida*? *Polar Biol.* **35**, 401-412.

Rijnsdorp, A. D., Peck, M. A., Engelhard, G. H., Möllmann, C. and Pinnegar, J. K. (2009). Resolving the effect of climate change on fish populations. *ICES J. Mar. Sci.* **66**, 1570-1583.
Robinson, E. (2008). Antarctic Fish: Thermal Specialists or Adaptable Generalists?, Ph.D., Christchurch: University of Canterbury.
Robinson, E. and Davison, W. (2008). The Antarctic notothenioid fish *Pagothenia borchgrevinki* is thermally flexible: acclimation changes oxygen consumption. *Polar Biol.* **31**, 317-326.
Ruck, K. E., Steinberg, D. K. and Canuel, E. A. (2014). Regional differences in quality of krill and fish as prey along the Western Antarctic Peninsula. *Mar. Ecol. Prog. Ser.* **509**, 39-55.
Ruud, J. T. (1954). Vertebrates without erythrocytes and blood pigment. *Nature* **173**, 848-850.
Sandersfeld, T., Davison, W., Lamare, M. D., Knust, R. and Richter, C. (2015). Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. *J. Exp. Biol.* **218**, 2373-2381.
Schaefer, J. and Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *J. Fish Biol.* **69**, 722-734.
Schloss, I. R., Abele, D., Moreau, S., Demers, S., Bers, A. V., González, O. and Ferreyra, G. A. (2012). Response of phytoplankton dynamics to 19-year (1991–2009) climate trends in Potter Cove (Antarctica). *J. Marine Syst.* **92**, 53-66.
Scholander, P., Flagg, W., Walters, V. and Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zool.* **26**, 67-92.
Secor, S. M. (2009). Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. B* **179**, 1-56.
Seebacher, F., Davison, W., Lowe, C. J. and Franklin, C. E. (2005). A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fishes. *Biol. Letters* **1**, 151-154.
Shama, L. N., Strobel, A., Mark, F. C. and Wegner, K. M. (2014). Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. *Funct. Ecol.* **28**, 1482-1493.
Sidell, B. D. and O'Brien, K. M. (2006). When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. *J. Exp. Biol.* **209**, 1791-1802.
Sidell, B. D., Crockett, E. L. and Driedzic, W. R. (1995). Antarctic fish tissues preferentially catabolize monoenoic fatty acids. *J. Exp. Zool.* **271**, 73-81.
Sidell, B. D., Vayda, M. E., Small, D. J., Moylan, T. J., Londraville, R. L., Yuan, M.-L., Rodnick, K. J., Eppley, Z. A. and Costello, L. (1997). Variable expression of myoglobin among the hemoglobinless Antarctic icefishes. *Proc. Natl. Acad. Sci. USA* **94**, 3420-3424.
Sleadd, I. M. and Buckley, B. A. (2013). The CCAAT/enhancer-binding protein δ (C/EBP-δ) transcription factor is heat inducible in the cold-adapted antarctic fish *Trematomus bernacchii*. *Polar Biol.* **36**, 335-342.
Sleadd, I. M., Lee, M., Hassumani, D. O., Stecyk, T. M., Zeitz, O. K. and Buckley, B. A. (2014). Sub-lethal heat stress causes apoptosis in an Antarctic fish that lacks an inducible heat shock response. *J. Therm. Biol.* **44**, 119-125.
Smale, D. A., Brown, K. M., Barnes, D. K., Fraser, K. P. and Clarke, A. (2008). Ice scour disturbance in Antarctic waters. *Science* **321**, 371-371.
Smith, C. R., Grange, L. J., Honig, D. L., Naudts, L., Huber, B., Guidi, L. and Domack, E. (2011). A large population of king crabs in Palmer Deep on the west Antarctic Peninsula shelf and potential invasive impacts. *Proc. R. Soc. Lond. B* **279**, 1017-1026.
Sokolova, I. M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr. Comp. Biol.* **53**, 597-608.

Somero, G. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**, 912-920.
Somero, G. N. (2012). The physiology of global change: linking patterns to mechanisms. *Ann. Rev. Mar. Sci.* **4**, 39-61.
Somero, G. N. and DeVries, A. L. (1967). Temperature tolerance of some Antarctic fishes. *Science* **156**, 257-258.
Somero, G. N., Giese, A. C. and Wohlschlag, D. E. (1968). Cold adaptation of the Antarctic fish *Trematomus bernacchii*. *Comp. Biochem. Physiol.* **26**, 223-233.
Stammerjohn, S. E., Martinson, D. G., Smith, R. C. and Iannuzzi, R. A. (2008). Sea ice in the western Antarctic Peninsula region: Spatio-temporal variability from ecological and climate change perspectives. *Deep-Sea Res. Pt II* **55**, 2041-2058.
Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65-65.
Strobel, A., Graeve, M., Pörtner, H. O. and Mark, F. C. (2013). Mitochondrial acclimation capacities to ocean warming and acidification are limited in the Antarctic nototheniid fish, *Notothenia rossii* and *Lepidonotothen squamifrons*. *PloS One* **8**, e68865.
Strobel, A., Bennecke, S., Leo, E., Mintenbeck, K., Pörtner, H. O. and Mark, F. C. (2012). Metabolic shifts in the Antarctic fish *Notothenia rossii* in response to rising temperature and PCO_2. *Front. Zool.* **9**, 28-28.
Sundby, S. and Nakken, O. (2008). Spatial shifts in spawning habitats of Arcto-Norwegian cod related to multidecadal climate oscillations and climate change. *ICES J. Mar. Sci.* **65**, 953-962.
Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C. and Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proc. R. Soc. Lond. B* **274**, 2935-2943.
Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C. and Chown, S. L. (2011). Ecologically relevant measures of tolerance to potentially lethal temperatures. *J. Exp. Biol.* **214**, 3713-3725.
Tewksbury, J. J., Huey, R. B. and Deutsch, C. A. (2008). Putting the heat on tropical animals. *Science* **320**, 1296-1297.
Timmermann, R. and Hellmer, H. H. (2013). Southern Ocean warming and increased ice shelf basal melting in the twenty-first and twenty-second centuries based on coupled ice-ocean finite-element modelling. *Ocean Dynam.* **63**, 1011-1026.
Trivelpiece, W. Z., Hinke, J. T., Miller, A. K., Reiss, C. S., Trivelpiece, S. G. and Watters, G. M. (2011). Variability in krill biomass links harvesting and climate warming to penguin population changes in Antarctica. *Proc. Natl. Acad. Sci. USA* **108**, 7625-7628.
Turner, J., Barrand, N. E., Bracegirdle, T. J., Convey, P., Hodgson, D. A., Jarvis, M., Jenkins, A., Marshall, G., Meredith, M. P., Roscoe, H. et al. (2014). Antarctic climate change and the environment: an update. *Polar Rec.* **50**, 237-259.
Van der Veer, H. W., Berghahn, R., Miller, J. M. and Rijnsdorp, A. D. (2000). Recruitment in flatfish, with special emphasis on North Atlantic species: progress made by the Flatfish Symposia. *ICES J. Mar. Sci.* **57**, 202-215.
Van Dijk, P. L. M., Tesch, C., Hardewig, I. and Pörtner, H. O. (1999). Physiological disturbances at critically high temperatures: A comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). *J. Exp. Biol.* **202**, 3611-3621.
Vestfals, C. D., Ciannelli, L., Duffy-Anderson, J. T. and Ladd, C. (2014). Effects of seasonal and interannual variability in along-shelf and cross-shelf transport on groundfish recruitment in the eastern Bering Sea. *Deep-Sea Res. Pt II* **109**, 190-203.
Voss, R., Clemmesen, C., Baumann, H. and Hinrichsen, H.-H. (2006). Baltic sprat larvae: coupling food availability, larval condition and survival. *Mar. Ecol. Prog. Ser.* **308**, 243-254.

Wells, R. (1978). Respiratory adaptation and energy metabolism in Antarctic nototheniid fishes. *New Zeal. J. Zool.* **5**, 813-815.
White, C. R., Alton, L. A. and Frappell, P. B. (2012). Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. *Proc. R. Soc. Lond. B* **279**, 1740-1747.
Whitehouse, M., Meredith, M., Rothery, P., Atkinson, A., Ward, P. and Korb, R. (2008). Rapid warming of the ocean around South Georgia, Southern Ocean, during the 20th century: forcings, characteristics and implications for lower trophic levels. *Deep-Sea Res. Pt. I* **55**, 1218-1228.
Wieser, W. (1994). Cost of growth in cells and organisms: general rules and comparative aspects. *Biol. Rev.* **69**, 1-33.
Wilderbuer, T., Hollowed, A., Ingraham, W., Spencer, P., Conners, M., Bond, N. and Walters, G. (2002). Flatfish recruitment response to decadal climatic variability and ocean conditions in the eastern Bering Sea. *Prog. Oceanogr.* **55**, 235-247.
Wilson, R. S., Franklin, C. E., Davison, W. and Kraft, P. (2001). Stenotherms at sub-zero temperatures: thermal dependence of swimming performance in Antarctic fish. *J. Comp. Physiol. B* **171**, 263-269.
Wittenberg, J. B. and Wittenberg, B. A. (2003). Myoglobin function reassessed. *J. Exp. Biol.* **206**, 2011-2020.
Wohlschlag, D. E. (1960). Metabolism of an Antarctic fish and the phenomenon of cold adaptation. *Ecology* **41**, 287-292.

6. Appendix

Other papers and contributions developed from this thesis:

Papetti, C., Harms, L., Windisch, H. S., Frickenhaus, S., Sandersfeld, T., Jürgens, J., Koschnick, N., Knust, R., Pörtner, H.O. and Lucassen, M. (2015). A first insight into the spleen transcriptome of the notothenioid fish Lepidonotothen nudifrons: Resource description and functional overview. *Mar. Genomics,* in press

Sandersfeld T., Davison W., Lamare M.D., Richter C., Knust R. (2015) Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. Main Meeting of the Society for Experimental Biology 2015, Prague (conference talk)

Sandersfeld T., Davison W., Lamare M.D., Richter C., Knust R. (2014) Do Antarctic fish like it hot? What energy allocation can tell us about distribution shifts. 11th International Congress on the Biology of Fish, Edinburgh (conference talk)

Sandersfeld T., Knust R. (2013) Temperature dependent metabolic rates of benthic marine fish species from temperate to polar regions. Scientific Committee on Antarctic Research Biology Symposium, Barcelona (conference talk)

Cohen-Rengifo M., Crafton R.E., Hassenrück C., Jankowska E., Koenigstein S., Sandersfeld T., Schmid M.S., Schmidt M., Simpson R., Sheward R.M. (2013) Module 1, Marine Ecosystems and Climate Change. In: Dummermuth A. and Grosfeld K. (eds.) Climate change in the marine realm: an international summer school in the framework of the European Campus of Excellence, Reports on Polar and Marine Research, Bremerhaven, Alfred Wegener Institute for Polar and Marine Research, **662**, 75 p

Supplementary material - Manuscript I

To enhance transparency of the conducted data analysis, text files with original data (displayed as tables in the following) and 'R' scripts for data analysis were submitted as electronic supplementary material (ESM) and are referred to as 'Online Resource' in manuscript I.

ESM 1

Summary respiration data *L. nudifrons* [mg O_2 kg^{-1} h^{-1}]

	Temperature [°C]				
Fish ID	**0**	**1**	**2**	**3**	**4**
1	26.42	12.20	37.81	39.65	46.06
2	24.94	21.97	-	33.55	34.62
3	21.96	27.46	29.26	45.19	47.86
4	18.76	14.87	21.51	25.04	25.71
5	20.31	20.22	23.76	26.25	28.63
6	14.35	19.06	25.24	21.51	30.10

ESM 2

Summary respiration data *L. squamifrons* [mg O_2 kg^{-1} h^{-1}]

	Temperature [°C]					
Fish ID	**2**	**3**	**4**	**5**	**6**	**7**
1	47.73	48.47	52.27	58.02	69.95	-
2	44.03	49.41	47.41	-	-	-
3	37.53	35.00	44.73	52.55	60.95	-
4	-	-	-	-	-	-
5	26.10	31.39	48.15	52.48	52.48	62.29
6	26.83	30.39	37.95	45.31	54.07	54.90
7	25.72	36.02	40.69	44.86	48.10	-
8	43.92	45.37	63.98	82.89	-	-
9	23.63	29.80	50.05	50.07	47.65	55.80

ESM 3

Summary respiration data *T. hansoni* [mg O_2 kg^{-1} h^{-1}]

	Temperature [°C]					
Fish ID	**0**	**1**	**2**	**3**	**4**	**5**
1	26.89	32.61	37.66	43.34	48.85	56.59
2	47.13	53.58	59.49	65.79	76.35	-
3	32.35	39.39	55.89	76.03	92.56	-
4	25.48	32.11	49.71	54.23	59.50	68.01
5	41.31	41.94	54.59	56.45	61.30	78.51

ESM 4

Complete data set (species abbreviation: L. nud = *L. nudifrons*, L. squami = *L. squamifrons*, T.han = *T. hansoni*)

Species	**Temperature [°C]**	**MO_2 [mg O_2 kg^{-1} h^{-1}]**
L.nud	0	26.42
L.nud	1	12.20
L.nud	2	37.81
L.nud	3	39.65
L.nud	4	46.06
L.nud	0	24.94
L.nud	1	21.97
L.nud	3	33.55
L.nud	4	34.62
L.nud	0	21.96
L.nud	1	27.46
L.nud	2	29.26
L.nud	3	45.19
L.nud	4	47.86
L.nud	0	18.76
L.nud	1	14.87
L.nud	2	21.51
L.nud	3	25.04
L.nud	4	25.71

L.nud	0	20.31
L.nud	1	20.22
L.nud	2	23.76
L.nud	3	26.25
L.nud	4	28.63
L.nud	0	14.35
L.nud	1	19.06
L.nud	2	25.24
L.nud	3	21.51
L.nud	4	30.10
L. squami	2	47.73
L. squami	3	48.47
L. squami	4	52.27
L. squami	5	58.02
L. squami	6	69.95
L. squami	2	44.03
L. squami	3	49.41
L. squami	4	47.41
L. squami	2	37.53
L. squami	3	35.00
L. squami	4	44.73
L. squami	5	52.55
L. squami	6	60.95
L. squami	2	26.10
L. squami	3	31.39
L. squami	4	48.15
L. squami	5	52.48
L. squami	6	52.48
L. squami	2	26.83
L. squami	3	30.39
L. squami	4	37.95
L. squami	5	45.31
L. squami	6	54.07

L. squami	2	25.72
L. squami	3	36.02
L. squami	4	40.69
L. squami	5	44.86
L. squami	6	48.10
L. squami	2	43.92
L. squami	3	45.37
L. squami	4	63.98
L. squami	5	82.89
L. squami	2	23.63
L. squami	3	29.80
L. squami	4	50.05
L. squami	5	50.07
L. squami	6	47.65
T.han	0	26.89
T.han	1	32.61
T.han	2	37.66
T.han	3	43.34
T.han	4	48.85
T.han	0	47.13
T.han	1	53.58
T.han	2	59.49
T.han	3	65.79
T.han	4	76.35
T.han	0	32.35
T.han	1	39.39
T.han	2	55.89
T.han	3	76.03
T.han	4	92.56
T.han	0	25.48
T.han	1	32.11
T.han	2	49.71
T.han	3	54.23

T.han	4	59.50
T.han	0	41.31
T.han	1	41.94
T.han	2	54.59
T.han	3	56.45
T.han	4	61.30

ESM 5

R code for analysis of respiration data - Shapiro-Wilk test for normality distribution

```
L.nudi<-read.table("L.nudifrons_summary.txt",header=TRUE,sep="\t", as.is=TRUE)
L.nudi
plot(density(L.nudi[,2]))
shapiro.test(L.nudi[,2])
shapiro.test(L.nudi[,3])
shapiro.test(L.nudi[,4])
shapiro.test(L.nudi[,5])
shapiro.test(L.nudi[,6])


L.squami<-read.table("L.squamifrons_summary.txt",header=TRUE,sep="\t", as.is=TRUE)
L.squami
plot(density(L.squami))
shapiro.test(L.squami[,2])
shapiro.test(L.squami[,3])
shapiro.test(L.squami[,4])

shapiro.test(L.squami[,5])
L.squami[,5]
L.squami[8,5]=NA # check with outlier assumption
shapiro.test(L.squami[,5])

shapiro.test(L.squami[,6])
shapiro.test(L.squami[,7])


T.han<-read.table("T.hansoni_summary.txt",header=TRUE,sep="\t", as.is=TRUE)
T.han
shapiro.test(T.han[,2])
shapiro.test(T.han[,3])
shapiro.test(T.han[,4])
shapiro.test(T.han[,5])
shapiro.test(T.han[,6])
shapiro.test(T.han[,7])

shapiro.test(r$MO2)
```

ESM 6

R code for analysis of respiration data - linear model (for MO_2 data_complete data set)

```
r<-read.table("MO2 data_complete data set.txt",header=TRUE,sep="\t")
r=r[-61,] # outlier assumption for data point 82 L. squami(5°C)

for (s in levels(r$species)) {w=which(r$species==s) ; print( bartlett.test(r$MO2[w],r$temp[w]
) ) }
bartlett.test(r$MO2,factor(paste(r$species,r$temp)))

logM<-log(r$MO2)

# model selection: exclude interactive effect of temp & species
r.lm<-lm(logM~r$temp*r$species)
summary(r.lm)

#model selection:effect of species on MO2 - intercepts
r.lm.1<-lm(logM~temp+species,data=r)
summary(r.lm.1)
summary(r.lm.1)$coefficients -> co
levels(r$species)

# remove trends
y=logM-co[2,1]*r$temp
summary(aov(y~r$species)->aov.y)
par(oma=c(5,3,1,1))
plot(TukeyHSD(aov.y),las=2)

# full model, incl. species specific slopes
co1=summary(r.lm)$coefficients
co1
y1=r.lm$residuals
y1
y1[r$species=="L. squami"]=y1[r$species=="L. squami"]+co1[1,1]
y1[r$species=="L.nud"]=y1[r$species=="L.nud"]+co1[1,1]+co1[3,1]
y1[r$species=="T.han"]=y1[r$species=="T.han"]+co1[1,1]+co1[4,1]

summary(aov(y1~r$species)->aov.y)
par(oma=c(5,3,1,1))
plot(TukeyHSD(aov.y),las=2)
```

Supplementary material - Manuscript II

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

7. Erklärung nach §6 (5) PromO der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche (vom 14. März 2007)

Tina Sandersfeld
Graudenzer Straße 7
28201 Bremen

Bremen, den 11. August 2015

Hiermit erkläre ich, Tina Sandersfeld, dass ich die Doktorarbeit mit dem Titel:

„Sensitivity of Antarctic fish to ocean warming – an energy budget approach"

1. Ohne unerlaubte fremde Hilfe angefertigt habe.
2. Keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.
3. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche als solche kenntlich gemacht habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

_____

Tina Sandersfeld

8. Acknowledgements

An dieser Stelle möchte ich zuerst Claudio Richter und Rainer Knust für die Betreuung dieser Arbeit danken.

Claudio, vielen Dank für den stetigen Rat, vor allem für das Bestärken und Unterstützen neuer Ideen und deren Umsetzung sowie die andauernde Motivation.

Danke Rainer, von Dir habe ich sehr viel gelernt! Nicht nur die vielen wissenschaftlichen Grundlagen, sondern auch hinter der eigenen Arbeit zu stehen und Durchsetzungskraft zu entwickeln ;). Vielen Dank für das Vertrauen in meine Arbeit und die Freiräume, die du mir gegeben hast, um diese weiterzuentwickeln.

Thanks Prof. Myron Peck, for agreeing to review this thesis. Danke auch an Prof. Matthias Wolff für die Bereitschaft, mein Promotionskolloquium zu ergänzen.

Vielen Dank an das POLMAR-Team, das diese Arbeit erst ermöglicht hat.

Holger Auel und Hauke Flores – ich glaube nicht, dass ihr wisst, wie viel Motivationsarbeit ihr geleistet habt – vielen Dank dafür!

Without Miles Lamare and Bill Davison, a large part of this thesis would never have been possible.

Miles, thanks for taking a stranger on an expedition – I still cannot believe this actually worked out – as well as for your great support during the last months.

Bill, thanks a lot for letting me work in your lab and your amazing advice and help.

Thanks to Mike, Sven, Shawna, Kate, Sam and the staff of Antarctica New Zealand for help with the fishing and a great time at Scott Base. Thanks as well to the Fish Physiology group at UC for a very nice time in Christchurch. Renny and Alan, thanks for the water (!), your support and a great fishing trip! Jan, thanks for your help, coordination of lab work and ovens!

Magnus, danke für die Möglichkeit mit euch auf Expedition zu fahren. Nils, danke für deine Unterstützung auf Expeditionen und in Bremerhaven, wie auch beim Präppen und während des unendlichen Probennehmens, sowie für die Fisch-Lektionen und den Kaffeevorrat. Ein riesiges Dankeschön an die Crews der Polarstern für die Hilfe beim Fischfang, das 'am Laufen halten' des Aquariencontainers und unglaublich tolle Expeditionen! Laura, Malte, Raphaela, Shobhit, Sabine, Stephie, Nils, Timo, Lena, Katja, Erik, Chiara, Micha, Anneli, Anette, den Leipzigern und vielen anderen ANT 27-3&4 und ANT 28-4&5'lern - danke für die tolle Zeit, kaputte Stühle, echte Punker, ABC, zu kurze Röcke, guten Kaffee, Knicklichter im Whirlpool, Doppelkopfrunden, Sundowner und Falcon Tubes!

Danke der ganzen Sektion BPP für eine tolle Zeit! Besonders Nan, Joy, Luisa, Erik, Theresa, Gerti und Santiago für die besten Mittagspausen und tolle Kochabende. Ulrike, vielen Dank für deine Unterstützung im Aquarium und mit allen Laborproblemen und -fragen! Jessi, vielen Dank für deine Hilfe beim Fischefüttern und deine nie endende Motivation bei sonstigen Aktionen unter 0°C! Fredy, vielen Dank für die Fischbetreuung. Christiane Lorenzen, Sandra Murawski, Stephan Frickenhaus, Sander Holthuijsen und Henk van der Veer, vielen Dank für die Hilfe bei Laborarbeit und Statistik. Felix und Anneli, danke für euren steten Rat und die Motivation. And Cyril - thank you so much for your endless reading!

Danke - Kristina, May und Laura für zahlreiche Diskussionen über Fische – und das echte Leben. Und danke, Theresa, für deinen Knall und die morgendlichen Zugfahrten!

Ein großes Dankeschön geht an meine Familie, besonders an Ma, Anne, Jan und Papa, für die nie fehlende Unterstützung und Motivation.

Und auch an meine Freunde, insbesondere Ena, Lisa, Lena, Maria und Wiebke – danke für unzählige tolle Tage und Abende der Ablenkung, Beratungsrunden, Unterstützung und eine unglaublich schöne Zeit in Bremen und überall.

Ein riesiges Dankeschön an Julian, für immerwährende Unterstützung, Motivation, Rat, Spaß, fürs Kartenlesen und Kochen können;) – aber am meisten fürs einfach da sein!

This version of the thesis includes corrections of misspellings and a revision of Figure 2A (Manuscript III, page 54).