Einfluss von Temperatur auf die Energie-Budgetierung bei antarktischen und borealen Fischen

---

Influence of temperature on energy budgets in Antarctic and boreal fish

Dissertation

zur Erlangung des akademischen Grades
– Dr. rer. nat. –
dem Fachbereich 2 (Biologie / Chemie)
der Universität Bremen

vorgelegt von

Eva Brodte

Diplom-Biologin

Bremen 2006

Gutachter:

1. Gutachter: **Prof. Dr. W. E. Arntz, Universität Bremen**

Alfred-Wegener-Institut für Polar- und Meeresforschung

Columbusstrasse, 27570 Bremerhaven

2. Gutachter: **Prof. Dr. H. O. Pörtner, Universität Bremen**

Alfred-Wegener-Institut für Polar- und Meeresforschung

Am Handelshafen 12, 27570 Bremerhaven

Ichthyologie

Er is in zee een coelacanth gevonden,
De missing link tussen twee vissen in.
De vinder weende van verwondering.
Onder zijn ogen lag voor 't eerst verbonden

De eeuwen onderbroken schakeling.
En allen die om deze vis heenstonden
Voelden zich op dat ogenblik verslonden
Door de millioenen jaren achter hen.

Rangorde tussen mens en hagedis
En van de hagedis diep in de stof,
Verder dan onze instrumenten reiken.

Bij dit besef mogen wij doen alsof
De reeks naar boven toe hetzelfde is
En kunnen zo bij God op tafel kijken.

(Achterberg, 1953)

Contents

Summary

Annual growth rates of Antarctic fish species are lower than rates of comparable species in temperate regions. Growth and limits of growth influence the distribution of fish species. To explain these limits, a comparison of energy budgets of two related fish species was conducted with reference to temperature and growth patterns.

The Antarctic eelpout, *Pachycara brachycephalum*, and the boreal eelpout, *Zoarces viviparus*, were chosen as model species because the former shows a more stenothermal adaptation and the latter more eurythermal. Both fish were compared in combination with field data and experimental work. Age, growth and fecundity in the field were investigated. The energy demands of metabolism, growth and excretion as well as body composition and lipids were measured and analysed at different temperatures in long-term acclimation experiments.

The field growth patterns of the Antarctic eelpout are different to those of the boreal eelpout. The Antarctic eelpout matures at five years, reaches a maximum age of 14 years and in its natural habitat the growth rate is lower than that of the boreal eelpout population in the Wadden Sea. However, during the experiments, growth of the Antarctic eelpout was higher than the growth of the boreal eelpout as measured with maximum food availability and at each optimum temperature for growth. The Antarctic species showed a comparable high food conversion efficiency reflected in the large capacity of the stomach of *Pachycara brachycephalum*. The ability to ingest and absorb energy in short periods of local appearance and to store this energy for starving periods is crucial in an environment where food supply is unstable and pulsed. Limitation of food is at least one reason for the slow growth of *Pachycara brachycephalum* in the field compared to its boreal relative.

Temperature influences the experimental growth rate of both fish species towards a clear optimum; for the Antarctic eelpout it was at 4 °C. This might be a relict of the deep sea origin of the genus *Pachycara* which invaded the Antarctic after cooling probably via the Scotia Ridge and has implications for their distribution in field in water layers above or about 0 °C. The growth optimum of *Zoarces viviparus* from the Wadden Sea is at 12 °C and matches the annual mean in the natural habitat. In evolutionary terms, a close matching of optimum and habitat temperature is useful; a habitat temperature below the thermal optimum for growth might therefore also cause the slow field growth of the Antarctic eelpout.

SUMMARY

The energy budgets of both fish provide insights for the underlying energy allocation patterns. In the Antarctic eelpout energy allocated to growth increases at 4 °C, because of high energy efficiency in diet absorption and of lower cellular level respiration rates at this temperature, whereas the respiration rates of the whole animal stay constant over the thermal range. In the boreal eelpout the growth optima at 12 °C is reflected in the whole animal respiration rates and due to reduced baseline metabolism. *Zoarces viviparus* is able to cover a broad range of habitat temperatures, but beyond and below the optimal temperature the cost for baseline metabolism increases. This eurythermal ability requires high energy demands for thermal attentiveness. For *Zoarces viviparus* eurythermy is evident in different populations inhabiting regions with different habitat temperature averages, resulting in a wide distribution area and in single populations (White Sea and Wadden Sea populations) inhabiting environments with high annual temperature amplitudes.

Lipids are of different importance in the stenothermal Antarctic eelpout and the eurythermal boreal eelpout, but there is apparently an enhanced importance of lipids in the cold in both species. In *Zoarces viviparus* functional differences in lipids between higher and lower temperatures were found. In the permanent cold adapted *Pachycara brachycephalum* the predominant lipid class in muscle and liver tissue over the whole temperature range are storage lipids (triaclyglycerids). The pronounced lipid metabolism in this Antarctic eelpout may be the result of evolutionary processes of high mitochondrial densities and low metabolic rates and the high availability of a lipid enriched diet indicated by the stable isotope analyses. The boreal eelpout changes the lipid composition to these storage lipids in the cold. This switch in metabolic preferences seen in eurytherms allows increased use of lipids at colder (winter) temperatures in the habitat and enables storage of energy.

In conclusion, the data presented here show that eurythermal ability requires high energy demands and coincide with a metabolic change in lipid function. The cold stenothermal adaptation in the Antarctic results in a highly efficient use of resources. The slow field growth rates of the Antarctic eelpout are due to habitat temperatures below the thermal growth optimum and to food limitation. The underlying mechanisms need to be further investigated.

Zusammenfassung

Die Fähigkeit zu wachsen und die Grenzen dieses Wachstums wirken sich auf die Verbreitung von Fischarten aus. Verbreitungsgebiete von Arten sind Regionen, in denen Wachstum im ausreichenden Maß möglich ist. Im Vergleich wachsen Fische aus antarktischen Regionen im Jahresdurchschnitt langsamer als ökologisch ähnliche oder verwandte Arten in gemäßigten Breiten. Um die Hintergründe dieses unterschiedlichen Wachstums zu untersuchen, wurden die Energie-Budgets zweier verwandter Fischarten im Hinblick auf Temperatureinfluß und Wachstumsmuster untersucht.

Als Modellarten wurden die antarktische Aalmutter, *Pachycara brachycephalum*, und die boreale Aalmutter, *Zoarces viviparus*, gewählt - erstere als Vertreter mit einer eher stenothermen Adaptation, letztere als eurytherme Art. Beide Fischarten wurden sowohl anhand von Felddaten als auch anhand von experimentellen Ergebnissen verglichen. Alter, Wachstum und Fruchtbarkeit der Tiere wurden in Feldstudien untersucht; innerhalb eines viermonatigen experimentellen Aufbaus bei unterschiedlichen Temperaturen wurde der Energiebedarf vom Metabolismus, vom Wachstum und von der Exkretion bestimmt. Die Zusammensetzung des Tiergewebes und der Anteil von verschiedenen Lipiden wurde in Muskel und Leber untersucht.

Das Wachstum der antarktischen Aalmutter im Feld unterschied sich von dem der Art aus den gemäßigten Breiten. Die antarktische Aalmutter erreicht ein maximales Alter von 14 Jahren, ist ab einem Alter von fünf Jahren fähig zur Fortpflanzung und zeigt im natürlichen Habitat ein wesentlich langsameres Wachstum als die Aalmutter aus dem Wattenmeer. Im Experiment jedoch konnte ich höhere Wachstumsraten bei Fütterung im Überfluß und der jeweiligen optimalen Temperatur bei der antarktischen Art im Vergleich zur Wattenmeer-Aalmutter feststellen. Die antarktische Aalmutter zeichnet sich durch gute Futterverwertung aus, die sich in einer großen Magenkapazität wiederspiegelt. Diese Fähigkeit, innerhalb von kurzen Zeiträumen Energie aufzunehmen, zu absorbieren und zu speichern für Hungerperioden, kann in einer Umgebung mit unregelmäßigem Nahrungsangebot entscheidend für das Überleben sein. Teilweise ist das verlangsamte Wachstum der antarktischen Aalmutter unter Feldbedingungen auf Nahrungslimitierung zurückzuführen.

Bei beiden Arten ist ein klares Temperaturoptimum für das Wachstum festzustellen. Das Optimum der antarktischen Aalmutter liegt bei 4 °C und ist wahrscheinlich noch ein Erbe des Tiefsee-Ursprungs der Gattung *Pachycara*. Diese

Präferenz spiegelt sich in der Verbreitung dieser Art in der Antarktis wider, wo sie hauptsächlich in Wasserschichten über oder um 0 °C vorkommt.

Das Temperaturoptimum für das Wachstum der borealen Aalmutter stimmt mit 12 °C mit dem durchschnittlichen jährlichen Mittel im lokalen Habitat überein. Vom evolutiven Standpunkt ist eine enge Übereinstimmung der Habitattemperatur mit dem Wachstumsoptimum sinnvoll. Für die antarktische Aalmutter ist das langsame Wachstum unter Feldbedingungen also wesentlich durch eine Habitattemperatur bedingt, die unter der optimalen Wachstumstemperatur liegt.

In den Energie-Budgets kann man die zugrunde liegende Verteilung hinter diesen Wachstumsprozessen erkennen. In der antarktischen Aalmutter wird bei 4 °C durch eine effiziente Energieaufnahme, gute Nahrungsverwertung und geringe zelluläre Stoffwechselraten mehr Energie für Wachstum zur Verfügung gestellt, während auf Ganztierebene keine Veränderung in der Atmung zu sehen ist. Die boreale Aalmutter zeigt auf Ganztierebene geringe metabolische Kosten, so dass mehr Energie fürs Wachstum zur Verfügung steht. *Zoarces viviparus* ist in der Lage, einen weiten Bereich an Habitattemperaturen abzudecken. Deutlich wird dies innerhalb von Populationen in Gebieten mit einer hohen jährlichen Amplitude (Wattenmeer und Weißes Meer), aber auch durch das große Verbreitungsspektrum entlang den borealen und gemäßigten Küsten. Allerdings steigen die Kosten für diesen thermal flexiblen Stoffwechsel unterhalb und oberhalb des Temperaturoptimums stark an.

Sowohl in der stenothermen antarktischen als auch in der eurythermen borealen Art nimmt die Bedeutung der Fette mit zunehmender Kälte zu. Die Funktion der Lipide ist bei beiden Arten unterschiedlich. Während bei der antarktischen Aalmutter die Speicherlipide die wichtigste Gruppe im Muskel und in der Leber über den gesamten Temperaturbereich sind, gewinnen die Speicherlipide in der borealen Aalmutter erst in der Kälte an Bedeutung. In *Pachycara brachycephalum* ist der Stoffwechsel offensichtlich mit einer Betonung auf den Lipidmetabolismus angepasst. Dies mag das Resultat eines evolutiven Prozesses sein, der zu hohen Mitochondriendichten und geringen Stoffwechselraten geführt hat und gleichzeitig die hohe Verfügbarkeit an Lipiden aus der Nahrung nutzt. In der eurythermen Art ist das Umschalten zu einen verstärkten Lipidstoffwechsel verknüpft mit der Nutzung von Fetten bei kälteren (Winter) Temperaturen und ermöglicht die Speicherung von Energie.

Eurythermie hat also einen hohen Energiebedarf zur Folge, der Reaktionen auf veränderte Temperaturen ermöglicht, wie zum Beispiel eine Veränderung in der Lipidfunktion. Die Stenothermie der antarktischen Aalmutter macht einen effizienten Gebrauch von Ressourcen möglich. Obwohl die zugrunde liegenden Mechanismen noch weiterer Nachforschungen bedürfen, ist das langsame Wachstum der antarktischen Aalmutter zum einen auf Habitattemperaturen unter dem Wachstumsoptimum sowie zum anderen auf Nahrungslimitierung im Feld zurückzuführen.

1. Introduction

Temperature focused research using aquatic organisms is frequently associated with the possible consequences of global climate change on ecosystems. The expected rise in seawater temperature will presumably affect organisms from cellular up to whole animal level. This rise will change faunistic communities within and will probably rearrange interactions (e.g., feeding links) between them. For the evaluation of possible consequences in complex systems it is important to know certain universalities. The main foci of this thesis are the general constraints on energy budgets of fish and the resulting implications on their ecology and distribution. I compared the energy allocation in different climatic regions over a variety of temperatures. For this comparison I combined ecology and physiology and used experimental methods as well as field approaches.

1.1 Temperature tolerance as an attribute for species distribution

Temperature is one of the most important abiotic factors influencing aquatic ecosystems. Marine ectotherms are directly affected by temperature in reproduction (Wood and McDonald, 1997; Marshall et al., 2000), recruitment (Houde, 1989; Dippner and Ottersen, 2001), mortality (Pauly, 1980; Thomas et al., 1986), behaviour (Shekk et al., 1990; Malloy and Targett, 1991), metabolism (Fonds et al., 1989, 1992; Johnston and Battram, 1993; Van Dijk et al., 1999), growth (Pauly, 1980; Fonds et al., 1989; Malloy and Targett, 1991; Fonds et al., 1992) and other processes. On population level growth of fish is one factor determining the temperature dependence of species and influencing the biogeographical patterns of distribution on macro-ecological scale. Changes in ambient temperatures or extreme climatic events have influenced the biogeography of aquatic ectotherm species in the past and present (Cushing, 1982, Arntz and Tarazona, 1990; Beamish, 1995; Pearsons and Lear, 2001; Perry et al., 2005). Thermally induced shifts in distribution boundaries of species disperse among various faunistic groups from plankton (Southward et al., 1995) to nekton, e.g. cod (O'Brien et al., 2000) and other fish species (Rocha et al., 2005). In the light of global warming, the significance of thermal tolerance becomes evident. Not just a continuous change of habitat temperature, but also the expected higher amplitudes and climate extremes as consequences of global warming (Houghton et al., 2001) will challenge the thermal plasticity of populations and species.

Shelford (1931) illustrated response and range of responses of organisms to abiotic conditions such as temperature in a general theoretical model, developing the consecutive stages of tolerance in ectothermal organisms towards abiotic factors. This theoretical optimum curve enhanced the "Law of the Minimum" by Liebig to a maximum limit. Frederich and Pörtner (2000) applied Shelford's model to explain the role of oxygen and the capacity of aerobic metabolic energy provision over a temperature range (Figure 1). The limits of tolerance, critical temperatures (T_{C-I} and T_{C-II}) were associated with a drastic increase in oxygen demand and declining aerobic scopes (for review, see Pörtner, 2001). The temperatures above and below, at which aerobic scope declines, are the upper and lower *pejus* temperatures (T_{P-I} and T_{P-II}). Although physiological tolerance also extends into the *pejus* range, in which short-term survival is still possible, the ecological tolerance range is likely to be reflected by the optimal aerobic scope between T_{P-I} and T_{P-II}. It has been hypothesized that this temperature optimum may reflect high abundance or biomass on population scale and high growth rates on individual scale (cf. Pörtner et al., 2005).

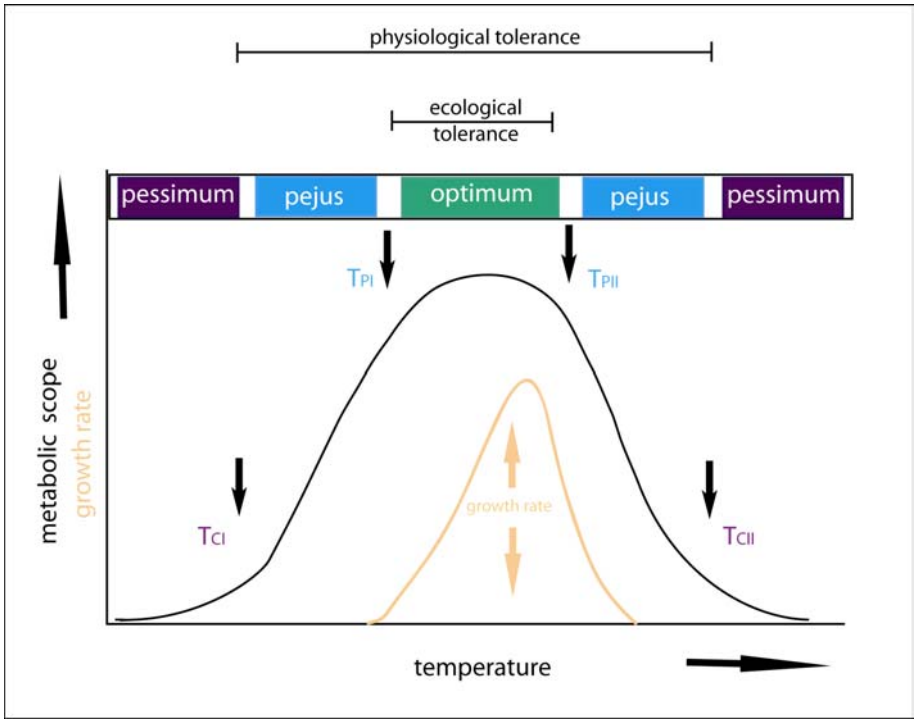


Figure 1:

Modified model of oxygen limited thermal tolerance (after Frederich and Pörtner, 2000). For explanations see text.

The thermal ranges are not invariant. The different energy demands of larvae, juveniles and reproducing animals often lead to a shifted tolerance window with ontogeny or thermal acclimatization (Shekk et al., 1990; Pepin et al., 1997; Otterlei et al., 1999).

Throughout latitudinal clines the environmental demands on metabolism most likely differ. Cold stenothermal fish are observed to possess rather narrow thermal tolerance windows and are not able to support life functions at higher temperatures (Clarke and Crame, 1989; Johnston and Bennett, 1995; Pörtner et al., 2000). In eurythermal temperate fish, which often live in fluctuating conditions, the margins of thermal tolerance are wider, but nonetheless mark species-specific temperature ranges in which the organisms can survive (Brett and Groves, 1979). The temperature curve of growth rate as one beneficiary of an increased metabolic scope is seldom bell shaped, but shortened to the upper thermal limit (Figure 1). The applicability of the oxygen limitation concept to individual growth performance has been adressed conceptually (Pörtner et al., 2004) but never been elaborated experimentally.

1.2 Growth directed by temperature

Ecological tolerance with sufficient energy remaining to support high activity, growth and reproduction has been hypothesized to determine species-specific ecological and geographical distribution boundaries (Pörtner, 2001). If a temperature tolerance model sets the ecological survival range between the *pejus* temperatures and is able to predict a possible optimum curve for growth, the range of growth is still the sum of several processes. Growth is a complex result of different, partly competing, partly concurring processes at several organisational levels. Thus foraging behaviour and assimilation of the diet (Lee et al., 2000) as well as protein biosynthesis, which is closely linked to growth (Houlihan, 1991) are at the same time energy demanding processes essential for individual growth. One very strong relation concerning growth is the triangle of "food – metabolism – temperature" (Brett, 1979).

Marine polar ectotherms display lower annual rates in somatic and reproductive growth than comparable species found in warmer regions (Clarke, 1983, Arntz et al., 1994; Peck, 2002). The obvious uncompensated temperature effect on biochemical reactions is most likely overcome by temperature compensation in evolutionary terms (Hochachka and Somero, 1984). Other explanations that are still discussed including a cost intensive pattern of metabolic adaptations required for successful living in the cold

(Metabolic Cold Adaptation Hypothesis: MCA) (Hochachka, 1988; Clarke and North, 1991; Kawall and Somero, 1996; Clarke and Johnston, 1999, Pörtner et al., 2000; Pörtner, 2002; Steffensen, 2002)), as well as a food limitation due to insufficient food availability or a strictly seasonal food supply and therefore strictly seasonal growth periods (Clarke and North, 1991; Cavalli et al., 1997; Coggan, 1997a,b; North, 1998; Peck, 2002).

The description of fish growth, not just on an individual basis, but in populations became crucial to fisheries biologists for stock assessments. A variety of different growth models are in use, often based on logistic curves. One of the most common models is the Von Bertalanffy equation (Pitcher and Hart, 1982), which was based on the work of Pütter (1920). It includes a counter-effect of assembly and degradation (Bertalanffy, 1934) taking aspects of energy allocation patterns into account. Bertalanffy (1938) assumed the former as a function of surface and the latter as a function of volume based on morphological and physiological arguments. Pauly (1979) developed on the basis of the Von Bertalanffy parameters an index of growth performance to compare fish species despite different growth or terminal length. This index widened the taxonomic and latitudinal range of species comparison and opened it to comparisons between fish communities from climatically different regions.

Individual growth and therefore the population parameters for growth seem to be influenced by temperature. In the EU project “Clicofi” (Effects of climate induced temperature change on marine coastal fish) differences between fish populations from different thermal regimes and latitudes were investigated (Pörtner et al., 2001). The ecological and physiological studies elaborate a clear significant correlation between climate induced temperature fluctuations and the recruitment of cod stocks and a decrease in temperature specific growth rates at higher latitudes (Pörtner et al., 2001). The authors suggest a cold-induced shift in energy budget. To investigate these possible shifts in energy budget is one topic of this thesis. There might be general underlying concepts of growth in environments with thermal fluctuations in contrast to thermally stable habitats in addition to species-specific ranges and limits of response to thermal challenge.

1.3 Energy budgets

When in 1878 J. W. Gibbs introduced the equation

$$\Delta G = \Delta H - T\Delta S$$

(ΔG = sum of free energies; ΔH = energy in form of heat;
T = temperature; ΔS = disorder of the system)

to thermodynamic studies, he applied the first and second theorem describing an energy state useful e.g. for cells and organisms. Although biological systems are not closed units, they are amenable to principles of steady state (Wieser, 1986). As long as the system stays constant – concerning the integral whole and the components (Bertalanffy, 1951) a true equilibrium in a closed system and the steady states in open systems illustrate certain similarities.

An organism maintains itself in a dynamic state by constant energy influx. This flow of (food) energy in and out of compartments can be viewed as an energy budget (Kooijman, 2000). An energy budget traces the energy allocation to participating parts and processes. Energy budgets can be looked at on different levels – ranging from below cellular level up to ecosystems. The organism conforms to the laws of thermodynamics at all levels. In the hierarchical organisation of biological systems (Bertalanffy et al., 1977) energy serves three main functions: maintenance, activity and production (Wieser, 1986).

Whereas the term "ecological energetics" describes the energy transfer from one level to another (Odum, 1971; Winberg, 1970), in this thesis the emphasis is on the individual level ("physiological energetics", Brett and Groves, 1979). As organisational unit the individual is separated from its environment by physical barriers and at the same time linked to tissue and cellular levels, as well as the population level (Kooijman, 2000). Energy allocation within the organism is not arbitrary, but underlies certain constraints and deals with competing demands. Only after the requirements for maintenance are met, surplus energy can be used for growth and reproduction (Brett and Groves, 1979).

Although recent investigations of cellular metabolism show that allocation patterns in cells are relatively robust against temperature influences (Mark et al., 2005), temperature acutely modulates energy budgets at higher organisational levels (Cui and Wooton, 1990) and therefore should affect growth patterns. Eurythermal animals will most likely react differently over a broad thermal range in energy allocation when

compared to stenothermal animals. This response might even differ between the organisational levels.

1.4 Lipids as stored energy in fish

Assimilated energy, which is not used for maintenance, can be stored in a collection of organic compounds. In omnivorous and carnivorous fish the major storage components are lipids (Pitcher and Hart, 1982; Urich, 1990), which are an important structural cell component as well (Urich, 1990; Stryer, 1994). The advantage of lipids as storage material is a high density of energy coupled with relatively low volume (Urich, 1990). Lipid metabolism along with protein metabolism is the most important catabolism in these fishes. Seasonal shifts in total lipid content of fish reflect sexual maturation and reproduction state, but also food availability and diet source as well as temperature (Ackman, 1989). In the heterogeneous substance group of lipids function is reflected in the lipid classes and fatty acids. Analyses of composition enlighten the role lipids take in the tissues.

The lipid classes of neutral lipids (mainly triacylglycerols (TAG) and wax esters) are the main storage components. Therefore the amount of triacylglycerols varies during ontogenetic states, e.g. during postlarval development of *Sebastes jordani* (Norten et al., 2001). At the same time TAGs are the most responsive lipid class to changes in feeding (Fraser et al., 1987).

Phospholipids, glycolipids and sterols are integral parts of the cellular membranes. In the model of the structure of membranes these molecules create the lipid bilayer and contribute to more specific functions (Urich, 1990; Stryer, 1994). The composition and ratios of polar lipids (especially of phosphatidyl ethanolamine and phosphatidyl choline), as well as the fatty acid composition affect the biophysical attributes of membranes (Hazel, 1995).

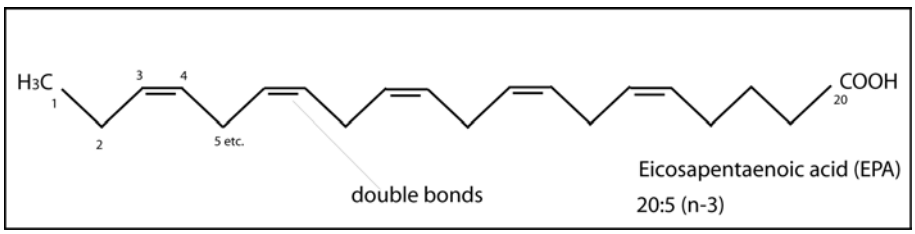


Figure 2:
Schematic figure of the polyunsaturated fatty acid 20:5 (n-3) or eicosapentaenoic acid, to illustrate the nomenclature of the fatty acids.

Fatty acids are distinguished in saturated, mono – and polyunsaturated acids depending on the existence and number of double bounds. The numeric nomenclature, apart from a few trivial names, conforms to the number of C-atoms, the number of double bonds, and the specification of the nearest methyl group in the region of the first double bond (Figure 2). Typical fatty acids of animals are unbranched and even-numbered (Urich, 1990). The biosynthesis of fatty acids in all organisms is very similar and occurs in the multi-complex of the fatty acids synthase, elongation of the C-atom chain is the usual conversion route of fatty acids (Urich, 1990). A negative correlation between the content of unsaturated fatty acids (UFA) and environmental temperature has been confirmed in many fish species, both freshwater and marine (Ackman, 1989). As a structural adaptation, a change in fluidity of the membranes through changing components with temperature and ontogeny in common carp have been observed (Farkas et al., 2001).

An increase in lipid accumulation from low to high latitudes is found in interspecific comparisons (Sidell, 1991). The high amount of lipids in Antarctic fish might therefore be due to storage, turnover or structural purposes and form an integral part of the energy budget. It is unclear if the different breadths of thermal tolerance windows affect lipid composition or vice versa.

1.5 Eelpout as model organism

For the present study zoarcid fish were chosen as model organisms among marine ectotherms. The organisational complexity of fish (circulatory system, cardiologic response and different functional tissues) enables studies on different hierarchical levels and the investigation of energy allocation patterns at these hierarchies. Fish are of major importance for marine food webs (Kock and Shimadzu, 1994; Barrera-Oro, 2003), therefore for energy fluxes in ecosystems and important as anthropogenic exploited resource.

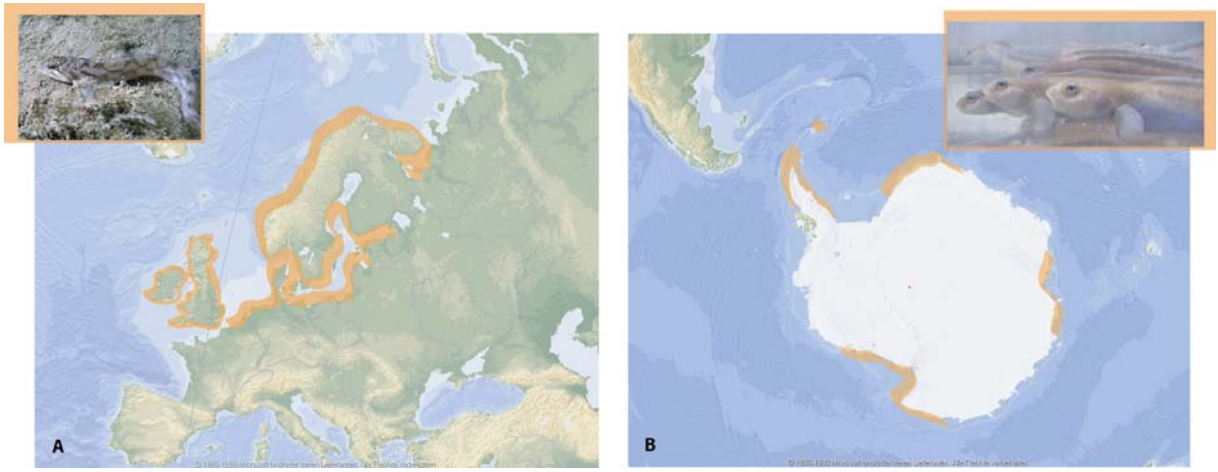


Figure 3:
Map of distribution of the eelpout *Zoarces viviparus* (A) and *Pachycara brachycephalum* (B).

Zoarcidae, eelpouts, are a cosmopolitan group of perciform fish with a widespread, mostly benthic distribution. It is a monophyletic family with four subfamilies according to Anderson (1994). Most of the 240 species inhabit the continental shelves and slopes of the boreal seas, eleven species are known from the intertidal areas (Anderson, 1984) and 24 are endemic to the Antarctic (Anderson, 1990; 1991). In this study *Zoarces viviparus* (Linnaeus, 1758) and *Pachycara brachycephalum* (Pappenheim, 1912) (Figure 3 A, B) were investigated. Both species have already been subjects of physiological and molecular investigations (Bernardi and DeVries, 1994; Morescalchi et al., 1996; Hardewig et al., 1998; 1999; Van Dijk et al., 1999; Lannig et al., 2003; Lucassen et al., 2003; Mark et al., 2004b; Storch et al., 2005).

Zoarces viviparus belong to the subfamily Zoarcinae (Anderson, 1994) and inhabits the boreal coastal areas (Figure 3A) from the southern Wadden Sea along the coastline to the White Sea (Knijn et al., 1993) and the Baltic Sea (Netzel and Kuczynski, 1995; Ojaveer, 1997; Vetemaa, 1998; Ojaveer and Lärv, 2003). This eurythermal species is able to inhabit regions with high temperature amplitudes (White Sea, Kiel Bight and Wadden Sea, see Table 1), but still has a boreal distribution focus. This preference for colder temperatures is reflected in its southern distribution limit in the Wadden Sea (Knijn et al., 1993) and the presumption that *Zoarces viviparus* is a glacial relict species in the Baltic Sea (Ojaveer and Lankov, 1997).

The diet of *Zoarces viviparus* consists of polychaetes, crustaceans and molluscs (Knijn et al., 1993). It is an ovoviviparous species (Goetting, 1979) with a maximum fecundity of 296 offspring per year (Kristoffersson and Oikari, 1975). Ovulation and internal fertilization occurs in August, by September hatched embryos are found in the ovary of pregnant females and in January / February the small juveniles are released (Kristoffersson and Oikari, 1975; Goetting, 1976). The released juveniles closely resemble the adults (Figure 4) and are about 4 to 5 cm in total length. Although the yolk mainly nourishes the embryo, there is also a matrotrophic supply to the hatched embryos (Korsgaard, 1986; 1994).

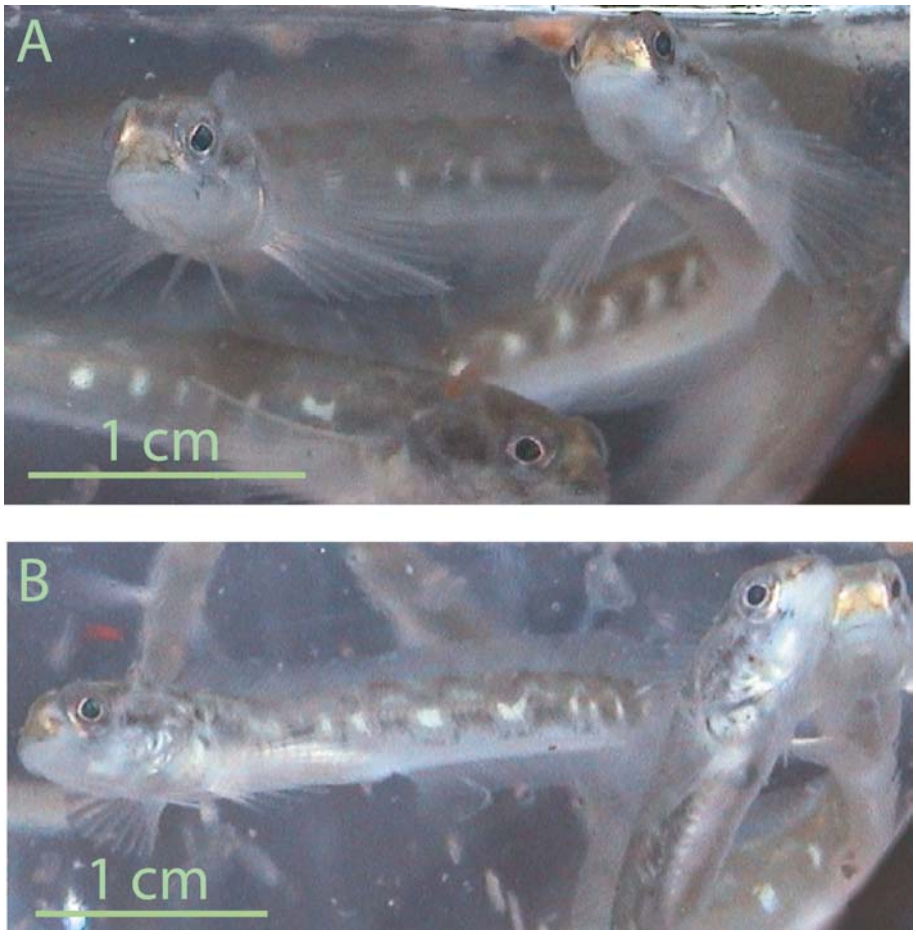


Figure 4 A and B:
Early juveniles of the North Sea eelpout, *Zoarces viviparus*, in aquaria. At this time, just one week had passed after release from the maternal ovary. Even in this early stage they closely resemble the adults.

The common eelpout is a non-migrating fish (Vetemaa, 1998) and tends to form local races (Schmidt, 1916; Christiansen et al., 1981). Therefore growth of different populations can vary with salinity and temperature regimes (Ojaveer et al., 1996; Wiecaszek, 1998).

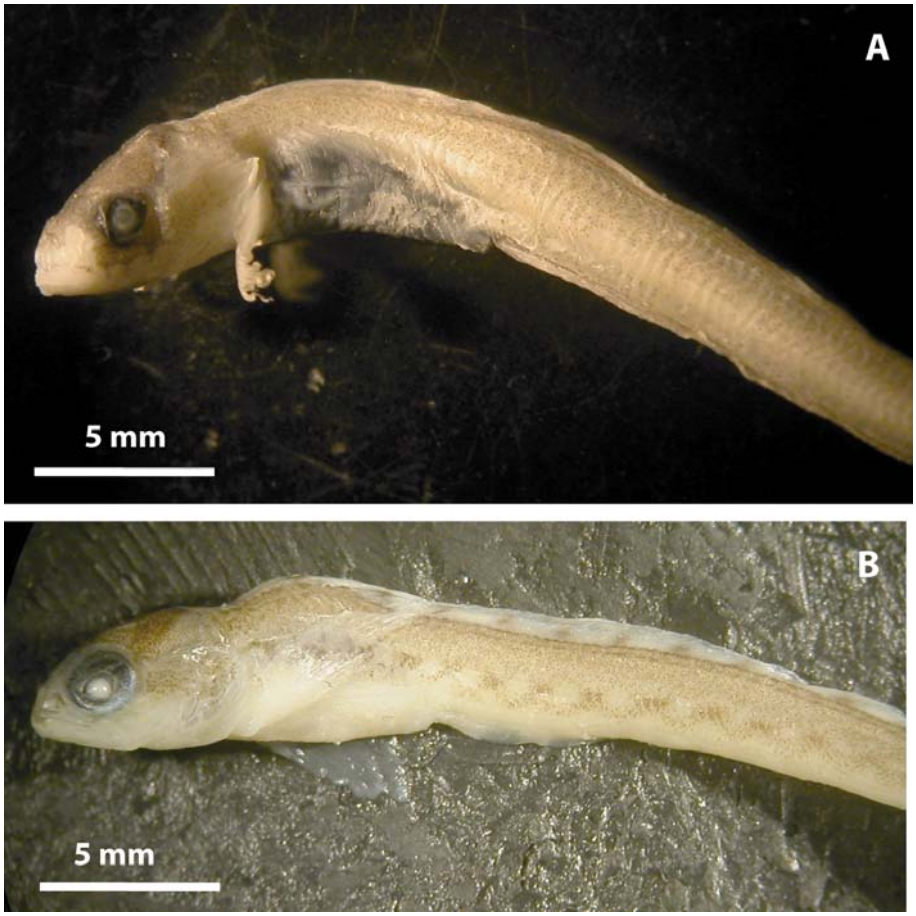


Figure 5:
A Picture of an early juvenile of *Pachycara brachycephalum*, which was fixed in 70% ethanol. This specimen is the youngest, caught so far (M.E. Anderson, Grahamstown, SAIAB, pers. comm.)
B Picture of an early juvenile of *Zoarces viviparus,* which was fixed in 70% ethanol.

Pachycara brachycephalum belongs to a more derived branch of the Zoarcidae, the Lycodinae (Anderson, 1994). It is an endemic Antarctic species with a circum-Antarctic distribution (Figure 3B) (Anderson, 1990). This eelpout is a sluggish benthic species with a stenotherm tolerance window (Van Dijk et al., 1999). Prior to this thesis even less was known about the biology and ecology of *Pachycara brachycephalum*. In

contrast to *Zoarces viviparus* it is oviparous (Anderson, 1984), and fecundity is comparatively low. The size of newly hatched larvae seems to be similar to other species, if we interpolate on the basis of the early juvenile we found (Figure 5A) (publication I). A qualitative investigation of the diet of *Pachycara brachycephalum* showed krill and amphipods as main prey items (Lipsky, 2001).

1.6 Thesis outline

The objectives of this thesis were to determine general patterns of energy allocation. Therefore the hypotheses focused on a comparative approach of both species from different thermal regimes and the temperature acclimation in use of a combination of field and experimental data.

The following aspects have been investigated:

(i) Do the growth patterns between the Antarctic and boreal eelpout differ from each other?

and

(ii) Is field growth in the Antarctic eelpout limited by insufficient food availability?

The comparison of field and experimental growth patterns for the boreal eelpout and for the Antarctic eelpout comprising populations from different latitudes, temperatures and salinities was designed to detect limits in growth and reasons for these limits.

(iii) Does energy allocation change with temperature?

The experiments to compile energy budgets over a broad temperature range were designed to compare inter- and intraspecific level shifts in energy allocation. The intention was to show shifts in preferred metabolic pathways and to investigate their thermal sensitivity.

(iv) Does lipid composition and function change with acclimation temperature?

The lipid composition of both species should provide information about the functional purpose of the high lipid content in Antarctic fish and of the thermal flexibility of the tissue composition in both species. The direct comparison benefits from maintaining both species at the same acclimation temperatures.

2. Material & Methods

In the following I would like to summarize briefly the materials used and methods applied. A detailed description of particular methods and sampling procedures is found in the publications.

2.1 Animals

The fish used in these investigations were the two eelpout species *Zoarces viviparus* and *Pachycara brachycephalum*. Fish were mainly collected from populations in the German Wadden Sea (*Zoarces viviparus*) and the Antarctic Peninsula (*Pachycara brachycephalum*). For the distribution range see Introduction (Figure 3A, B).

2.2 Sampling

The Antarctic eelpout *Pachycara brachycephalum* was caught during four RV "Polarstern" cruises from 1998 to 2004 at different sampling sites (see publication I). The fishes were obtained using baited fish traps and an epibenthic sledge (Figure 6).

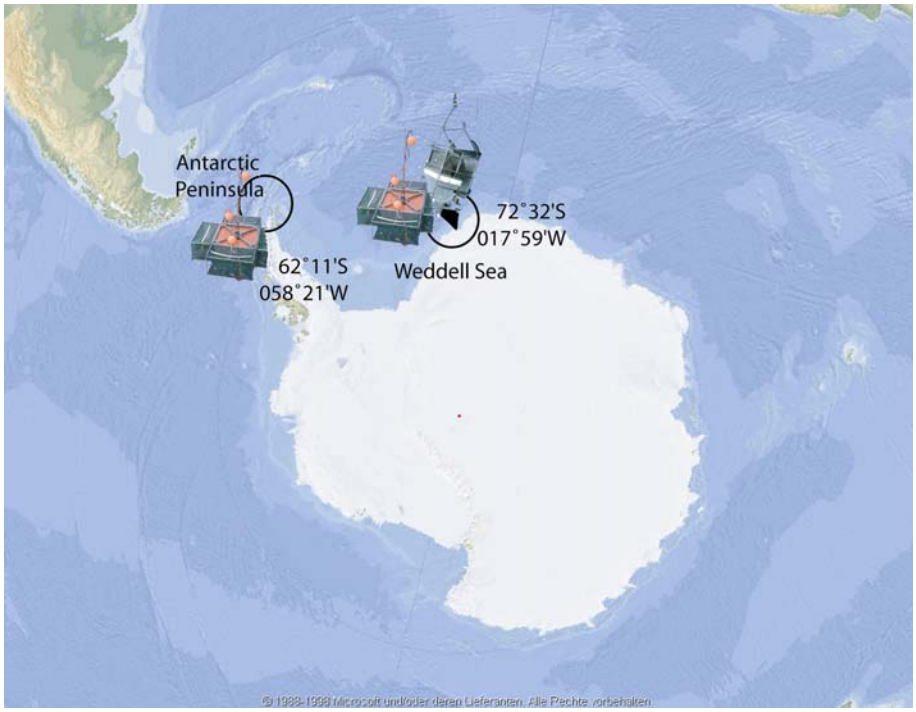


Figure 6:
Sampling sites and sampling gear in Antarctica. The eelpout *Pachycara brachycephalum* was sampled using fish traps and an epibenthic sledge. In the Weddell Sea the sampling depth was much deeper than at the Antarctic Peninsula (Table 1)

The traps were placed on the bottom for 24 to 48 hours and were recovered using acoustic releasers. At the Antarctic Peninsula the baited traps were deployed in Admiralty Bay and Maxwell Bay between 360 and 460 m depth, in the vicinity of the Antarctic Peninsula (62°11,2' S; 58°20,7' W and 62°10,9' S; 58°20,8' W). The temperatures (between 0 and 0.6 °C) and salinity (34.6 PSU) were provided by CTD data. Three individuals were caught in greater depth in the Weddell Sea with a trap and an epibenthic sledge (see Table1).

Table 1: Compilation of sampling data for both eelpout species.

Cruise code	Date	Gear	Location	Depth	Temperature (°C)	Salinity (PSU)	Source
Pachycara brachycephalum							
ANT XV –3	March 1999	baited trap	Admiralty Bay (King George Island)	405 to 437m	0.2	35	Arntz and Gutt, 1999
ANT XVII-3	May 2000	baited trap	Admiralty Bay (King George Island)	371 to 379m	0.2	35	Arntz and Brey, 2001
ANT XIX-5	April 2002	baited trap	Admiralty Bay (King George Island)	415 to 437m	0.2	35	Arntz and Brey, 2003
ANT XXI-2	January 2004	epibenthic sledge	Drescher Inlet (Weddell Sea)	804m	-0.4	34	Arntz and Brey, 2005
ANT XXI-2	January 2004	baited trap	Drescher Inlet (Weddell Sea)	835m	-0.4	34	Arntz and Brey, 2005
Zoarces viviparus							
From PO 01/93 to PO 02/03	March 1993 to July 2003	3m beam trawl	Wadden Sea (North Sea)	0.5 – 16m	-2 – 23**	27 - 31	Eva Brodte, 2001
-	Feb. to Aug. 1999; April to May 2000	trap	Kiel Bight (Baltic Sea)	6-10m	2 – 13**	14	Fischer et al., 1992; Böhnecke and Dietrich, 1951
-	Nov. to May 2000	trap	Hafrsfjord (Norwegian Sea)	3m	3– 15**	34	Eva Brodte, 2001; Böhnecke and Dietrich, 1951
-	July 1997; Aug. and Sep. 1998	trap	Bay of Kandalaksha (White Sea)	≈5m	-1 – 10**	19 – 24**	personal communication D. Lajus (Zoological Institute St. Petersburg) Babkov, 1982

**yearly amplitude of water temperatures

The eelpouts were sorted out of the hauls and traps. Fishes were identified using Gon and Heemstra (1990) and identification was confirmed by M. E. Anderson (J.L.B. Smith Institute of Ichthyology, Grahamstown). Fishes were either killed and frozen on board for field growth and fecundity data or kept alive and transferred to the Institute for Polar and Marine Research in Bremerhaven for further research.

The common eelpout *Zoarces vivparus* was sampled in the German Wadden Sea, Kiel Bight (54°27'N; 010°14'E), Hafrsfjord by Stavanger, Norway (58°53' N; 005°45' E) and in the Bay of Kandalaksha, White Sea (67°04' N; 32°31' E) (Figure 7). Sampling took place throughout the year (Wadden Sea), in spring (Kiel Bight, Stavanger), in summer (White Sea) and autumn (Stavanger). The eelpouts were collected with different gear depending on the character of the area, with a mesh size of 10 mm (Brodte et al., in prep.). The fish were killed and frozen immediately after sampling.

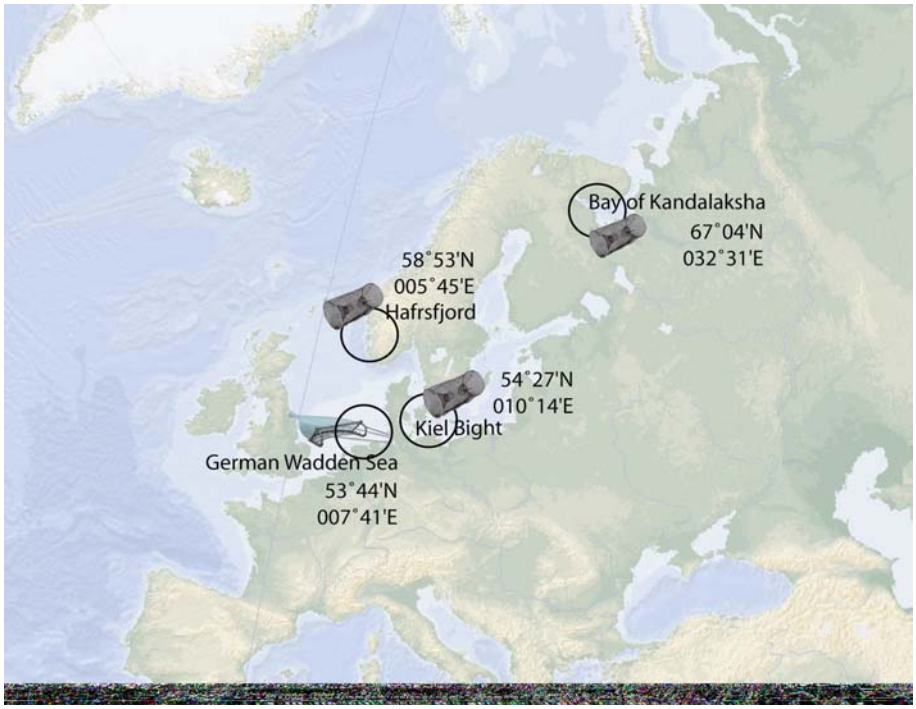


Figure 7:
Sampling sites and sampling gear for *Zoarces viviparus*. The common eelpout was sampled using fish traps of a commercial type at the White Sea, Norway and Kiel Bight. In the German Wadden Sea the eelpouts were sampled using a beam trawl.

To monitor long-term changes of the *Zoarces viviparus* population in the Wadden Sea an ongoing field programme has been active. Since 1992 investigations have been conducted at fixed sampling sites at given tidal restrictions in spring and summer (Figure 8). The fish were caught by beam trawl using standardized methods (Brodte et al., in prep.).

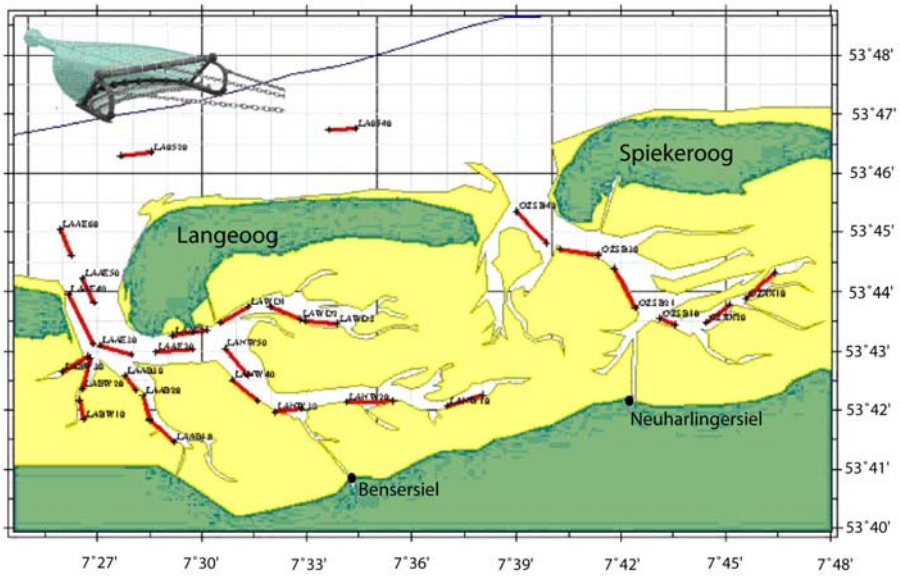


Figure 8:
Fixed sampling sites of the monitoring time series in the German Wadden Sea.

Zoarces viviparus used in the experimental studies and tissue analyses was caught in summer 2002 in the Otzumer Balje (53°44' N; 007°41'E) with a beam trawl at a depth range of 1.5 to 5.0 m, at temperatures between 17.5 and 19.5 °C and salinities from 30.6 to 31.4 PSU (Figure 9). The fish were transferred alive to the laboratory in Bremerhaven where experiments were conducted.

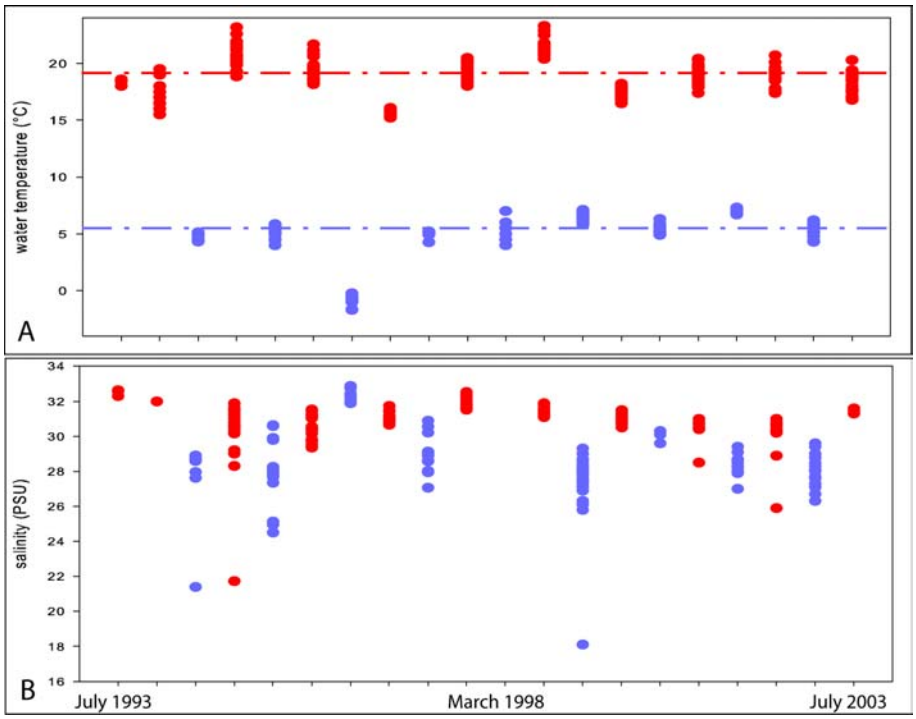


Figure 9:
Actual water temperatures (A) and salinities (B) measured during the sampling in the Wadden Sea area. Red circles are the summer values - measured in July, blue circles are the spring parameters (measured in March). The spread depicts the difference between the sampling sites. In the upper figure the lines indicate mean summer (red) and winter (blue) temperature values.

2.3 Maintenance

The Antarctic eelpout *Pachycara brachycephalum* was kept on board of RV "Polarstern" at 0 °C and 31.9 - 33.3 PSU seawater. Before starting the acclimation period animals were preconditioned by keeping them for three months at 10 °C (*Zoarces viviparus*) and at 0 °C (*Pachycara brachycephalum*), with a 12 hour dimmed light period and by feeding both species with cockle flesh (*Cerastoderma edule*) to get them used to this diet.

2.4 Field data

Total length and standard length were measured to the nearest 1 mm, in defrosted samples. Total body wet weights and gutted weights were determined to the nearest 1 g. Otoliths were sampled for age determination. Liver and gonads were weighed to the nearest 0.1 g. The population parameters were acquired by measuring the fish length

(total length, to the nearest 1 mm), the wet and the gutted weight (to the nearest 0.1g), determination of sex and maturity state (adult fish) and counting of the larvae or weight of the ovaries, respectively.

2.4.1 Age determination

For age determination the dried otolith was fixed onto a black plastic lid and ground to its focus with a grinding machine. The growth bands of the otolith were counted under a binocular and age was determined by the annulus count method (Pannella, 1974; 1980; North et al., 1980).

2.4.2 Fecundity studies

For fecundity studies the gonad maturation state of the ovaries was determined after Everson (1977) and the ovaries were dissected and fixed in 4% buffered formalin (Bleil and Oeberst, 1993). After one month they were weighed again, the eggs were separated from the ovary tissue, counted, each measured under a binocular using a microscope micrometer scale, dried with tissue paper and weighed on a fine scale. Eggs with a diameter below 3 mm were weighed in a batch of 20 eggs to reduce the error while weighing.

2.5 Experimental data

The following experiments were conducted with *Zoarces viviparus* and *Pachycara brachycephalum*.

2.5.1 Growth experiment

15 animals of nearly the same size (from 15 cm to 26.5 cm; mean size 20.1 cm ± 2.6 SD) were used per experimental temperature and species. The fishes were kept for four months under identical conditions (seawater, salinity from 31.9 to 33.3 PSU). Experimental temperatures included the range of habitat temperatures and above; they were 0, 2, 4 and 6 °C for the Antarctic eelpout and 4, 6, 12 and 18 °C for the North Sea eelpout. Temperatures were kept stable (± 0.4 °C) and water quality was monitored for 4 months. For identification of each individual, plastic gratings separated the fishes sharing the same seawater circulatory system. The animals were weighed (to the nearest 0.1 g) and measured (to the nearest 1mm below) at the beginning of the trial, after the

respiration measurement and at the end of the growth experiment. Fishes were fed *ad libitum* with mussel meat (*Cerastoderma edule*), every second day. Food intake and food portion of each fish were quantified by repeated weighing of the animal as well as of the unconsumed food.

2.5.2 Respiration measurements

Oxygen consumption measurements of the whole animals were conducted after at least two months of acclimaton to the experimental temperature and were started 24 hours after having the fish carefully transferred to the respiration chamber to avoid handling stress based artefacts in oxygen consumption (Steffensen, 2002). The fish were not fasted, but feeding was discontinued one day before the measurement started. The fishes had the opportunity to move in the respiration chambers to reflect conditions of the growth experiment but usually both species sat quietly on the ground. Measurements were carried out with a flow through respirometer at the acclimation temperature. A peristaltic pump (Ismatec, Midi-Digital, cassette MS/CA 4-6-, ISM 881/871) regulated the water flow through the chamber. Oxygen concentration of the water leaving the respiration chambers was measured by oxygen micro optodes (PreSens, type B2). Depending on the species, the size of the individual and temperature, the flow rate was set in a way that the out-flowing water displayed about 20% (± 5%) less oxygen than the in-flowing water. The fibre optic sensors (optodes) were calibrated prior to analysis and the calibration was checked at the end of the respiration measurements by use of 100% air saturated seawater from the basin and of nitrogen saturated water at experimental temperatures. Oxygen consumption of the fishes was monitored for 2 to 4 hours (MicroxTX oxygen meter, software TX 2.9, PreSens) at constant temperature and during the same period of the day to avoid differences due to diel patterns. The fishes were weighed and measured after the respiration measurements.

2.5.3 Excretion measurements

During respiration analyses ammonia excretion was determined in the flow through system (Rosas et al., 1999). Aliquots of 10ml were sampled from the out-flowing as well as the in-flowing water from each chamber and analysed after diluting the samples with distilled water. The photometric assay used an ammonium cell test based on the phenol hypochlorite method (Brockington, 2001).

2.6 CHN Analysis

At the end of the growth experiment the fishes were anaesthetized with MS 222 (0.25 g / l seawater for *Pachycara brachycephalum*, 0.13 g / l seawater for *Zoarces viviparus*, respectively) and killed. Liver, gonads, muscle tissue and the "rest" were separated and weighed (to the nearest 0.1 g). Each tissue subsample of the liver and the muscle as well as a subsample of the homogenised residual components of the fish (including bones, skin, gonads etc., excluding stomach and guts) were weighed and sampled in two Eppendorf tubes. These were frozen directly in liquid nitrogen and stored at -80 °C. The samples were lyophilised and the dry weight was determined. One replicate was ground to fine powder and redried for at least three days at 60 °C in a drying closet before starting the CHN analysis with three replicates of each sample (Euro EA 3000 series, Elemental Analyser, Euro Vector CHNS-O; Software: Callidus 1.0). The other replicate was used for ash content and composition determination (see publication II). Subsamples of the food (cockle tissue) were prepared and analysed in the same way.

2.7 Lipid analyses

2.7.1 Total lipid content

Tissue samples of muscle and liver were carefully separated and dissected, weighed, frozen in liquid nitrogen and transferred in separated glass vials filled with a mixture of dichlormethane:methanol (2:1; by vol.). These samples were stored at –80 °C. The tissue was lyophilised and after determination of the dry weight homogenised and extracted in dichlormethane:methanol (2:1; by vol.) after Folch et al. (1957). The total lipid content was determined gravimetrically.

2.7.2 Lipid class composition

Lipid classes were determined by high performance thin layer chromatography (HPTLC) densitometry (Olsen and Henderson, 1989). Pre-coated HPTLC silica gel 60 plates (20x10 cm, Merck) were pre-developed in hexane:diethylether (1:1; by vol.) to remove impurities. The plates were then dried in a vacuum desiccator for 1 h and spotted with samples (duplicates) and standards. For calibration, the following standards were used in concentrations of 0.1, 1, 2, 5, 10 and 15 $\mu g*\mu l^{-1}$: phospholipids (phosphatidylcholine, PL), sterols (cholesterol, ST), free fatty acids (oleic acid, FFA), triacylglycerols (triolein, TAG), wax esters (oleic acid palmityl ester, WE) (all from

Sigma) and 1-O-alkyldiacylglycerol ethers (DAGE) that were isolated from shark liver oil by preparative TLC. Five µl of sample extracts of muscle tissue, three µl of sample extract of liver tissue and five µl standard solutions were spotted on the HPTLC plates with a CAMAG-Linomat 4. The separation of lipid classes was performed in a CAMAG horizontal chamber with hexane:diethylether:acetic acid (80:20:2; by vol.). Thereafter the plate was dried in a desiccator under vacuum for 30 min. Lipid classes were visualised by submerging the plate in manganese (II)-chloride (4 H_2O), methanol and sulphuric acid reagent in a CAMAG immersion device for 5 sec followed by combustion at 120°C for 20 min. The quantification was performed with a TLC Scanner (CAMAG 3) combined with winCATS software. The measurement was carried out with a wolfram lamp at 550 nm.

2.7.3 Fatty acid composition

The fatty acid compositions were analysed by gas-liquid chromatography according to Kattner and Fricke (1986). Fatty acids of the total lipid extracts were converted to their methyl esters by transesterfication in methanol containing 3% concentrated sulphuric acid at 80°C for 4 hours. After extraction with hexane, fatty acid methyl esters were analysed with a Chrompack 9000 Series gas chromatograph with a DB-FFAP fused silica capillary column (30 m x 0.25 mm inner diameter, 0.25 µm film thickness) using temperature programming (160-240°C at 4°C min^{-1}, hold 15 min). For recording and integration, Class-VP software (4.3) (Shimadzu, Germany) was used. Fatty acids were identified with standard mixtures.

2.8 Stable Isotope analysis

Stable isotope analysis was conducted for selected samples. Samples of muscle tissue of *Pachycara brachycephalum*, the Antarctic species, were taken directly in the field in the Weddell Sea. The samples were stored at –30 °C, lyophilised for 24 hours and ground to a fine powder. According to Jacob et al. (2005) the samples were acidified with 1 molar hydrochloric acid (HCl) to remove the $CaCO_3$. After redrying at 60 °C the samples were ground again. The stable isotope analysis was conducted with an isotope-ratio mass spectrometer (Thermo/Finnigan Delta plus) by the GeoBioCenter in Munich.

2.9 Data analyses

2.9.1 Field growth parameters

Growth parameters for length and weight were obtained from a standard Von Bertalanffy growth equation and fitted using a statistics software package (STATISTICA, StatSoft Inc.). For *Zoarces viviparus* from different sampling sites the age of the fishes (t (a)) was normalized to the hypothetical catch event in April by adding or subtracting 0.083 per month ($^1/_{12}$ of a year) to the observed age in years (Brodte et al., in prep.).

$$L(t) = L_\infty * (1 - e^{-k*(t-t_0)}) \quad (1)$$

with L(t) = total length (cm) at time t

$$W(t) = W_\infty * (1 - e^{-k*(t-t_0)})^3 \quad (2)$$

with W(t) = total wet weight (g) at time t

W_∞ was calculated by the length-weight relationship constants a, b after Le Cren (1951), see below, and L_∞:

$$W_\infty = a * L_\infty^{\ b} \quad (3)$$

Using the total wet weight (W_{wet}) in g and the total length (TL) in cm the length-weight relationship was determined after Le Cren (1951):

$$W_{wet} = a * TL^{\ b} \quad (4)$$

(with a and b as constants)

The index of growth performance P after Pauly (1979) was computed by means of the Bertalanffy k and W_∞

$$P = \log k + \log W_\infty \quad (5)$$

Fulton's condition factor was calculated after Ricker (1975):

$$c = \frac{W_{wet}}{TL^{\ 3}} * 100 \quad (6)$$

Additionally, the hepatosomatic index (HSI):

$$HSI = \frac{Liver\_weight(g)}{gutted\_weight(g)} * 100 \quad (7)$$

and the gonadosomatic index (GSI) were computed:

$$GSI = \frac{gonad\_weight(g)}{gutted\_weight(g)} * 100 \quad (8)$$

To determine the adsorption efficiency of the digestive tract the relative stomach weight (STW) was calculated as ratio of the weight of the empty stomach and the gutted weight of the fish:

$$STW = \frac{stomach\_weight(g)}{gutted\_weight(g)} \tag{9}$$

2.9.2 Energy conversion

Weight gain and food were converted into amounts of protein, carbohydrate and lipid as well as into energy units, for whole body composition and muscle tissues or liver. Calculations used the procedure after Gnaiger and Bitterlich (1984).

Oxygen consumption was converted into energy units (kilo Joules) by use of the oxycaloric equivalent (Elliot and Davidson, 1975; Gnaiger and Bitterlich, 1984; Wieser, 1986) derived from the composition of the diet. Ammonia excretion was converted with the approximation that 1 mg NH4 –N equals 24.87 J (Elliot and Davidson, 1975). Faecal excretion was approximated based on the equation developed for brown trout (Elliott, 1976a). Elliott (1976a) developed an equation which determines the faecal deposition depending on temperature and diet quantity. The relationship between the energy losses in faecal excretion (F), temperature and ration sizes was defined by a multiple regression equation. At maximum rations energy losses in faecal excretion can be calculated by use of the following equation (cf. Elliott, 1976a) at experimental temperature (T in °C).

$$F = 0{,}212 * T^{-0{,}222} * e^{0{,}631} \tag{10}$$

Food conversion efficiency (Con_{gross}) was determined applying the energy equivalents (Gnaiger and Bitterlich, 1984; Wieser, 1986) to the mass units of the weight gain and the food to minimize the error resulting from different body composition (Brett and Groves, 1979):

$$Con_{gross} = \frac{E_{growth}}{E_{ingest\_food}} * 100\,. \tag{11}$$

The E_{growth} is the energy content of the daily weight gain and E_{ingest_food} is the energy content of the daily food intake, both in kJ.

2.9.3 Statistics

If not indicated as single values, results are presented as means with standard deviation (SD), standard error of the mean (SEM) or confidence interval (95%). The results were tested using Prism 4.0 (GraphPad), Sigma Plot 9.0 (SysstatSoftware) or Statistica (StatSoft) and were considered significant at $p < 0.05$, using the t-test or ANOVA with a post hoc test for unequal N (Tukey Honest Significant Difference test for unequal N).

The fatty acid composition of the fish tissues was analysed with a principal component analysis (PCA) with the programme Primer (version 5.2, PRIMER-E Ltd) using single values. The variety of variables used in the PCA was restricted to the twelve most abundant fatty acids. Therefore the proportions of the fatty acids were ranked cumulatively – over temperature and species. The results of the PCA were plotted using the two new coordinates (PC1 and PC2) describing the largest and the second largest variance among the samples.

3. Publications

List of publications and declaration of my contribution towards them

Publication I

E. Brodte, R. Knust, H.-O. Pörtner, W. E. Arntz (2005). Biology of the Antarctic eelpout *Pachycara brachycephalum*.

Deep-Sea Research II, Special Issue EASIZ Symposium, accepted

I developed the initial idea and concept of this paper. I did the field and laboratory work and conducted the data analyses. The first draft of the manuscript was written by myself and revised together with the co-authors.

Publication II

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

Polar Biology, submitted

Together with the second and third author, I planned the concept, the experimental design and outline of this study. I carried out the experiments and data analysis and wrote the manuscript. The manuscript was revised in cooperation with the second and third author.

Publication III

E. Brodte, M. Graeve, U. Jacob, R. Knust, H.-O-Pörtner (2006). Temperature dependent lipid levels and components in polar and temperate eelpout (Zoarcidae)

Fish Physiology and Biochemistry, submitted

The concept of this study was planned together with the second author. The laboratory work was conducted and analysed by myself, in cooperation with the second author. The stable isotope data were analysed by the third author. I wrote the manuscript, which was improved in cooperation with the co-authors.

Biology of the Antarctic eelpout *Pachycara brachycephalum*

E. Brodte*, R. Knust, H.O. Pörtner, W. E. Arntz

Authors Affiliations: Alfred – Wegener- Institute for Polar- and Marine Research, Marine Animal Physiology and Marine Animal Ecology, D-27515 Bremerhaven, FRG

Corresponding author:

Eva Brodte

Alfred – Wegener – Institute for Polar- and Marine Research

Marine Animal Physiology

Postfach 12 01 61

D - 27515 Bremerhaven

Germany

Telephone ++49 471 4831 2014

Fax ++49 471 4831 1048

ebrodte@awi-bremerhaven.de

Abstract

The aim of this study was to associate the distribution of the Antarctic eelpout, *Pachycara brachycephalum*, with available physiological data and to detect constraints in life history and biology. A population of *Pachycara brachycephalum* from the Antarctic Peninsula was investigated with regard to its growth and fecundity. At this sampling site the species occurs in water depths around 400m, much shallower than where it is found in high Antarctic waters. The age of this species was determined by applying the annulus count method to the otoliths. Growth parameters, which show no strong sexual dimorphism are L_∞ =36.0 cm; k = 0.12; t_0 =-1.75 (male) and L_∞ =36.0 cm; k = 0.10; t_0 = -2.99 (female). The maximum age found was 14 years and first maturity is reached after five years. At the end of July, females spawn about 80 eggs with a diameter of 9 mm. The smallest fish found so far was an early juvenile of 4.4 cm. The life style characteristics of this species are similar to other polar and boreal eelpout species, supporting the uniformity of the Zoarcidae in general traits.

Keywords

Growth, fecundity, eelpout, temperature, Zoarcidae, reproduction

Regional terms

King George Island, Antarctic Peninsula, Antarctica

Introduction

Pachycara brachycephalum, Pappenheim 1912, is a fish species of the family Zoarcidae, a monophyletic group comprising about 240 species, many of them still undescribed (Anderson, 1984; M. E. Anderson, J.L.B. Smith Institute of Ichthyology, Grahamstown, pers. comm.). It is a benthic, relatively stenothermal species (Anderson, 1984; van Dijk et al., 1999). The genus *Pachycara* belongs to the more derived branch of the Zoarcidae, the Lycodinae (Anderson, 1994). The zoarcids originated in the North Pacific probably in Palaeocene time (Anderson, 1994) and may have colonized Southern Ocean and Antarctic waters after the Miocene. Entry to the Antarctic may have occurred via the deep sea several times.

Pachycara brachycephalum is one among 24 endemic Antarctic eelpout species (Anderson, 1990; 1991). The zoarcids together with the liparids (snail fishes) represent the two most common demersal, non-notothenioid teleost families in the Antarctic. The

cosmopolitan distribution of the zoarcids and their existence in various climatic zones makes them intriguing subjects for comparative studies. Hence *Pachycara brachycephalum* has become the subject of physiological and molecular investigations, with the goal of identifying general traits which enabled the zoarcids to colonize the Antarctic benthos and to become dominant within the non-notothenioid Antarctic fish fauna (Anderson, 1994).

Bernardi and DeVries (1994) characterized antifreeze peptides (AFPs) in *Pachycara brachycephalum* and Morescalchi et al. (1996) investigated its karyological characters. Hardewig et al. (1999; 1998), Lannig et al. (2003), Lucassen et al. (2003), Storch and Pörtner (2003) and Mark et al. (2004b) investigated different aspects of molecular and metabolic physiology as well as gene expression patterns of *Pachycara brachycephalum* in comparison with the temperate species *Zoarces viviparus*. Van Dijk et al. (1999) studied the standard metabolic rate of *Pachycara brachycephalum* and found its upper critical temperature at 9 °C, characterized by transition to anaerobic mitochondrial metabolism. Mark et al. (2002, 2004a) identified an earlier thermal limit by use of NMR and cellular studies. This so called pejus temperature at 6 °C reflects onset of a reduced aerobic scope. A thermal window of this species, which ranges up to 6° C, might reflect the widespread distribution patterns of the genus *Pachycara*, its deep sea origin as well as the distribution of this species in deep Antarctic waters (Anderson, 1994). Possible limits of *Pachycara brachycephalum* with respect to an extension of its range of Antarctic distribution may rather result from its limited level of cold tolerance and increased metabolic cost in the cold, an aspect which has not been closely investigated. Empirical observations would suggest, however, that this species does not tolerate habitat temperatures much below 0°C in permanence (see below).

As a corollary, little is known about the biology and ecology of *Pachycara brachycephalum*. This information would be important to put the recent physiological results into a clearer ecological context. Some qualitative data on diet and fecundity are found in the cruise and field season report of the Antarctic Marine Living Resources Program of 2000/2001 (Lipsky, 2001) which showed both krill and amphipods as main prey items. There are also a few length data available for fish from different museum collections from 1912 onward (Hureau, 1991; Anonymous, 1993; 2000a; 2000b; 2001). Accordingly, the aim of the present study was to identify major traits of the life history of *Pachycara brachycephalum*, to determine age and growth parameters, to study

fecundity and thus give a rough outline of the life history of this species and discuss the ecological parameters with regard to available physiological information. The question is also addressed whether the thermal window identified in the physiological studies is related to the distribution range of this species in the Antarctic.

Material and methods

Sampling

Fish were caught during RV "Polarstern" cruises in 1998/1999 (PS-48), 2000 (PS-56), 2002 (PS-61) and 2003/2004 (PS-65) (ANT XV-3 (EASIZ II), ANT XVII-3 (EASIZ III), ANT XIX-5, ANT XXI-2) with different gears at different Antarctic sampling sites (Figure 1;Table 1). Samples were obtained using baited fish traps (BT), Agassiz trawls (AGT) and otter trawls (OT), and occasionally small individuals were collected with an epibenthic sledge (EBS). The traps were placed on the bottom for 24 hours and recovered using acoustic releasers. The Agassiz trawl, otter trawl and epibenthic sledge were towed between 15 and 30 minutes depending on area and ground characteristics. The temperature and salinity information were provided by CTD data which were ascertained according to Gerdes et al. (2005).

Growth studies

Eelpouts were sorted from the hauls and traps. Fish were identified using Gon and Heemstra (1990). The identification was confirmed by M. E. Anderson (J.L.B. Smith Institute of Ichthyology, Grahamstown). Most fishes were killed and frozen on board. Few fish were kept alive and transferred to the Institute for Polar and Marine Research in Bremerhaven. These fish were kept at 0 °C temperature in sea water (salinity from 31.9 to 33.3 PSU) and 12 hours dimmed light. At the institute a diet of alive shrimps was supplied once a week. They were anaesthetized with MS 222 (0.25g / l) and killed for tissue sampling needed in other analyses and were provided for this investigation after processing. For all fish total length and standard length were measured to the nearest 1 mm, in defrosted samples. Total body wet weight and gutted weight were determined to the nearest 1 gram. Otoliths were sampled for age determination. Liver and gonads were weighed to the nearest 0.1 gram and the sex of each individual was determined.

For age determination the dried otolith was fixed onto a black plastic lid and ground to its focus. The growth bands of the otolith were counted under a binocular

microscope and age was determined by the annulus count method (Pannella, 1974; 1980; North et al., 1980).

Fecundity studies

For fecundity studies the gonad maturation state of the ovaries was determined after Everson (1977) and the ovaries were dissected and fixed in 4% buffered formalin (Bleil & Oeberst, 1993). After one month they were weighed again, the eggs were separated from the ovarian tissue, counted, each measured under a binocular using a microscope micrometer scale, dried with tissue paper and weighed on a fine scale. Eggs with a diameter below 3 mm were weighed in a batch of 20 eggs to reduce the error while weighing.

Mathematical & statistical analysis

Results are presented as means and standard error of the mean (SEM). Growth parameters for length and weight were obtained from a standard Von Bertalanffy growth equation (Ricker, 1979; Pitcher & Hart, 1982; Lozan, 1985) and fitted using a statistics software package (STATISTICA, StatSoft Inc.).

$$L(t) = L_{\infty} * (1 - e^{-k*(t-t_0)})$$

with L(t) = total length (cm) at time t

$$W(t) = W_{\infty} * (1 - e^{-k*(t-t_0)})^3$$

with W(t) = total wet weight (g) at time t

L_{∞} was approximated by the maximum observed length (L_{max}), assuming L_{max} as L_{∞}:

$L_{\infty} \cong L_{\max}$

W_{∞} was calculated by the length-weight relationship constants a, b after Le Cren (1951), see below, and L_{∞} :

$$W_{\infty} = a * L_{\infty}^{\ b}$$

The index of growth performance P after Pauly (1979) was computed by means of the Bertalanffy k and W_{∞}

$$P = \log k + \log W_{\infty}$$

Using the total wet weight (W_{wet}) in gram and the total length (TL) in cm the length-weight relationship was determined after Le Cren (1951):

$$W_{wet} = a * TL^{\ b}$$

(with a and b as constants)

and Fulton's condition factor was calculated (Ricker, 1975):

$$c = \frac{W_{wet}}{TL^{3}} * 100$$

Additionally, the hepatosomatic index (HSI):

$$HSI = \frac{Liver\_weight[g]}{gutted\_weight[g]} * 100$$

and the gonadosomatic index (GSI) were computed:

$$GSI = \frac{gonad\_weight[g]}{gutted\_weight[g]} * 100$$

Results were tested using Prism 4.0 (GraphPad), Statistica (StatSoft) or Sigma Plot 9.0 (SysstatSoftware) and were considered significant, if the p value were <0.05.

Results

A total of more than 690 individuals of *Pachycara brachycephalum* were caught during four RV "Polarstern" cruises (Table 1). For age and growth studies 95 males and 103 females of *Pachycara brachycephalum*, for fecundity studies 20 females were used, randomly chosen or provided after tissue sampling. Both studies were conducted exclusively on the Antarctic Peninsula population. Most specimens of this population were caught in Admiralty Bay near King George Island (Antarctic Peninsula) between 370 m and 440 m depth (Table 1) with baited traps, where the temperature ranged between 0 and 0.5 °C. Our observations as well as literature data show an increasing depth of areas inhabited by *Pachycara brachycephalum* from low to high latitudes, especially in the western Antarctic (Figure 1).

Growth studies

The largest individual found was a female with a total length of 36 cm, the largest male was 34.3 cm. The maximum age was 14 years (a female fish), the oldest male was 10 years old. The most abundant year class was 5 years, the most abundant length class ranged from 22 cm to 24 cm. The smallest individual, an early juvenile, was 4.4 cm in total length and was caught with the epibenthic sledge in the Drescher Inlet (Weddell Sea) at 800 m depth (Collection of the J.L.B. Smith Institute of Ichthyology (SAIAB), Grahamstown, South Africa, catalogue number 75221).

The condition factors of males and females did not differ significantly (Table 2). The length - weight relationship can be approximated with a power equation for male fish (r^2=0.88) as

$$W_{wet} = 0.022 * TL^{2.414}$$

and for female fish (r^2=0.90) as

$$W_{wet} = 0.011 * TL^{2.658}$$

The Bertalanffy parameters of the growth curve built on body length (Table 3; Figure 2a) as well as the growth curve built on body weight did not differ between males and females (male W_∞ =125.7g, k =0.15, t_0 =-1.34, r^2=0.76; female W_∞ =150.7g, k =0.12, t_0 =-2.08, r^2=0.75) (Figure 2b). Therefore the Pauly growth index P is nearly the same for both sexes (male 1.27; female 1.26). The HSI of males differed significantly between January (1.49±0.09 SEM), March (2.14±0.16 SEM) and June (2.03±0.12 SEM) ($p<0.049$, One way ANOVA), the difference for females was not significant.

Fecundity studies

Pachycara brachycephalum matures at an age of five years (both sexes). Females of a total body weight of 41 g start to show ripe oocytes (Figure 3). The fecundity of mature females increases with age (Figure 4). The females develop very large eggs with a diameter of up to 9 mm and display a generally low fecundity of 40 to 79 ripe eggs.

The egg diameter in July is larger (t-test, $p<0.001$) than the diameter in May and October (Figure 5). The GSI of fish heavier than 30 g reflects this trend in both sexes (Table 2) indicating that peak spawning time of this Antarctic eelpout seems to be close to the end of July.

Discussion

Limitation of data in ecological Antarctic studies, especially concerning the annual range, is a known problem to researchers, but nevertheless good assumptions could be made of biology of *Pachycara brachycephalum*.

Generally, the occurrence of *Pachycara brachycephalum* is circum-Antarctic (Anderson, 1990) with an emphasis in the West Antarctic where a depth-dependent distribution is found (Figure 1). At increasing latitudes the species is found in deeper waters. In these water layers temperatures were between 0.76 and –1.8 °C, but on average, closer to the upper limit of this window. Compiled from our data and the literature data (Hureau, 1991; Anonymous, 1993; 2000a; 2000b; 2001) *Pachycara brachycephalum* of the Antarctic Peninsula inhabits in high abundance comparatively shallow depths with a temperature between 0 and 0.6 °C. Even when considering the

important aspect of gear selectivity (MacLennan, 1993; Myers & Hoenig, 1997) and of high sampling effort in the vicinity of Antarctic research stations, the distribution of *Pachycara brachycephalum* seems to be correlated with "warmer" water temperatures. Whereas in the Weddell Sea the catches were taken at depths with temperatures around –0.4 °C.

The physiological data available for this species support the conclusion that these Antarctic fish display a higher thermal optimum than other Antarctic fish. Van Dijk et al. (1999) found an upper critical temperature at 9 °C, but the pejus temperature was determined at 6 °C (Mark et al., 2002; 2004a). A recent experiment, which compared growth, metabolism and excretion rates at temperatures between 0 and 6 °C, showed the highest growth rate for *Pachycara brachycephalum* at 4 °C (Brodte et al., in prep.), implying a thermal optimum for the fish at around 4 °C. This might lead to a preferred temperature even above 0 °C. This preference may relate to the origin of the genus *Pachycara* in the deep sea (Anderson, 1989) and its current distribution in deep water. The zoarcids in general are mostly deep -sea species (Anderson, 1994). At 2500 m depth *Pachycara brachycephalum* was found in the deep sea region of the Scotia Ridge west off the South Orkney Islands (Anonymous, 2001; South African Museum catalogue number 34531, 1996). This distribution pattern and the lack of pelagic larvae and, therefore, of pelagic dispersal, which occurs only in two zoarcid genera (*Lycodapus* and *Melanostigma*) (Anderson, 1984) sustains the hypothesis that *Pachycara brachycephalum* colonized Antarctic waters via the deep sea once the Antarctic reached sufficient temperatures during the post- Eocene cooling process.

Growth studies

Growth and fecundity of this species was investigated in the population living at the Antarctic Peninsula. The comparison of the growth rate of *Pachycara brachycephalum* with that of other Antarctic eelpouts shows that this species attains a greater body size than any other species, which mostly reach an average maximum body length of below 30 cm (Anderson, 1990).

Von Bertalanffy parameters are unavailable for other Antarctic eelpouts. Thus for comparison, data were used of Dorrien (1993) on the Arctic eelpout *Lycodes reticulatus* and by Nash (1986) on *Lycodes vahlii* from the Oslofjord. Whereas Dorrien (1993) found no dimorphic differences in Von Bertalanffy growth parameters of the Arctic eelpout *Lycodes reticulatus*, Nash (1986) revealed sexual dimorphism in the

growth rate of Vahl's eelpout. Dorriens two Arctic populations of *Lycodes reticulatus* differed in L_∞ by about 12 cm. The computed k value and the Pauly index of growth performance differed accordingly (Dorrien, 1993)(see Table 3). This comparison between both populations may simply reflect a temperature effect on growth parameters and condition (Table 3). *Lycodes vahlii* inhabiting "warmer" waters shows a higher k value despite the strong sexual dimorphism with respect to growth (Nash, 1986). Temperature seems to modulate the growth rate of zoarcids in the field (Table 3). The growth curves of *Lycodes vahlii* of both sexes split in the fourth year age-class, indicating a potential shift to a high energy - demanding sexual maturation process in the females (Nash, 1986). The Antarctic eelpout *Pachycara brachycephalum* seems to show a growth pattern similar to *Lycodes reticulatus*, displaying a similar Pauly growth index and k value, although on the basis of its maximum age, it resembles *Lycodes vahlii*, which has a maximum life span of 10 years (Nash, 1986).

Anderson (1990) assumed that zoarcids are generally slow growing and relatively short lived, with a life span between 9 and12 years. The shortest lived eelpout group he found matured in the second or third year and lived less than 8 years. Brodte et al. (in prep) found an eelpout population of *Zoarces viviparus* in the Wadden Sea with a maximum age of 4 years, 4 years less than in other populations of the same species. A short life span may characterize the population at the southern distributional boundary of *Zoarces viviparus*. Declining maximum age at elevated habitat temperatures is found in different populations of *Zoarces viviparus* (Brodte et al., in prep.), ranging from eight years in the White Sea population at temperatures of 10 to –1 °C to four years in the Wadden Sea population at 4 to 18 °C. This trend is also reflected in the maximum age of *Lycodes reticulates*, which reaches 35 years in northeastern Greenland at temperatures below 0 °C and 19 years in the Barents Sea at temperatures between 0 and 6 °C (Dorrien, 1993). Comparison of *Lycodes vahlii* and *Pachycara brachycephalum* also suggests an exponential decline of maximum age with increased temperatures (maximum age= $22.29e^{-0.10 \text{upper habitat temperature}}$, $r^2= 0.86$).

Fecundity studies

Pachycara brachycephalum matures at an age of five years, but fecundity increases with age to up to 80 eggs per female. Fecundity is higher than for *Lycodes vahlii* with 18-40 eggs (Nash, 1986), but still in the range given by Anderson (1990) for the Zoarcidae, between 4 and 150 maturing ova. Eggs of 9 mm in diameter found in the

Antarctic Peninsula population is very large for fish eggs and even larger than the diameter given by Lipsky (2001) for *Pachycara brachycephalum* at 6 mm in diameter, corresponding to a fecundity of 52 to 63 eggs per ovary. Eggs and larvae of species living at low temperatures are generally larger than those at high temperatures (Bagarinao & Chua, 1986).

Zoarcid egg sizes range from about 4 to 9 mm (Anderson, 1990). The intertidal South American eelpout *Austrolycus depressiceps* has an egg size (9.2 to 9.8 mm) similar to *Pachycara brachycephalum* (Matallanas et al., 1990), but the Arctic *Lycodes vahlii* has a smaller egg size (2.4 to 6.6 mm; Nash, 1986). The ripe ovary of *Pachycara brachycephalum* comprises 15% to 30 % of total body wet weight (increasing with total body weight in females). The average somatic weight gain according to Von Bertalanffy parameters is 8 g per year, therefore the reproductive effort per spawning event corresponds to one to three somatic year productions, even neglecting the higher energy content of the eggs compared to muscle tissue. The observed egg size and fecundity of *Pachycara brachycephalum* and the high energy demand of its reproductive mode suggests that this species cannot afford to spawn and guard its eggs more than three times in a life time. Anderson (1990) assumed that eelpouts in general do not spawn more often than 1-3 times during their lifespan and suggested in line with other authors (Kendall et al., 1983; Nash, 1986; Matallanas et al., 1990) that parental care and nest guardianship is very common as was found in *Phucocoetes latitans, Iluocoetes spp.* (Gosztonyi, 1977) and some *Lycodes* species.

Peak spawning in *Pachycara brachycephalum* seems to take place in late July. The incubation time of eggs is closely linked to egg size and ambient temperature (Kock, 1992). The incubation time of other zoarcids ranges thus: from one to two months of egg development until hatching (in the inner abdominal cavity) at 8 to 16 °C in *Zoarces viviparus* (Goetting, 1976) and in *Zoarces elongatus* (Koya et al., 1994), two months in *Iluocoetes spp.* (Gosztonyi, 1977) at about 7 °C and two and a half to three and a half months in *Zoarces americanus* (Olsen & Merriman, 1946) at temperatures below 10°C. Pepin et al. (1997) reported that the incubation time of cod eggs resembles the Q_{10} rule. Taken the similar egg size of *Austrolycus depressiceps* at 7 °C habitat temperature (Matallanas et al., 1990) into account and following the Q_{10} rule, an incubation time for *Pachycara brachycephalum* eggs of four to five months can be assumed. The hatched larvae strongly resembles adults, therefore during incubation the

differentiation process, which is most likely not cold –compensated, plays a major part. The larvae would hatch at the end of October / beginning of November of the same year in the Antarctic spring. Suggesting a short yolk- absorbing period like in *Zoarces americanus* (Anderson 1984), they could start feeding on infaunal evertebrates profiting of an early planktonic bloom, which might start in November, but develops to a maximum peak in January (Scott et al., 2000). During this time our early juvenile, which is the smallest known individual of *Pachycara brachycephalum* (M.E. Anderson, SAIAB Grahamstown, pers. comm.), caught in January and probably hatched two months before reached a body length of 4.4 cm (Collection of the J.L.B. Smith Institute of Ichthyology (SAIAB), Grahamstown, South Africa catalogue number 75221), similar to the earliest juveniles of *Macrozoarces* and *Zoarces* (Kristofferson et al., 1973; Goetting, 1976; Anderson, 1984).

The life history of *Pachycara brachycephalum* seems to parallel the known life history traits of other eelpout species, which have a moderate maximum age and length, relatively slow and narrow dispersal reproduction modes and a sluggish benthic life style. This allows meaningful comparisons of data sets within this fish family even in species from different climatic regions.

Acknowledgements

The authors would like to thank M.E. Anderson, T. Hirse, K. Mintenbeck, B. Klein, and the crew of RV „Polarstern“ for their assistance in catching, sorting and identification of the fishes, D. Gerdes for providing the CTD data and U. Jacob for her helpful comments.

References

Anderson, M.E., 1984. Zoarcidae: development and relationship. Special publications-American Society of Ichthyologists and Herpetologists, 17, 578-582.

Anderson, M. E. , 1989. Review of the eelpout genus *Pachycara* Zugmayer 1911 (Teleostei:Perciformes) with descriptions of six new species. Proc. Calif. Acad. Sci., 46(10), 221-242.

Anderson, M.E., 1990. Zoarcidae.

In: Gon, O. and Heemstra, P. C. (Eds.), Fishes of the Southern Ocean. J. L. B. Smith Institute of Ichthyology, Grahamstown , South Africa pp. 256-276.

Anderson, M. E. , 1991. Studies on the Zoarcidae (Teleostei: Perciformes) of the Southern hemisphere. V. Two new species from the Weddell Sea, Antarctica. Cybium 15 (2),151-158.

Anderson, M.E., 1994. Systematics and Osteology of the Zoarcidae (Teleostei: Perciformes). Ichthyological Bulletin, 60,120pp.

Anonymous, 1993. Computerized Catalogue of the Fish Collection. California Academy of Science, San Francisco, California.

Anonymous, 2000a. Fish Collection of the Institut für Seefischerei (ISH), BIOLAB Forschungsinstitut, Hohenweststedt, Germany.

Anonymous, 2000b. Fish Collection Database of the J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.

Anonymous, 2001. Fish Collection Database of the National Museum of Natural History (Smithsonian Institution - Division of Fishes).

Bagarinao, T. and Chua, T.E., 1986. Egg size and larval size among teleosts: implications to survival potential. Proceedings of the first Asian fisheries forum, 651-656.

Bernardi, G. and DeVries, A.L., 1994. Cytochrome b gene sequences from two eelpouts (Perciformes, Zoarcidae) from McMurdo Sound (Antarctica): Implications on the antifreeze gene structure. Antarctic Journal of the United States,29 (5), 159-161.

Bleil, M. and Oeberst, R., 1993. On the accuracy of cod fecundity estimations. ICES council meeting papers, CM D 48, 1-14.

Collection of the J.L.B. Smith Institute of Ichthyology (SAIAB), Grahamstown, South Africa, catalogue number 75221.

Dorrien, C.F. von, 1993. Ecology and Respiration of Selected Arctic Benthic Fish Species. Reports on polar research, 125, 104pp.

Ekau, W., 1988. Ecomorphology of nototheniid fish from the Weddell Sea, Antarctica. Reports on Polar Research, 51, 140pp.

Everson, I., 1977. The living Resources of the Southern Ocean. FAO Southern Ocean Fisheries Survey Program, Rome, GLO/SO/77/1, 156 pp.

Gerdes, D., J.-M. Gili, E. Isla, M. Lavaleye, J. Michels, A. Pasternak, A. Rodriguez y Baena, S. Rossi, B. Vendrell, and T. Brey, 2005. Multidisciplinary spring bloom study. Reports on Polar- and Marine Research 503, 36-37.

Goetting, K.J., 1976. Reproduction and oocyte-development in *Zoarces viviparus* (Pisces, Osteichth.). Helgoländer Wissenschaftliche Meeresuntersuchungen, 28 (1), 71-89.

Gon, O. and Heemstra, P.C.(Eds.), 1990. Fishes of the Southern ocean. J. L. B. Smith Institute of Ichthyology, Grahamstown, 462pp.

Gosztonyi, A.E., 1977. Results of the research cruises of FRV 'Walther Herwig' to South America. 48 Revision of the South Armican Zoarcidae (Osteichtyes, Blennioidei) with the description of three genera and five new species. Archiv für Fischereiwissenschaft, 27 (3), 191-249.

Hardewig, I., Van Dijk, P.L.M., and Pörtner, H.O., 1998. High-energy turnover at low temperatures: Recovery from exhaustive exercise in Antarctic and temperate eelpouts. American Journal of Physiology, 43, R1789-R1796.

Hardewig, I., Van Dijk, P.L.M., Moyes, C.D., and Pörtner, H.O., 1999. Temperature-dependent Expression of Cytochrome-C Oxidase in Antarctic and temperate fish. American Journal of Physiology, 277, R508-R516.

Hureau, J.-C., 1991. La base de données GICIM: Gestion informatisee des collections ichtyologiques du Muséum.

In: Anonymus (Eds.), Atlas Préliminaire des Possions dČaux douces de France. Conseil Supérieur de la Pêche, Ministère de l'Environment, CEMAGREF et Muséum National d'Histoire Naturelle, Paris, pp. 225-227.

Kock, K.-H. 1992. Antarctic Fish and Fisheries. University Press, Cambridge.

Kendall, A.W., Jennings, C.D., Beasley, T.M., Carpenter, R., and Omayajulu, B.L.K., 1983. Discovery of a cluster of unhatched fish eggs of a zoarcid buried 10 to 12 cm deep in continental slope sediments off Washington State, USA. Marine Biology, 75 (2-3), 193-199.

Koya, Y., Ikeuchi, T., Matsubara, T., Adachi, S., and Yamauchi, K., 1994. Embryonic growth during gestation of the viviparous eelpout, *Zoarces elongatus*. Japanese Journal of Ichthyology, 41 (3), 338-342.

Kristoffersson, R., Broberg, S., and Pekkarinen, M., 1973. Histoloy and physiology of embryotrophe formation, embryonic nutrition and growth in the eel-pout, *Zoarces viviparus* (L.). Annales Zoologici Fennici, 10 (3), 467-477.

Lannig , G., Storch, D., and Pörtner, H.O., 2003. Aerobic mitochondrial capacities in Antarctic and temperate eelpout (Zoarcidae) subjected to warm versus cold acclimation. Journal of Experimental Biology, submitted.

Le Cren, E.D., 1951. The length - weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology, 20, 201-219.

Lipski, J. (Eds.), 2001. AMLR 2000/2001 Field Season Report - Objectives , Accomplishment and Tentative Conclusions. Southwest Fisheries Science Center - Antarctic Ecosystems Research Division, NOAA-TM-NMFS-SWFSC-314, 187pp.

Lozan, J. L.,1985. Wachstum, Rekrutierung, Netzselektion, Fortpflanzung und Fruchtbarkeit. Fischereibiologische Methoden 3, 59-60.

Lucassen, M. , Schmidt, A., Eckerle, L.G., and Pörtner, H.-O., 2003. Mitochondrial proliferation in the permanent vs. temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 285, R1410-R1420.

MacLennan, D. N., 1993.Gear selectivity and the variation of yield. ICES council meeting papers, CM D 32, 11pp.

Mark, F.C., Bock, C., and Pörtner, H.O., 2002. Oxygen limited thermal tolerance in Antarctic fish investigated by magnetic resonance imaging (MRI) and spectroscopy (^{31}P-MRS). American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 283, R1254-R1262.

Mark, F.C., Hirse, T., and Pörtner, H.-O., 2004a. Thermal sensitivity of cellular energy budgets in Antarctic fish hepatocytes. Polar Biology, submitted.

Mark, F.C., Lucassen, M., and Pörtner, H.-O., 2004b. Are mitochondrial uncoupling proteins involved in thermal acclimation in temperate and polar fish. Physiological Genomics, submitted.

Matallanas, J., Rucabado, J., Lloris, D., and Olivar, M.P., 1990. Early stages of development and reproductive biology of the South-American eelpout *Austrolycus depressiceps* Regan, 1913 (Teleostei: Zoarcidae). Scientia Marina (Barcelona), 54 (3), 257-261.

Morescalchi, A., Morescalchi, M. A., Odierna, G., Stingo, V., and Capriglione, T., 1996. Karyotype and genome size of zoarcids and notothenioids (Teleostei, Perciformes) from the Ross Sea: Cytotaxonomic implications. Polar Biology, 16 (8), 559-564.

Myers, R.A. and Hoenig, J.M., 1997. Direct estimates of gear selectivity from multiple tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences, 54, 1-9.

Nash, R.D.M., 1986. Aspects of the general biology of Vahl's eelpout, *Lycodes vahlii gracilis* M. Sars, 1867 (Pisces, Zoarcidae), in Oslofjorden, Norway. Sarsia, 71(3-4), 289-296.

North, A.W., White, M.G., and Burchett, M.S., 1980. Age determination of Antarctic fish. Cybium, 8, 7-11.

Olsen, Y.H. and Merriman, D. , 1946. Studies on the marine resources of southern New England IV. The biology and economic importance of the Ocean pout , *Macrozoarces americanus* (Bloch and Schneider). Bulletin of the Bingham Oceanographic Collection 9, 175pp.

Pannella, G., 1974. Otolith growth patterns: An aid in age determination in temperate and tropical fishes.

In: Bagenal, T. B. (Ed.), The Aging of Fish. Unwin Brother Ltd., Surrey, pp. 28-39.

Pannella, G. 1980. Growth patterns in fish sagittae.

In: Rhoads, D. C. and Lutz, R. A. (Eds.), Skeletal Growth of Aquatic Organisms. Pleum Press, New York, pp. 519-560.

Pauly, D., 1979. Gill size and temperature as governing factors in fish growth: a generalization of von Bertalanffy's growth formula. Berichte des Instituts für Meereskunde, Christian – Albrecht - Universität, Kiel, 63, 156pp.

Pepin, D.C., Orr, D.C., Anderson, J.T., 1997. Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 54, Suppl. 1, 2-10.

Pitcher, T. J. and Hart, P. J. B.,1982. Fisheries Ecology. Croom Helm. London & Canberra.

Ricker, W.E. 1975. Computations and Interpretations of Biological Statistics of Fish Populations. Bulletin of the Research Board of Canada, 191, 1-382.

Ricker, W. E.,1979. Growth rates and models. Fish physiology. Bioenergetics and growth.8. 677-743.

Scott, F.J., Davidson, A.T., and Marchant, H.J., 2000. Seasonal variation in plankton, submicrometre particles and size-fractionated dissolved organic carbon in Antarctic coastal waters. Polar Biology, 23, 635-643.

South African Museum, 1996. Catalogue number 34531 from Polarstern , AGT 111, 14. December 1996 in 2227-2234m by P. A. Hulley and K.-H. Kock.

Storch, D. and Pörtner, H.-O., 2003. Temperature dependent protein synthesis capacities in stenothermal (Antarctic) and eurythermal (North Sea) fish (Zoarcidae). American Journal of Physiology, submitted.

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). Journal of Experimental Biology, 202 (24), 3611-3621.

Figure 1:

A: Depth-dependent occurrence of *Pachycara brachycephalum* plotted against latitude south (in degrees) (data collected from Hureau 1991, Anonymous 1993, 2000a, 2000b, 2001). Vertical bars indicate statistically significant groups ($p<0.002$) separated by the Antarctic polar circle. The data point below 2500m depth refers to an occurrence of *Pachycara brachycephalum* on the Scotia Ridge and was excluded from both groups.

B: Our sampling sites

Figure 2:

A: Growth of *Pachycara brachycephalum* according to total length, fitted with the Von Bertalanffy growth equation (males: filled squares, continuous regression, females: open squares, dashed regression; for parameters see Table 4).

B: Growth of *Pachycara brachycephalum* according to total body weight, fitted with the Von Bertalanffy growth equation (males: filled squares, continuous regression curve; females: open squares, dashed regression; for parameters see text).

Figure 3:

Onset of the reproductive period in female *Pachycara brachycephalum*.

Mean egg diameter and number of eggs in ovaries (standard error expressed by horizontal bars) versus total body weight of females. The dashed vertical line indicates the transition to maturity with increasing egg size and decreasing number of eggs per ovary.

Figure 4:

Number of eggs per ovary versus age of mature females.

Figure 5:

Egg diameters observed over the course of a year, an asterisk depicts significant differences of means. “n.d.” indicates months where no data were available. The diameter of ripe oocytes (above the dashed horizontal line) reaches a peak in July, whereas egg sizes in immature ovaries with pre-vitellogenic growth (below the dashed horizontal line) remain virtually unchanged over the course of the year. The maturation process starts in females of 41 g total weight (see Figure 3) which conforms with an age of five years.

Table 1:

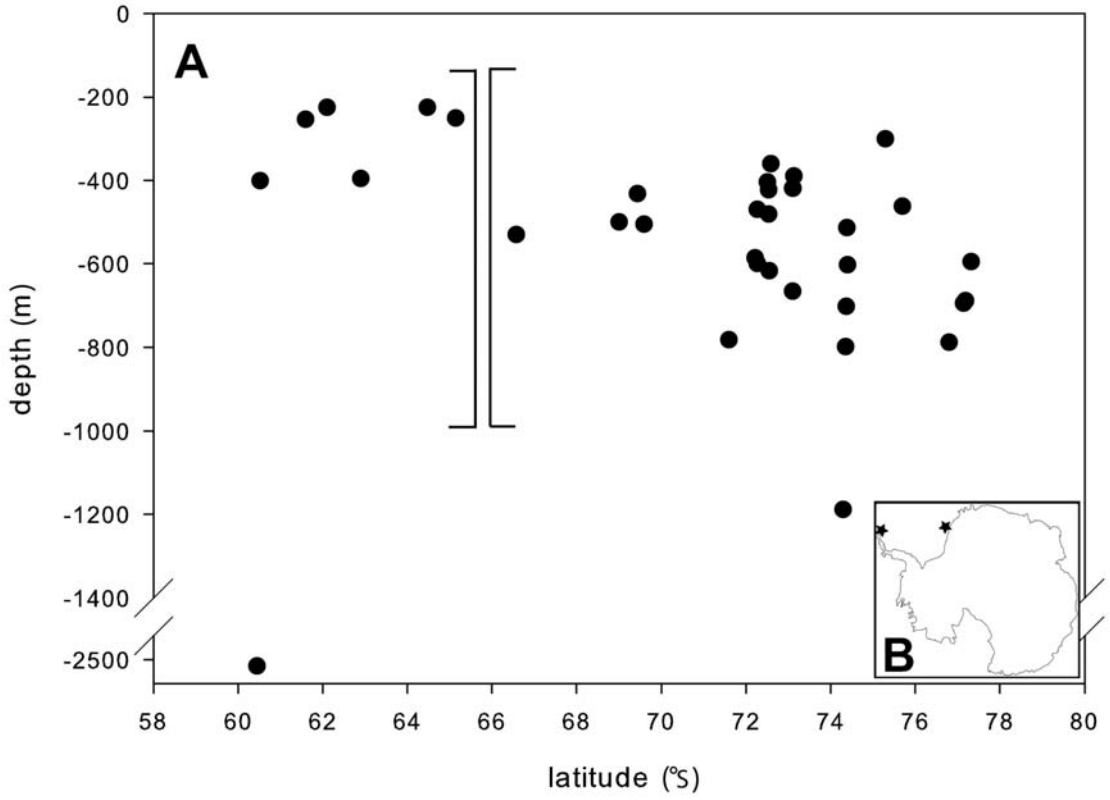
Compilation of all eelpout caught during the RV "Polarstern" cruises ANT XV-3, ANT XVII-3, ANT XIX-5 and ANT XXII-2 (Abbreviations: BT: baited trap, AGT-Agassiz trawl, OT- otter trawl, EBS – epibenthic sledge).

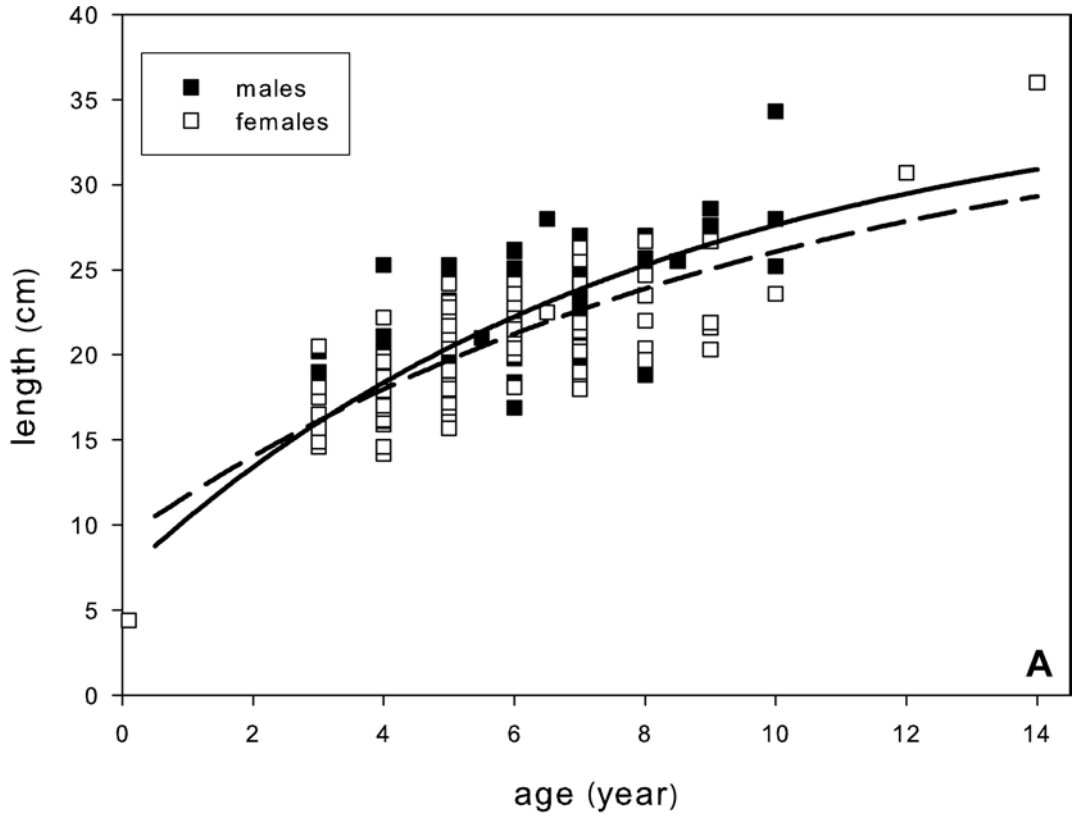
Table 2:

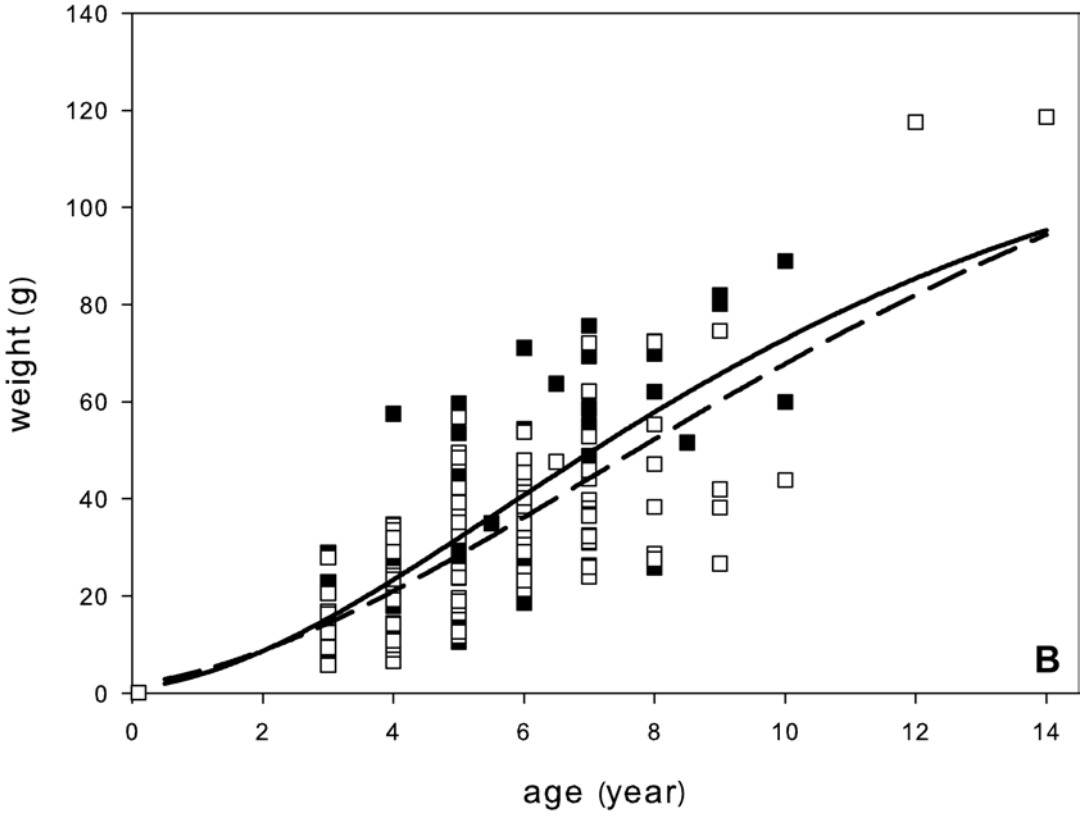
Gonadosomatic indices of *Pachycara brachycephalum* (>30g wet weight) over the course of the year (SEM = Standard error).

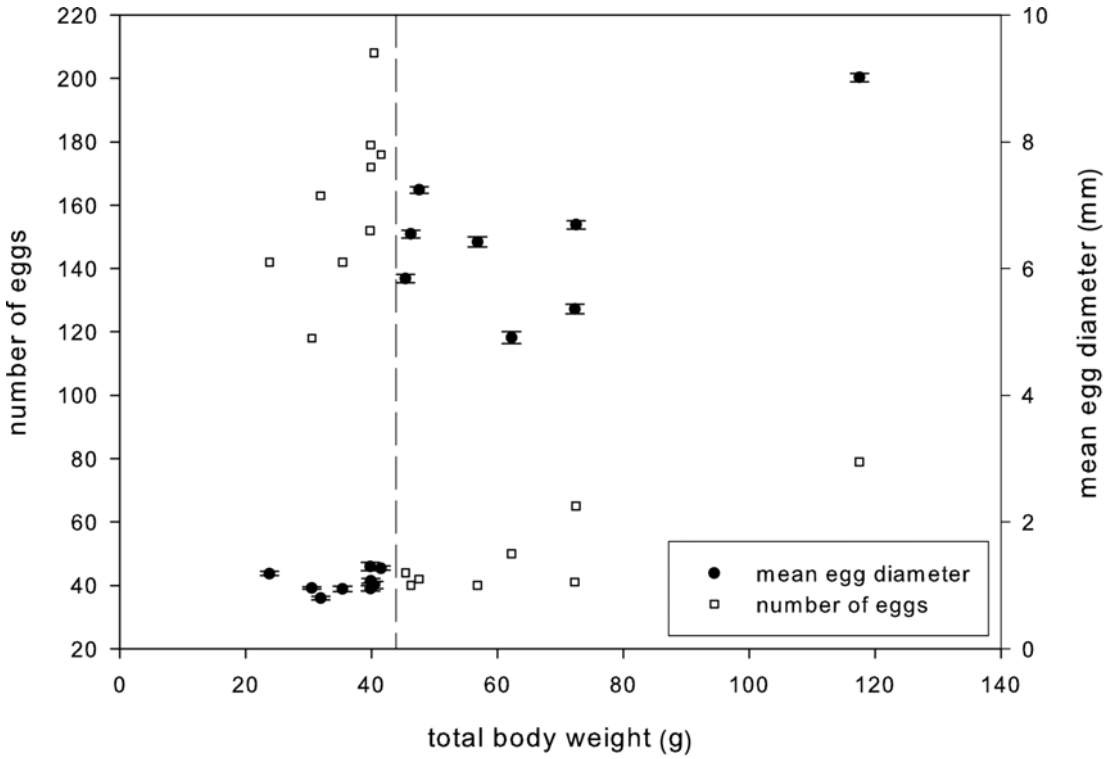
Table 3:

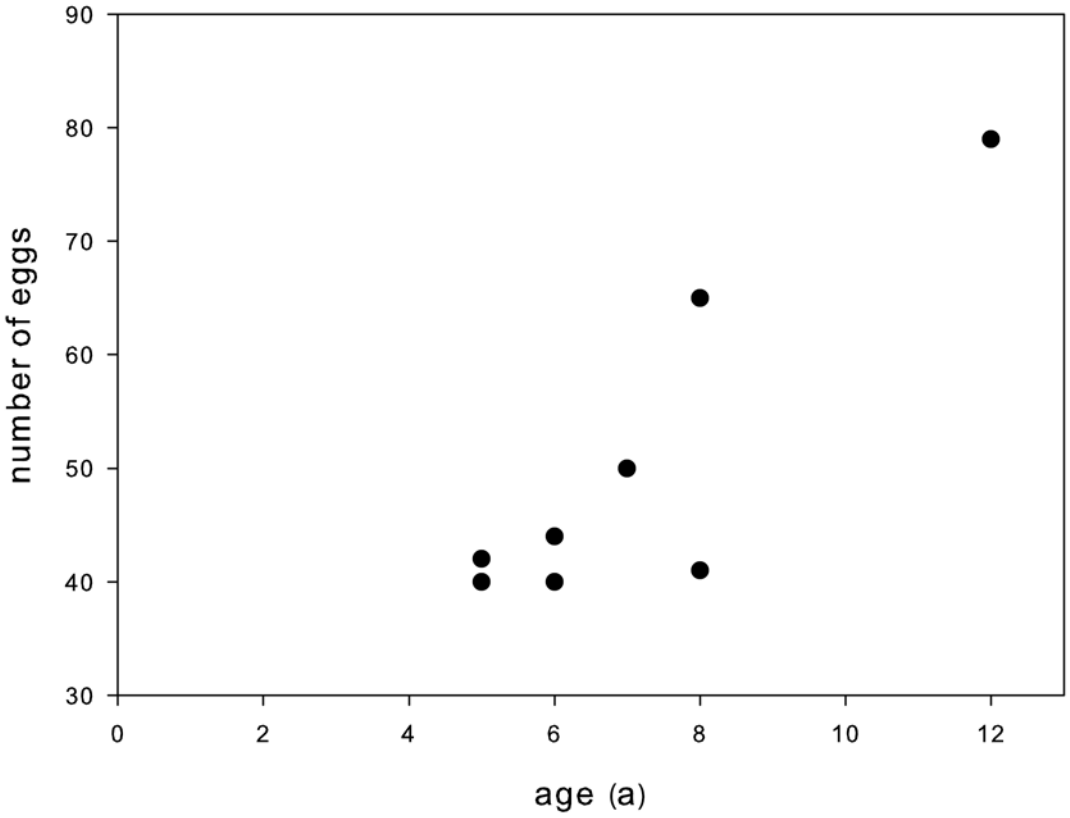
Von Bertalanffy growth parameters and condition factors obtained in the present study, listed with literature data for comparison (Note that the condition factor for *Lycodes reticulates* was calculated differently, from standard length and gutted weight of the fishes).


A
B
depth (m)
0
-200
-400
-600
-800
-1000
-1200
-1400
-2500
58
60
62
64
66
68
70
72
74
76
78
80
latitude (°S)


males
females
length (cm)
age (year)
A
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
14


weight (g)
age (year)
B
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14


number of eggs
mean egg diameter (mm)
total body weight (g)
mean egg diameter
number of eggs
220
200
180
160
140
120
100
80
60
40
20
10
8
6
4
2
0
0
20
40
60
80
100
120
140


90
80
70
60
50
40
30
number of eggs
0
2
4
6
8
10
12
age (a)

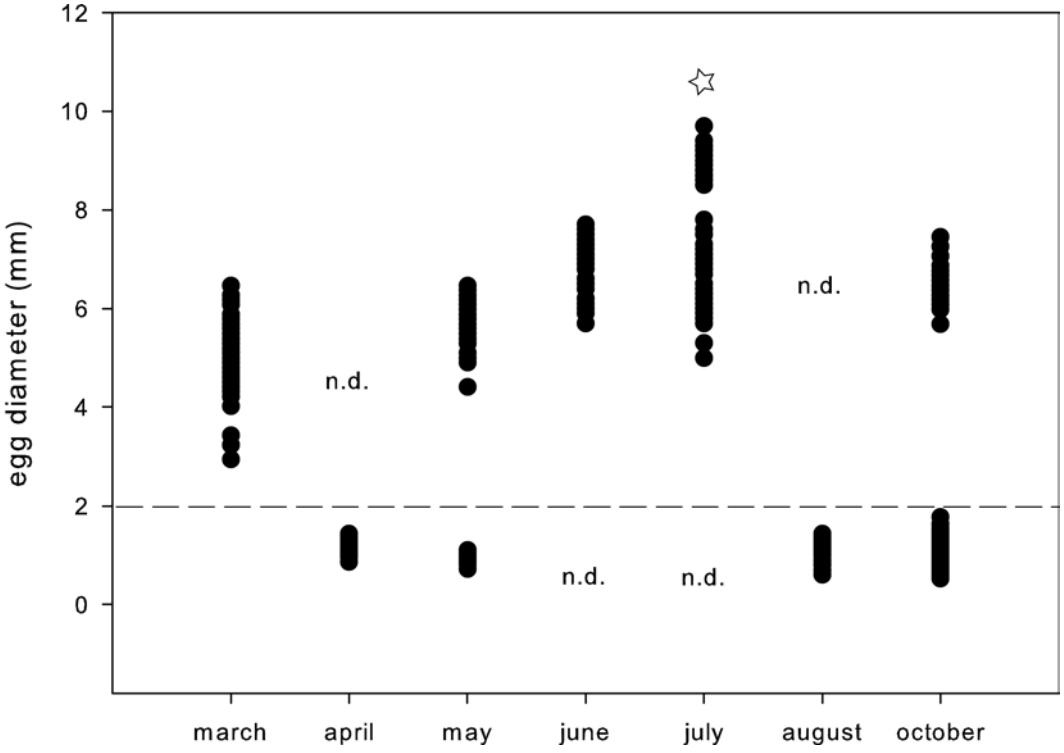

egg diameter (mm)
12
10
8
6
4
2
0
n.d.
n.d.
n.d.
n.d.
march
april
may
june
july
august
october

Table 1

Cruise	Date	Station	Gear	Location	Depth	Number of caught eelpouts	
						Total number of eelpouts	Number of *Pachycara brachycephalum*
ANT XV –3	Feb. 1999	150 / 154 / 167 / 168	OT	Halley Bay	746m / 666m / 392m / 247m	6	-
ANT XV –3	Feb. 1999	263	OT	Drescher Inlet	411m	1	-
ANT XV –3	Feb. 1999	206	AGT	Kapp Norvegia	518m	4	-
ANT XV –3	Feb. 1999	077	AGT	North of Kapp Norvegia	433m	2	-
ANT XV –3	March 1999	293 /294	BT	Admiralty Bay (King George Island)	405m / 437m	> 500	> 500
ANT XV –3	March 1999	338b	AGT	Drake Passage	417m	15	-
ANT XVII-3	April 2000	164 / 166	AGT / OT	Bransfield Starit	858m / 666m	2	-
ANT XVII-3	April 2000	173	OT	West of Deception Island	352m	3	-
ANT XVII-3	May 2000	191 / 192	BT	Admiralty Bay (King George Island)	379m / 371m	} > 400	} > 400
ANT XVII-3	May 2000	195 / 197	BT	King George Island	512m / 497m		
ANT XVII-3	May 2000	178	OT	West of Deception Island	804m	9	-
ANT XVII-3	May 2000	183 / 184	OT	West of Deception Island	204m / 374m	4	-
ANT XIX-5	April 2002	153	OT	Burdwood Bank	296	1	-
ANT XIX-5	April 2002	174	OT	Northwest of South Georgia	278	1	1
ANT XIX-5	April 2002	258 / 259 / 261	BT	Admiralty Bay (King George Island)	418m / 415m / 437m	184	184
ANT XXI-2	Jan. 2004	284	EBS	Drescher Inlet	804m	3	1
ANT XXI-2	Jan. 2004	289	BT	Drescher Inlet	835m	2	2

Table 2

		January	March	June	July	August
Female (>30g weight)	fish wet	0.67	0.46 ±0.18 SEM	6.35 ±3.51 SEM	8.11 ±7.13 SEM	0.59 ±0.07 SEM
Male (>30g weight)	fish wet	0.30 ±0.11 SEM	0.36 ±0.09 SEM	0.67 ±0.43 SEM	0.98 ±0.35 SEM	0.72 ±0.35 SEM

Table 3

Species	Area	Sex	L∞ (cm)	k	t_0	r²	Condition c	Pauly growth index	Habitat temperature (°C)	Source
Pachycara brachycephalum	Antarctic Peninsula	male	36.0	0.12	-1.75	0.79	0.32 ± 0.01SEM	1.27	0 – 0.6	this paper
Pachycara brachycephalum	Antarctic Peninsula	female	36.0	0.10	-2.99	0.76	0.31 ± 0.01SEM	1.26	0 – 0.6	this paper
Lycodes reticulatus	Northeast Greenland	-	41.5	0.06	-1.59	0.98	0.6 ± 0.02SEM	1.55	> 0 - < 0	Dorrien, 1993
Lycodes reticulatus	Barents Sea	-	28.8	0.13	-0.42	0.91	0.05 ± 0.01SEM	1.04	< 0 - 6	Dorrien, 1993
Lycodes vahlii	Oslofjord	male	28.2	0.15	-0.12	-	0.27-0.40	-	5.75 – 6.7	Nash, 1986
Lycodes vahlii	Oslofjord	female	15.8	0.40	0.24	-	0.28-0.42	-	5.75 – 6.7	Nash, 1986

Temperature dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae)

E. Brodte*, R. Knust, H.O. Pörtner

Authors Affiliations: Alfred–Wegener-Institute for Polar- and Marine Research, Physiology of marine animals, D-27515 Bremerhaven, FRG

*Corresponding author:

Eva Brodte

Alfred–Wegener–Institute for Polar- and Marine Research

Physiology of marine animals

Postfach 12 01 61

D - 27515 Bremerhaven

Germany

Telephone ++49 471 4831 2014

Fax ++49 471 4831 1048

ebrodte@awi-bremerhaven.de

Abstract

Antarctic fishes display slower annual growth rates than congeneric species from temperate zones. For an analysis of growth in relation to energy turnover, body composition was analysed in two benthic fish species to establish a whole animal energy budget. The Antarctic eelpout, *Pachycara brachycephalum*, was maintained at 0, 2, 4 and 6 °C and the boreal eelpout, *Zoarces viviparus* at 4, 6, 12 and 18 °C. At maximum food supply the weight gain was highest for *Pachycara brachycephalum* at 4 °C. Routine metabolic rate in acclimated Antarctic eelpouts did not differ between temperatures, whereas in *Zoarces viviparus* maximized growth benefited from a reduction of metabolic energy demands at 12 °C. The lipid content of liver declined with increasing temperature in both species. The thermal window for growth is based on food conversion efficiency and the level of metabolic energy demand and is limited according to the level of aerobic scope available between pejus temperatures.

Introduction

It has become common knowledge that most Antarctic fish species display a slower growth performance compared to congeneric species or ecotype equivalents in temperate zones (Burchett, 1983; Radtke et al., 1989; Radtke and Hourigan, 1990; Morales–Nin et al., 2000; La Mesa and Vacchi, 2001). Temperature is known to be a factor directing fish growth (Pitcher and Hart, 1982; Fonds et al., 1989a; 1992; Otterlei et al., 2000; Hansen and Falk-Petersen, 2001) as it affects all physiological processes in aquatic ectotherms (Pörtner et al., 1998). Accordingly, it has been debated whether this difference in growth rate is related to

i) a plain uncompensated temperature effect on biochemical reactions (Clarke and North, 1991),
ii) a cost intensive pattern of metabolic adaptation required for successful living in the cold (Metabolic Cold Adaptation Hypothesis : MCA) (Hochachka, 1988; Kawall and Somero, 1996; Clarke and North, 1991; Clarke and Johnston, 1999) or to
iii) food limitation due to insufficient food availability or strictly seasonal food supply in the polar cold and therefore to seasonal growth periods (Clarke and North, 1991; North, 1998; Peck, 2002; Cavalli et al., 1997; Coggan, 1997a,b).

Especially the metabolic cold adaptation hypothesis has been discussed controversially (Holeton, 1974; Clarke, 1980; Hochachka, 1988; Torres and Somero, 1988; Crockett and Sidell, 1990; Kawall and Somero, 1996; Peck, 2002 s.o.). Recently, Clarke and Johnston (1999) as well as Peck and Conway (2000) rejected this theory for both Antarctic fish and invertebrates. Among Antarctic notothenioid fishes annual growth performance varies depending on mode of life and increases with falling energy turnover (Pörtner et al., 2005). Antarctic ice fishes (Channichthyidae), for example, show very low rates of energy turnover and thus, values of "growth performance" (Pauly, 1979) similar to temperate fishes (La Mesa and Vacchi, 2001; Pörtner et al., 2005). Extremely low standard metabolic rates as seen in Antarctic compared to temperate invertebrates and in Antarctic demersal versus pelagic fishes indicate that growth performance is enhanced when growth efficiency is high due to low standard metabolic rates and associated low baseline costs and energy saving modes of life (Pörtner et al., 2005). At the same time, the relatively low and sometimes pulsed food availability in the Antarctic (Arntz et al., 1994; 1997; Walker, 2005) may contribute to set the level of energy turnover low and thereby growth efficiency relatively high.

Flow of food energy (input / output balance) in an animal can be viewed as an energy budget (Kooijman, 2000), which is a powerful tool for identifying relevant aspects of life and associated energy demand in animals in different habitats (Scofiani and Hawkins, 1985). Energy allocation to growth is subject to constraints and competing demands and depends on the thermal tolerance window of a species. Frederich and Pörtner (2000) applied Shelford's law of tolerance and elaborated that the thermal window of an animal is defined by the window where significant scope for aerobic metabolism is available. This window is bordered by lower and upper "pejus" temperatures, which indicate onset of a decline in aerobic scope. A phenotypic expression of this range is the temperature range where growth is still possible. Growth thus depends on the availability of surplus aerobic energy (Pörtner et al., 2005) beyond the baseline costs of maintenance. Only within this window of aerobic scope (Pörtner et al. 2005) is surplus energy available for growth and reproduction (cf. Brett and Groves, 1979).

Temperature and food supply acutely modulate energy budgets at the whole organism level. Cui and Wooton (1990) found increasing energy allocation to growth of the minnow, *Phoxinus phoxinus*, with rising temperature at maximum food supply and

the opposite with restricted ratios. The individual thus is a good basis to test how growth performance is determined by food limitation in relation to metabolic costs and costs of adaptation to various thermal regimes. As an organisational unit it is separated from its environment by physical barriers and might control responses to these physical barriers and factors, such as temperature.

At the organismic level, the energy budget includes the body composition of storage compounds, i.e. higher molecular structures like carbohydrates, proteins, lipids, which may change at different acclimation temperatures (Elliott, 1976; Fonds et al., 1989a; Lyytikaeinen et al., 1997). These changes would indicate metabolic situations where excess substrate from available food is stored or where food limitation or the thermal limitation of food uptake leads to the net use of endogenous substrate stores.

We hypothesized that shifts in energy allocation are temperature dependent and mirror the changes in growth optima of whole organisms. To test this hypothesis temperature dependent changes in energy turnover and energy budget components were investigated in fishes from different thermal habitats. Two species of the fish family Zoarcidae were chosen as model organisms, the Antarctic eelpout, *Pachycara brachycephalum* (Pappenheim), and the boreal eelpout, *Zoarces viviparus* (Linne). Both species are quite similar with respect to behaviour, ecology and feeding type (Brodte, 2001; Brodte et al., 2006) and have previously been studied in comparative physiological research projects (Hardewig et al., 1998; 1999; Van Dijk et al., 1999; Lannig et al., 2005; Storch et al., 2005, Mark et al., 2005). Both species could therefore be fed on the same diet. For long-term acclimation experimental temperatures were chosen which reflect the thermal range in the natural habitat of the two eelpout species, but go beyond this range by including warmer temperatures for the Antarctic species (0, 2, 4 and 6 °C for the Antarctic eelpout and 4, 6, 12 and 18 °C for the North Sea eelpout).

Material and methods

The Antarctic eelpout *Pachycara brachycephalum* was caught by use of baited traps in Admiralty Bay, Antarctic Peninsula, at between 360 and 460m depth, ambient temperatures of between 0 and 0.6 °C and salinity levels of 34.6 PSU (CTD data; Gerdes, AWI, Bremerhaven, pers. comm.). The North Sea eelpout *Zoarces viviparus* was caught in July with a beam trawl in the German Wadden Sea in the vicinity of the

islands Spiekeroog and Langeoog at a depth range of 1.5 to 5.0 m, at temperatures between 17.5 and 19.5 °C and salinities from 30.6 to 31.4 PSU. The fish were transferred alive to the laboratory in Bremerhaven where experiments were conducted. Before starting the growth experiment animals were preconditioned by keeping them for three months at 10 °C (*Zoarces viviparus*) and at 0 °C (*Pachycara brachycephalum*) and by feeding both species with cockle flesh (*Cerastoderma edule*).

Growth experiment

15 animals of nearly the same size (from 15.0 cm to 26.5 cm; mean size 20.1 cm ± 2.6 standard deviation) were used per experimental temperature and species. The fishes were kept for four months under identical conditions (seawater, salinity from 31.9 to 33.3 PSU). For clear identification of each individual, plastic gratings separated the fishes sharing the same seawater circulatory system. The animals were weighed (to the nearest 0.1 g) and measured (to the nearest 1mm below) at the beginning, after the respiration measurement (see below) and at the end of the growth experiment. Experimental temperatures comprised the range of habitat temperatures and beyond and were 0, 2, 4 and 6 °C for the Antarctic eelpout and 4, 6, 12 and 18 °C for the North Sea eelpout. Temperatures were kept stable (± 0.4 °C) for 4 months. Fishes were fed ab libitum with cockle meat (*Cerastoderma edule*) on every second day. Food intake by each fish was quantified by weighing of the diet as well as of the unconsumed rests of the food.

Respiration measurements

Respiration analyses were conducted after two months of acclimatisation to the experimental temperature and were started 24 hours after transferring the fish to the respiration chambers to avoid artefacts in oxygen consumption due to handling stress (Steffensen, 2002). The fish were not fasted, but feeding was discontinued one day before the measurement started. Thereby, the measurements reflect the routine (or intermediate) respiration rate according to Jobling (1994) who defined the metabolism of fish fed daily, with normal activity and without stress, as routine or intermediate. Routine metabolism includes a component of "specific dynamic action" (SDA), i.e. 24 hours after the last meal, metabolic rate was elevated due to digestion and anabolic processes (Johnston and Battram, 1993). SDA is largely attributable to the cost of

protein synthesis and, therefore, the metabolic cost of growth (Brown and Cameron, 1991).

The fishes were unrestrained in the respiration chambers to reflect similar conditions during the growth experiment but usually both species were resting quietly on the ground. With respect to temperature and salinity experimental conditions were also the same as in the growth experiment. Measurements were carried out in a flow-through respirometer. The four chambers were partly covered to avoid algal production and were placed in a water basin, which was set to constant temperature by a recirculated thermostat. Three fishes and one blank were measured in parallel to take the influence of bacterial respiration into account. A peristaltic pump (Ismatec, Midi-Digital, cassette MS/CA 4-6-, ISM 881/871) regulated the water flow through the chamber. Oxygen concentration of the water leaving the respiration chambers was measured by oxygen micro-optodes (PreSens, type B2). The flow rate was set depending on species, size of the individual and temperature, such that the out-flowing water displayed about 20% (± 5%) less oxygen than the in-flowing water.

Optodes were calibrated prior to analysis and the calibration was checked at the end of the respiration measurements by use of 100 % air saturated seawater from the basin and of nitrogen saturated water at experimental temperatures. Oxygen consumption of the fishes was monitored for 2 to 4 hours (Microx TX oxygen meter, software TX 2.9, PreSens) at constant temperature and during the same period of the day to avoid differences due to diurnal patterns. Body weight and length were determined after respiration measurements and the fish were then transferred back to the growth experiment.

Excretion measurements

During respiration analyses ammonia excretion was determined in the flow through system (Rosas et al., 1999). Aliquots of 10ml of the out-flowing as well as of the in-flowing water were sampled from each chamber and analysed after diluting the samples with distilled water. The photometric assay used an ammonium cell test based on the phenol hypochlorite method (Brockington, 2001).

CHN Analysis

At the end of the growth experiment the fishes were anaesthetized with MS 222 (0.25g / l seawater for *Pachycara brachycephalum*, 0.13g / l seawater for *Zoarces viviparus*, respectively) and killed. Sex and maturity were determined after Everson (1977). Otoliths were taken for analyses of age. Liver, gonads, muscle tissue and the "rest" were separated and weighed (to the nearest 0.1 g). Each tissue subsample of the liver and the muscle as well as a subsample of the homogenised residual components of the fish (including bones, skin, gonads etc., excluding stomach and guts) were divided between two Eppendorf tubes. After determination of tissue wet weight the tubes were frozen directly in liquid nitrogen and stored at -80 °C. The samples were lyophilised, dry weight was determined and the content of one tube was ground to fine powder and redried for at least three days at 60 °C in a drying closet before starting the CHN analysis with three replicates of each sample (Euro EA 3000 series, Elemental Analyser, Euro Vector CHNS-O; Software: Callidus 1.0). The contents of the other tubes were burnt to ash in a muffle oven at 580 °C for two hours. The ashed samples were dried for at least three days at 60 °C and analysed for CHN content as mentioned above. Subsamples of the food (cockle tissue) were prepared and analysed in the same way.

Stoichiometric and statistical analysis

Weight of the whole body, of muscle and liver tissue as well as of the food was converted into amounts of protein, carbohydrate and lipid and into energy units using the procedure after Gnaiger and Bitterlich (1984).

Oxygen consumption was converted into energy units (kilo Joules)(oxycaloric equivalent: Elliot and Davidson, 1975; Gnaiger and Bitterlich, 1984; Wieser, 1986) derived from the composition of the diet. Ammonia excretion was converted with the approximation that 1 mg NH_4 –N equals 24.87 J (Elliot and Davidson, 1975). Faecal excretion was approximated based on the equation developed for brown trout (Elliott, 1976). Elliott (1976) developed an equation, which determines the faecal deposition depending on temperature and diet quantity. The relationship between the energy losses in faecal excretion (F), temperature and ration sizes was defined by a multiple regression equation. At maximum rations energy losses in faecal excretion can be calculated by use of the following equation (cf. Elliott, 1976) at experimental temperature (T in °C).

$$F = 0.212 * T^{-0.222} * e^{0.631}$$

Fulton's condition factor (c) was calculated as (Ricker, 1975):

$$c = \frac{wet\_weight[g]}{total\_length[cm]^3} * 100$$

The hepatosomatic index (HSI) was determined as

$HSI = \frac{Liver\_weight[g]}{gutted\_weight[g]} * 100$.

The food conversion efficiency (Con_{gross}) was determined applying the energy equivalents (Gnaiger and Bitterlich, 1984; Wieser, 1986) to the mass units of the weight gain and of the food to minimize the error resulting from differences in body composition (Brett and Groves, 1979):

$$Con_{gross} = \frac{E_{growth}}{E_{ingest\_food}} * 100 .$$

E_{growth} is the energy content of the daily weight gain and E_{ingest_food} is the energy content of the daily food intake, both in kJ.

The Arrhenius activation energy (E_a) of whole animal respiration was approximated by the slope (m) of the respiration rate plotted against temperature in an Arrhenius plot

$$E_a = -R * m$$

with R as the gas constant (8.314 [J / mol*K]) (Wieser, 1986).

Results were displayed as means and standard error (SEM). Means and the 95% confidence interval were depicted in the scatter plots of figure 3B where a regression was fitted. Results were tested using statistical software (Prism 4.0 (GraphPad) or Statistica (StatSoft)) and were considered significant at $p < 0.05$, using the t-test or ANOVA with a post hoc test for unequal N (Tukeys Honest Significant difference test for unequal N).

Results

Fishes used in the experiments had a total body length of between 15.0 and 26.5 cm at an age of either 5 to 9 years (*Pachycara brachycephalum*) or 1 to 2 years (*Zoarces viviparus*). Average weight gain was 0.03 g/d for *Pachycara brachycephalum* (without any differences between age classes) and 0.02 g/d for *Zoarces viviparus*. Length increments ranged from 0.04 to 0.07 mm/d (*Pachycara brachycephalum*) and from 0.02 to 0.05 mm/d (*Zoarces viviparus*). For further analysis only those fishes were taken into

consideration, which never refused feeding and survived until the end of the experiment. Food intake by both species was continuous and ranged between 80 and 96% of the offered food. The amount of food taken in was statistically the same at all experimental temperatures and between both species (Figure 1a). Mean growth rates per day were significantly different between both species, with a maximum weight gain at 4 °C for *Pachycara brachycephalum* and, albeit non-significantly, at 12 °C for *Zoarces viviparus* (Figure 1b). The food conversion efficiency was highest in *Pachycara brachycephalum* at 4 °C and significantly higher than in *Zoarces viviparus* (which displayed no significant differences between experimental temperatures), except at 6 °C (Figure 1c). Growth in body length at different temperatures is compiled in Table 1. The length increment in the Antarctic species was larger than in the temperate species. Gain in length was highest for *Pachycara brachycephalum* at 4 °C.

The relative liver weight (hepatosomatic index, HSI) (Figure 2a) did not show temperature dependent changes in *Pachycara brachycephalum* between 0 and 6 °C (0 °C: 3.3 ± 0.27 SEM and 6 °C: 2.9 ± 0.13 SEM). Liver weight increased at colder temperatures in *Zoarces viviparus*, with a significant difference ($p<0.02$) between HSI levels at 4 °C (2.5 ± 0.19 SEM) and at 12 °C (1.0 ± 0.16 SEM) (Figure 2a). The condition factor, indicating nutritional status, was stable for *Pachycara brachycephalum,* with no significant differences between 0 and 6 °C (Figure 2b). In *Zoarces viviparus* the highest value (significant at $p<0.007$) was found at the lowest temperature (4°C), the lowest condition was measured at 12°C (Figure 2b). Long term acclimated respiration rates (Figure 3a) of whole animals showed no temperature dependence at experimental temperatures in *Pachycara brachycephalum*. In contrast, the respiration rate of *Zoarces viviparus* increased significantly with temperature (with a significant difference between the highest and lowest temperatures). The linear regression of the data is depicted in the Arrhenius plot (Figure 3b,c). For *Pachycara brachycephalum* the slope was not significantly different from zero, with a slight trend best described by a linear equation:

$$MO_2 = -2.37 - 0.49T$$

($R^2=0.0033$).

For *Zoarces viviparus* the linear regression was significant for both coefficients with $p< 0.01$. The linear equation was

$$MO_2 = 10.44 - 3.93T$$

(R^2=0.3923).

MO_2 is the rate of oxygen consumption [ln (µmol O_2/min*g wet weight)] and T is the temperature [K]. Accordingly, the slopes of the plots yielded a low Arrhenius activation energy (E_a) of 4.1 kJ/mol for the Antarctic eelpout and a higher level of 32.7 kJ/mol for the North Sea eelpout.

The energy budgets of *Pachycara brachycephalum* (a, c) and *Zoarces viviparus* (b, d) at different temperatures are shown in Figure 4. Measured parameters were converted by use of bioenergetic conversion factors (Elliott and Davidson, 1975; Gnaiger and Bitterlich, 1984; Wieser, 1986). The ingested energy was set to 100% and is indicated by a dashed line (Figure 4). Ammonia excretion did not change significantly with temperature in both species. The main fraction of ingested energy was allocated to routine metabolism. This amount of energy remained more or less unchanged in the budget of *Pachycara brachycephalum*. Nonetheless, energy allocated to growth showed a temperature dependent maximum in *Pachycara brachycephalum* at 4°C (Figure 4a). The results suggest that the total energy demand of investigated processes exceeded the energy content of the food at 2 and 4 °C and remained below nutritional energy content at 6 °C.

For *Zoarces viviparus* temperature dependent changes in the energy budget were more pronounced (Figure 4b), with a minimum fraction of available energy allocated to routine metabolism at 12 °C and a maximum at 6 °C. In contrast to the respective observations in *Pachycara brachycephalum*, the amount of energy allocated to routine metabolism dropped upon warming in *Zoarces viviparus*. In this species the summed energy quantities used for growth, metabolism, ammonia excretion and faecal deposition closely matched the energy content of consumed food (Figure 4b).

Water and ash content of muscle and of liver tissue as well as of the compiled "rest" showed no significant change with temperature. The average water content of muscle and residual tissue was about 80% of tissue wet weight in both species. The composition of the food source cockle flesh, when related to dry weight, was 62% proteins, 18% carbohydrates and 12% lipids.

In both species the composition of the liver tissue showed more pronounced changes with temperature than the muscle tissue (Figure 5a, c). The lipid content of liver declined with increasing temperature. In *Pachycara brachycephalum* liver tissue at 2 °C, the carbohydrate level was significantly reduced and the protein level was

elevated when compared to higher temperatures. In contrast, muscle tissue protein levels remained constant, but carbohydrate levels showed a significant drop with rising temperature. In *Pachycara brachycephalum* the lowest carbohydrate fraction in muscle was found at 4 and 6 °C. For *Zoarces viviparus* the lowest fraction of carbohydrate in muscle was found at 18 °C. Additionally, there was a slight but significant increase of lipid contents in the muscle of the boreal eelpout with temperature rising above 6 °C (Figure 5d). Such pattern was not observed in the Antarctic eelpout.

Discussion

Using summed values in the energy budget, when individual processes were determined independently, is susceptible to bias due to potentially unidirectional methodological errors. Neither the energy demanding processes for *Pachycara brachycephalum* at 2 and 4 °C, nor for *Zoarces viviparus* at 6 °C were met by the nutritional energy uptake. Metabolic demands were calculated by using the oxycaloric coefficient. The stoichiometric calculation of this coefficient neglects structural atomic parameters, which modify the energy content of molecules (Wieser, 1986). This leads to overestimations of the heat of combustion by about 2-5% for shorter fatty acids and to an underestimation by 6% for glycogen (Wieser, 1986). The higher amount of lipids than carbohydrates consumed from the diet as well as a major importance of fatty acids and lipids as the diet of carnivorous fish (Brett and Groves, 1979), may contribute to an overestimation of the energy fraction allocated to metabolism when approximated by the oxycaloric coefficient.

The functional difference of muscle and liver may be mirrored in larger temperature dependent changes in the contents of proteins, carbohydrates and lipids in liver than in muscle tissue. The liver has storage (Larsen et al., 2001) as well as anabolic functions and shows higher fractional rates of protein synthesis than white muscle (Houlihan et al., 1988). In contrast, muscle is a “working” and growing tissue and its structural protein content depends on growth rate and on the condition of the fish (Couture et al., 1998). In our study the total protein content of both muscle and liver was not significantly affected by growth rate. In cod the protein content of the liver decreases with increasing growth rate, while in muscle the sarcoplasmatic protein content increases (Couture et al., 1998).

Among investigated compounds the lipids displayed major changes over the investigated thermal range. In both eelpout species the lipid contents of the liver increased in the cold. This trend coincided with increasing HSI values in *Zoarces viviparus*. Increasing liver weight with decreasing temperature has previously been reported for this temperate eelpout (Lannig et al., 2005). The fraction of lipids in tissue dry weight was higher in *Pachycara brachycephalum* than in *Zoarces viviparus*, in line with a three-fold higher lipid content of the whole animal (Brodte, 2001). The high amount of lipid in liver and muscle seems to reflect a typical preference for lipid anabolism in Antarctic species due to cold (Giardina et al., 1998; Colella et al., 2000; Pörtner, 2002). This may mirror a cold induced shift to a more storage oriented metabolic situation in both eelpout species. In the boreal eelpout enhanced storage of lipids seems to occur when growth is reduced. At higher acclimation temperatures the boreal eelpout displayed no pronounced changes in lipid composition. This reminds of the situation in young brown trout, which use most of the surplus nutritional energy for protein synthesis and only small fractions to accumulate energy reserves in the form of lipid stores (Jonsson and Jonsson, 1998). In muscle tissue of the Antarctic species a decrease in carbohydrates upon warming to 4 and 6 °C indicates an increased net use of storage compounds at higher temperatures. In *Zoarces viviparus* liver tissue a significant decrease of protein content at 4 °C may reflect the beginning of accelerated protein catabolism in the cold and the potential conversion of protein into lipid.

In the light of the growth rates observed in both species, the compiled energy budgets lead to some possible explanations of differences and constraints in growth performance in the field. Growth rate depends on age and ontogeny – as considered in the Von Bertalanffy growth equation. Therefore, we chose fishes of nearly the same age (intraspecific) or of the same ontogenetic life phase, approximated by size (interspecific) in our experimental growth experiments. At 0 °C which is close to the habitat temperatures of the studied population of *Pachycara brachycephalum*, experimental growth is 0.16 cm/a and approached the observed growth rate in the field (Brodte et al., 2006). Investigations of growth in Arctic eelpout (*Lycodes reticulatus*) also found similar growth rates in the field and in the laboratory (Dorrien, 1993). The field growth rate of our Antarctic eelpout is similar to the growth performance of other Antarctic fishes like *Trematomus eulepidotus* (Table 1) and is higher than the growth rate of Arctic eelpouts (Dorrien, 1993).

The experimental growth of *Pachycara brachycephalum* was faster than in *Zoarces viviparus* and contrasts the situation in the field, where *Zoarces viviparus* displays two fold faster growth than *Pachycara brachycephalum*, either at the same age or at the same size (Table 1). For *Zoarces viviparus* our observed experimental growth rate was 1.5 times lower than the field growth of this population. In contrast, Fonds et al. (1989a) found an experimental growth rate 8.5 fold faster than field growth at annual mean habitat temperature. Higher experimental than field growth is expected from excessive food availability and reduced costs of prey collection under laboratory conditions. The phenomenon of accelerated growth under experimental conditions is known from Antarctic as well as from temperate fishes (Fischer, 2003; North, 1998).

In our experiment, the observed length increments for *Zoarces viviparus* were 10 times less than in the study by Fonds et al. (1989a, Table 1) despite summer growth conditions. The size range of the animals was similar in our study and the one by Fonds et al, but the condition factor recalculated for the data by Fonds et al. (1989a) was higher (0.39 ± 0.08 SD) than found in our study. In addition to the difference in condition factor, the population parameters concerning maximum age, length and weight (Iedema, 1989) differed between the fishes used in the two experiments. Therefore, the fishes which Fonds and co-workers used in their study likely belong to a different population which displays higher field growth than the population used in the present study (Iedema, 1989; Brodte et al., in preparation). In our experiment, growth was slower, but food consumption was the same as in the experiment by Fonds et al. (1989a) indicating low growth efficiency with the diet provided in our study. The diet supplied by Fonds et al. (1989a) consisted of chopped shrimp (*Crangon crangon*) in one trial and mussel meat from *Mytilus edulis* in another trial. Both diets contain more proteins and fewer lipids than the food we used. Fonds (pers. comm.) assumes freshly cut *Mytilus* meat and live *Crangon crangon* as optimal diet of the temperate eelpout. The composition of the diet is similarly important as the amount of food. Concerning the lipid to protein ratio in ash free dry weight cockle diet displays a value of 0.21, a diet prepared from *Mytilus* meat ranges (depending on the season) between 0.11 to 0.18 (Fonds et al., 1989b) and a diet consisting of *Crangon crangon* displays of a ratio of 0.08 (Fonds et al., 1989b). Similarity in the composition of prey and predator is considered an energetic advantage (Kooijman, 2000), because it reduces the cost of conversion into required substances. On average, the composition of muscle tissue of

the Antarctic eelpout displays a high lipid to protein ratio of 0.29 (Figure 5). For the Antarctic eelpout the cockle flesh may thus be a more suitable diet than for the temperate eelpout. In fact, muscle tissue in *Zoarces viviparus* displays an average lipid to protein ratio of 0.01 and the cockle diet may have provided too little protein for optimal growth. Specific food quality of cockle tissues may thus have been lower for the temperate than for the Antarctic species leading to lower growth in the common eelpout and a higher food conversion efficiency of the Antarctic eelpout at the same rate of food intake.

In general, a high, above optimal ingestion rate reduces food adsorption efficiency (Elliott, 1976; Jobling, 1994) and leads to an increase in faecal deposition (Jobling, 1994). Although the dependence of gastric evacuation rates on energy content and particle size of the diet (Jobling, 1986, 1987; Andersen, 2001) and, therefore, on the rates of food adsorption and amount of faecal deposition is still discussed, the positive correlation of faecal deposition and temperature is accepted (Elliot, 1976). Low temperature conditions in the Antarctic might increase the ability of the Antarctic eelpout to convert food at high assimilation rates, similar to findings in northern cod populations (Nicieza et al., 1994; Purchase and Brown, 2000). A comparison of subtropical, temperate and Antarctic fish showed 3 to 4 times longer periods of specific dynamic action (SDA) in cold water compared to warm water fishes (Johnston and Battram, 1993). This finding indicates extended periods of food passage through the digestive tract, thereby supporting higher assimilation rates in Antarctic fish. High food conversion efficiency and a cold compensated capacity for protein synthesis in the Antarctic when compared to the temperate species (Storch et al., 2005) might then explain the higher growth rates of *Pachycara brachycephalum* observed in the cold, at same rate of food uptake.

It needs consideration that high food conversion efficiency of the Antarctic eelpout may also relate to different levels of food availability in the two natural habitats. While *Zoarces viviparus* experiences high food availability in the Wadden Sea throughout the year with a peak in spring and summer, food supply for the scavenging Antarctic eelpout may be even more seasonal with a peak in the summer (Arntz et al., 1994; 1997; Walker, 2005). Quantity and quality of food in the Antarctic can be assumed to be lower and more seasonal than in the boreal Wadden Sea area.

However, there are no data available for food consumption or seasonal changes in food composition of *Pachycara brachycephalum* in the field. *Zoarces viviparus* experiences continuous food supply during the year. The food spectrum is more diverse in winter / spring than in summer when crustaceans are highly abundant in the diet (Ulleweit, 1995). Crustacean diet might in fact be required for optimum growth performance of *Zoarces viviparus* (see above). The lower protein content of polychaetes like *Nereis*, which form a main component of *Zoarces viviparus*' diet in winter (Ulleweit, 1995 and E. Brodte, personal observations), as well as the lower condition of mussels in winter compared to crustaceans in summer (Fonds et al., 1989a,b) may also contribute to the absence of winter growth. Additionally, the lack of winter growth may be due to partly cold compensated metabolic rates and higher baseline energy turnover under the effect of winter cold (van Dijk et al., 1999).

Nonetheless, the temperature dependence of growth was similar in our study and in the earlier experiments by Fonds and co-workers (1989a). In the present study, the optimum growth of boreal *Zoarces viviparus* from the German Wadden Sea was not significantly different from other temperatures, but likely close to 12 °C. Fonds et al. (1989a) found an optimum at 15 °C. Both optima match the habitat temperature range between 3 and 24 °C, with an annual mean of about 12 °C. Pörtner et al. (2001) reported that the growth rate of cod populations from various latitudes analysed in experimental studies decreased at high latitudes, but growth optima resulted similar between 11 and 12 °C, at least in the cod populations from the North Sea and from the Norwegian coast. Temperature dependent growth optima (at unlimited food availability) have also been found in other fish species (Houde, 1989; Fonds et al., 1992; Conover et al., 1997; Koskela et al., 1997; Billerbeck et al., 2000; Purchase and Brown, 2000; Ayala et al., 2001; Fischer, 2003; Hofmann and Fischer, 2003). These optima can shift with increasing body size as seen in Atlantic cod (Björnsson and Steinarsson, 2002).

Maximum growth of the Antarctic eelpout was found at 4 °C. This temperature may appear relatively high for an Antarctic species, but at the same time, may reflect the Deep Sea origin of the genus *Pachycara* (Anderson, 1984). The higher thermal optimum for this species may be one reason for the reduced density of this species in high Antarctic waters at temperatures below –1 °C (Brodte et al., 2006). This growth optimum matches results obtained from respiration measurements in isolated hepatocytes of *Pachycara brachycephalum* during acute temperature exposure between

0 and 21 °C. The measured cellular oxygen demand was lowest at 3 °C reflecting a minimum in baseline cellular energy demand (Mark et al., 2005). At the whole animal level no significant reduction in acclimated respiration rates was seen at this temperature. Therefore, the minimized metabolic costs seen in cell suspensions may support enhanced energy allocation to growth, at constant whole animal respiration rates. The relative contribution of energy demanding processes, namely metabolic maintenance, growth and excretion to the energy budget of both species is compiled in Figure 4. Some energy is lost in faecal deposition. This loss is higher at below optimum temperatures in both species. Surplus energy fractions allocated to growth shift in accordance with temperature dependent metabolic requirements for maintenance and species specific growth optima. In *Zoarces viviparus* maintenance costs are much higher below and above the average habitat temperature, thereby leading to an optimum of around 12 °C. Similarly, the surplus energy allocated to growth in *Pachycara brachycephalum* at 4 °C likely results from high (cellular) efficiency and minimized costs (Mark et al., 2005; see above) as well as high food adsorption capacity.

Van Dijk et al. (1999) compared the standard metabolic rates (SMR) of unfed *Pachycara brachycephalum* acclimated at 0 °C and of *Zoarces viviparus* acclimated at 3 and at 12 °C. They found 30 % lower oxygen consumption values in *Pachycara brachycephalum* acclimated at 0 than seen in our study. This difference between their and our present data likely reflects the SDA component contributing to our present analyses of routine metabolism (Ciu and Liu, 1990). The higher Arrhenius activation energy values found by Van Dijk and co-workers (1999) for respiration rates of Antarctic eelpout (99.4kJ/mol), and for warm acclimated *Zoarces viviparus* (82.9 kJ/mol) than for cold acclimated *Zoarces viviparus* (55.5kJ/mol) reflect the acute effect of short term warming after previous acclimation to one control temperature. In our present study the fishes were long term acclimated to each temperature prior to analysis. The reduced slope of the Arrhenius plot clearly indicates close to full temperature compensation of metabolic rate upon long term acclimation in the Antarctic eelpout. This finding is in line with recent insight that, in contrast to previous understanding, Antarctic fish are able to acclimate to higher than ambient temperatures (Lannig et al., 2005).

The acclimated respiration rate of *Pachycara brachycephalum* did not reach the one of *Zoarces viviparus* when compared at 4 and 6 °C. Higher growth at lower

metabolic rate thus again reflects the higher growth efficiency of the Antarctic eelpout, in line with the recent finding of higher growth efficiency and rate in benthic than in pelagic Antarctic fish elaborated by Pörtner et al. (2005). The mechanisms underlying the temperature dependent shifts in cellular and organismic energy efficiency require further investigation.

The thermal range where positive growth occurs corresponds with the tolerance window set by oxygen and capacity limitations according to a model developed by Frederich and Pörtner (2000) and updated by Pörtner et al. (2005). An acclimated window results for the Antarctic eelpout between 0 and 6 °C with a clear optimum at 4 °C. Pejus temperatures delineate the onset of a loss in aerobic scope. Former investigations during acute temperature changes determined the pejus temperature of this eelpout species at 7 °C (Mark et al., 2002). According to our present analysis of metabolic costs and of temperature dependent growth the upper pejus temperature of the Antarctic eelpout remained unchanged during long-term thermal acclimation.

In contrast to the more stenothermal Antarctic species the eurythermal boreal eelpout *Zoarces viviparus* displays a thermal window from at least 4 to 18 °C with a growth maximum around 12 °C. Zakhartsev et al. (2003) assumed an upper pejus temperature for boreal eelpout populations from the Baltic Sea, Norwegian Coast and from the North Sea (Helgoland) of between 13 and 15 °C. Our window of positive growth thus matches the concept of oxygen and capacity limited thermal tolerance. At 15 °C, as an upper pejus temperature, aerobic scope would progressively be lost and this loss leads to reduced energy allocation to growth seen at 18 °C. Positive growth thus reflects whether the organism is in the range between lower and upper pejus temperatures. The observed growth maximum would therefore be located close to the upper pejus temperature, where the temperature dependent increase in aerobic scope is still supported by the capacity of oxygen supply to tissues.

Conclusions

Temperature dependent growth in both species is reflected in a lop-sided bell shaped growth curve (at maximum food supply), modulated by physiological limits and capacities in line with a recent model of temperature dependent aerobic scope (Pörtner et al., 2005). In *Pachycara brachycephalum* acclimated overall metabolic rate is relatively stable over the measured temperature range and reflects, more or less, full

temperature compensation. The high fraction of assimilated energy allocated to growth at 4 °C indicates a high potential for growth due to excess energy availability from high energy efficiency. The bioenergetic strategy of the Antarctic species is supported by high food conversion efficiency in the cold. The low field growth rate of *Pachycara brachycephalum* was determined in a habitat with ambient temperatures below the experimental growth optimum and possibly close to lower pejus temperature (Brodte et al., 2006). Growth performance in the field is then restricted firstly by habitat temperatures below the thermal optimum and only secondarily by reduced energy availability from the diet or by higher metabolic rates resulting from higher costs of prey collection in the field.

In *Zoarces viviparus* the energy allocated to growth at an optimum temperature close to 12 °C is also supported by reduced maintenance costs. The higher acclimated metabolic rate seen at 6 °C indicates cold compensated baseline energy turnover at the expense of low growth. At 4 °C the onset of net protein catabolism may reflect unbalanced costs at the lower thermal limits of this species. At 18 °C, on the warm side of the thermal window, high metabolic costs of maintenance lead to abated growth. In the field the boreal eelpout supports growth by continuous feeding throughout the year in a habitat characterized by high thermal variability. The bioenergetic strategy of *Zoarces viviparus* likely comprises enhanced growth and a high energy turnover rate at high energy efficiency in summer which are supported by a high amount and quality of food at temperatures adequate for positive growth.

Acknowledgements:

The authors would like to thank Dr. Mark Fonds for his helpful comments, especially concerning the former growth experiments with *Zoarces viviparus*.

References

Andersen N G (2001) A gastric evacuation model for three predatory gadoids and implications of using pooled field data of stomach contents to estimate food rations. J. Fish Biol. 59(5): 1198-1217.

Anderson M E, (1984) Zoarcidae: development and relationship. Special Publications.

American Society of Ichthyologists and Herpetologists 17: 578-582.

Arntz W E, Brey T, and Gallardo V A (1994) Antarctic Marine Zoobenthos. Oceanography and Marine Biology Annual Reviews 32: 241-304.

Arntz W E, Gutt J, Klages M (1997) Antarctic marine biodiversity: an overview. In: Battaglia B, Valencia, J, Walton, D W H (eds) Antarctic Communities. Species, Structure and Survival. Cambridge University Press, Cambridge, pp 3-14.

Ayala M D, Lopez-Albors O, Gil F, Garcia-Alcazar A, Abellan E, Alarcon J A, Alvarez M C, Ramirez-Zarzosa G, Moreno F (2001) Temperature effects on muscle growth in two populations (Atlantic and Mediterranean) of sea bass, *Dicentrarchus labrax* L. Aquaculture 202(3-4): 359-370.

Billerbeck J M, Schultz E T, Conover D O (2000) Adaptive variation in energy acquisition and allocation among latitudinal populations of the Atlantic silverside. Oecologia 122(2): 210-219.

Bjornsson B, Steinarsson A (2002) The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci./J. Can. Sci. Halieut. Aquat. 59 (3): 494-502.

Brett J R, Groves T D (1979) Physiological energetics. Fish Physiology 8: 279-352.

Brockington S (2001) The seasonal energetics of the Antarctic bivalve *Laternula elliptica* (King and Broderip) at Rothera Point, Adelaide Island. Polar Biology 24: 523-530.

Brodte E (2001) Wachstum und Fruchtbarkeit der Aalmutterarten *Zoarces viviparus* (Linne) und *Pachycara brachycephalum* (Pappenheim) aus unterschiedlichen klimatischen Regionen. Diploma thesis im Fach Biologie, Universität Bremen, Fachbereich 2.

Brodte E, Knust R, Pörtner H O, Arntz W E (2006) Biology of the Antarctic eelpout *Pachycara brachycephalum*. Deep Sea Research II, in press.

Brodte E, Knust R, Ulleweit J, Fonds M, and Arntz WE *Zoarces viviparus* - aspects of growth in latitudinal and temporal scale. in preparation.

Brown C R, Cameron J N (1991) The relationship between specific dynamic action (SDA) and protein synthesis rates in the channel catfish. Physiological Zoology 64 (1): 298-309.

Burchett M S (1983) Age and growth of the Antarctic fish *Notothenia rossii* from South Georgia. Bull. Br. Antarct. Surv. 60: 45-61.

Cavalli L, Chappaz R, Bouchard P, Brun G (1997) Food availability and growth of the brook trout, *Salvelinus fontinalis* (Mitchill), in a French Alpine lake. Fish. Manage. Ecol. 4(3): 167-177.

Clarke A (1980) A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. Biol. J. Linn. Soc. 4(1): 77-92.

Clarke A, Johnston N M (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology 68: 893-905.

Clarke A, North A W (1991) Is growth of polar fish limited by temperature? In: Di Prisco G, Maresca B, Tota B (eds.) *Biology of Antarctic Fish* Springer Verlag, Berlin, pp 54-69.

Coggan R (1997a) Growth: ration relationships in the Antarctic fish *Notothenia coriiceps*, Richardson, maintained under different conditions of temperature and photoperiod. J. Exp. Mar. Biol. Ecol. 210(1): 23-35.

Coggan R (1997b) Seasonal and annual growth rates in the Antarctic fish *Notothenia coriiceps* Richardson. J. Exp. Mar. Biol. Ecol. 213(2): 215-229.

Colella A, Patamia M, Galtieri A, Giardina B (2000) Cold adaptation and oxidative metabolism of Antarctic fish. Ital. J. Zool. 67, Suppl: 33-36.

Conover D O, Brown J J, Ehtisham A (1997) Countergradient variation in growth of young striped bass (*Morone saxatilis*) from different latitudes. Can. J. Fish. Aquat. Sci./J. Can. Sci. Halieut. Aquat. 54(10): 2401-2409.

Couture P, Dutil J D, Guderley H (1998) Biochemical correlates of growth and condition in juvenile Atlantic cod (*Gadus morhua*) from Newfoundland. Can. J. Fish. Aquat. Sci./J. Can. Sci. Halieut. Aquat. 55 (7): 1591-1598.

Crockett E L, Sidell B D (1990) Some pathways of energy metabolism are cold adapted in Antarctic fishes. Physiol. Zool. 63(3): 472-488.

Cui Y, Liu J (1990) Comparison of energy budget among six teleosts - 2. Metabolic rates. Comp. Biochem. Physiol. A 97° (2): 169-174.

Cui Y, Wootton R J (1990) Components of the energy budget in the European minnow, *Phoxinus phoxinus* (L.) in relation to ration, body weight and temperature. Acta hydrobiologica sinica/Shuisheng Shengwu Xuebao. Wuhan 14(3): 193-204.

Dorrien C F von (1993) Ecology and respiration of selected Arctic benthic fish species. Berichte zur Polarforschung/Reports on Polar Research 125, 104 pp.

Elliott JM (1976) Energy losses in the waste products of brown trout (*Salmo trutta* L.). Journal of Animal Ecology 45: 561-580.

Elliot J M, Davidson W (1975) Energy equivalents of oxygen consumption in animal energetics. Oecologia 19: 195-201.

Everson I (1977) The living resources of the Southern Ocean. FAO Southern Ocean Fisheries Survey Program, Rome, GLO/SO/77/1 156 pp.

Fischer T (2003) The effects of climate induced temperature changes on cod (*Gadus morhua* L.): Linking ecological and physiological investigations. Berichte zur Polar- und

Meeresforschung 453.

Fonds M, Jaworski A, Iedema A, Puyl P V D (1989a) Metabolism, food consumption, growth and food conversion of shorthorn sculpin (*Myoxocephalus scorpius*) and eelpout (*Zoarces viviparus*). ICES COUNCIL MEETING 1989 (COLLECTED PAPERS), ICES, COPENHAGEN (DENMARK),G: 31, 19 pp.

Fonds M, Drinkwaard B, Resink J W, Eysink G G J, Toet W (1989b). Measurements of metabolism, food intake and growth of *Solea solea* (L.) fed with mussel meat or with dry food. Aquaculture- A biotechnology in progress: 851-874.

Fonds M, Cronie R, Vethaak A D, 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. Netherlands Journal of Sea Research 29 (1-3): 127-143.

Frederich M, Pörtner H O (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. Am. J Physiol. Regul. Integr. Comp. Physiol. 279: R1531-R1538.

Giardina B, Mordente A, Zappacosta B, Calla C, Colacicco L, Gozzo M L, Lippa S (1998) The oxidative metabolism of Antarctic fish: Some peculiar aspects of cold adaptation. Fishes of Antarctica. A biological overview: 129-138.

Gnaiger E, Bitterlich G (1984) Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62: 289-298.

Hansen T K, Falk-Petersen I B (2001) The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). Aquacult. Res. 32(5): 369-378.

Hardewig I, Van Dijk P L M, Pörtner H O (1998) High-Energy Turnover at Low Temperatures:

Recovery From Exhaustive Exercise in Antarctic and Temperate Eelpouts. Am J Physiol 43: R1789-R1796.

Hardewig I, Van Dijk P L M, Moyes C D, Pörtner H O (1999). Temperature-Dependent Expression of Cytochrome-C Oxidase in Antarctic and Temperate Fish. Am J Physiol 277: R508-R516.

Hochachka P W (1988) Channels and pumps-determinants of metabolic cold adaptation strategies. Comparative Biochemistry and Physiology B 90B(no. 3): 515-519.

Hofmann N, Fischer P (2003) Impact of temperature on food intake and growth in juvenile burbot. J. Fish Biol.63 (5): 1295-1305.

Holeton G F (1974) Metabolic cold adaptation of polar fish: fact or artefact. Physiol. Zool. 47(3): 137-152.

Houde E D (1989) Comparative growth, mortality, and energetics of marine fish larvae: Temperature and implied latitudinal effects. Fishery Bulletin 87(3): 471-495.

Houlihan D F, Hall S J, Gray C, Noble B (1988) Growth rates and protein turnover in Atlantic cod, *Gadus morhua* . Canadian Journal of Fisheries and Aquatic Sciences 45(6): 951-964.

Iedema A (1989) Metingen van groei, voedselopname en zuurstofconsumptie an de puitaal, *Zoarces viviparus* (Linne) ed. Stageverslag voor studie biologie aan de Rijkssuniversiteit Groningen

Jobling M (1986). Gastrointestinal overload - a problem with formulated feeds? Aquaculture 51: 257-263.

Jobling M (1987). Growth of Arctic charr (*Salvelinus alpinus* L.) under conidtion of constant light and temperature. Aquaculture 60: 243-249.

Jobling M (1994) Fish Bioenergetics. Chapman & Hall, New York.

Johnston I A, Battram J (1993) Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. Marine Biology 115(no. 1): 7-14.

Jonsson N, Jonsson B (1998) Body composition and energy allocation in life-history stages of brown trout. Journal of Fish Biology 53: 1306-1316.

Kawall H G, Somero G N (1996) Temperature compensation of enzymatic activities in brain of Antarctic fishes: Evidence for metabolic cold adaptation. Antarctic Journal of the United States 31(2): 115-117.

Kooijman S A L M (2000). Dynamic Energy and Mass Budgets in Biological Systems. University Press, Cambridge.

Koskela J, Pirhonen J, Jobling M (1997) Effect of low temperature on feed intake, growth rate and body composition of juvenile Baltic salmon. Aquacult. Int. 5 (6): 479-488.

La Mesa M, Vacchi M (2001) Age and growth of high Antarctic notothenioid fish. Antarctic Science 13(3): 227-235.

Lambert Y, Dutil J-D (1997) Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Can. J. Fish. Aquat. Sci. 54: 2388-2400.

Lannig G, Storch D, Pörtner H O (2005) Aerobic mitochondrial capacities in Antarctic and temperate eelpout (Zoarcidae) subjected to warm versus cold acclimation. Polar Biology 28: 575-584.

Larsen D A, Beckman B R, Dickhoff W W (2001) The Effect of Low Temperature and Fasting during the Winter on Metabolic Stores and Endocrine Physiology (Insulin, Insulin-like Growth Factor-I, and Thyroxine) of Coho Salmon, *Oncorhynchus kisutch*. General and Comparative

Endocrinology 123 (3): 308-323.

Lyytikaeinen T, Koskela J, Rissanen I (1997) The influence of temperature on growth and proximate body composition of under yearling Lake Inari arctic char (*Salvelinus alpinus*) (L.)). J. Appl. Ichthyol./Z. Angew. Ichthyol. 13 (4): 191-194.

Mark F C, Bock C, Pörtner H O (2002) Oxygen limited thermal tolerance in Antarctic fish investigated by magnetic resonance imaging (MRI) and spectroscopy (^{31}P-MRS). Am. J Physiol Regul. Integr Comp Physiol 283: R1254-R1262.

Mark F C, Hirse T, Pörtner H-O (2005) Thermal sensitivity of cellular energy budgets in Antarctic fish hepatocytes. Polar Biology 28: 805-814.

Morales-Nin B, Moranta J, Balguerias E (2000) Growth and age validation in high-Antarctic fish. Polar Biology 23: 626-634.

Nicieza A G, Reyes-Gavilan F G, Brana F (1994) Differentiation in juvenile growth and bimodality patterns between northern and southern populations of Atlantic salmon (*Salmo salar* L.). Canadian Journal of Zoology/Revue Canadien de Zoologie 72(9): 1603-1610.

North A W (1998) Growth of young fish during winter and summer at South Georgia. Polar Biology 19: 198-205.

Otterlei E, Folkvord A, Nyhammer G (2000) Temperature dependent otolith growth of larval and early juvenile Atlantic cod (*Gadus morhua*). Temperature- and Size-Dependent Growth of Larval and Early Juvenile Atlantic Cod (*Gadus morhua* L.), Department of Fisheries and Marine Biology, University of Bergen.

Pauly D (1979) Gill size and temperature as governing factors in fish growth: a generalization of von Bertalanffy's growth formula. Ber. Inst. Meereskd. Christian-Albrecht-Univ. Kiel 63, 156p.

Peck L S (2002) Ecophysiology of Antarctic marine ectotherms: Limits to life. Polar Biology 25, 31-40.

Peck L, Conway L Z (2000) The myth of metabolic cold adaptation:oxygen consumption in stenothermal Antarctic bivalves. In: Harper E M, Taylor J D, Crame J A (eds) The Evolutionary Biology of the Bivalvia, vol. 177, pp. 441-450. London: Geological Society, Special Publications.

Pitcher T J, Hart P J B (1982) Fisheries Ecology. Croom Helm, London & Canberra.

Pörtner H O (2002) Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. J. Exp. Biol. 205(15): 2217-2230.

Pörtner H O, Hardewig I, Sartoris F J, Van Dijk P L M (1998) Acid-based balance, ion regulation and energetics in the cold. In: Pörtner H O, Playle R C. (eds) Cold ocean physiology, University Press, Cambridge.

Pörtner H O, Berdal B, Blust R, Brix O, Colosimo A, De Wachter B, Giuliani A, Johansen T, Fischer T, Knust R, Lannig G, Naevdal G, Nedenes A, Nyhammer G, Sartoris F J, Serendero I, Sirabella P, Thorkildsen S, Zakhartsev M (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*). Continental Shelf Research 21: 1975-1997.

Pörtner H O, Lucassen M, Storch D (2005) Metabolic biochemistry, an integrative view. In: Steffensen J F, Farrell A P (eds). The Physiology of Polar Fishes vol. 22, Fish Physiology.

Purchase C F, Brown J A (2000) Interpopulation differences in growth rates and food conversion efficiencies of young Grand Banks and Gulf of Maine Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci./J. Can. Sci. Halieut. Aquat.57(11): 2223-2229.

Radtke R L, Hourigan T F (1990) Age and growth of the Antarctic fish *Nototheniops nudifrons*. Fishery Bulletin 88(3): 557-571.

Radtke R L, Targett T E, Kellermann A, Bell J, Hill, K T (1989) Antarctic fish growth: Profile of *Trematomus newnesi*. Mar. Ecol. Prog. Ser. 57(2): 103-117.

Ricker W E (1975) Computations and interpretations of biological statistics of fish populations. Bulletin of the Research Board of Canada 191: 1-382.

Rosas C, Martinez E, Gaxiola G, Brito R, Sanchez A, Soto L A (1999) The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (Linnaeus) juveniles. J. Exp. Mar. Biol. Ecol. 234(1): 41-57.

Scofiani N M, Hawkins A D (1985) Field studies of energy budgets. Fish Energetics : new perspectives 283-307.

Steffensen J F (2002) Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artefact? Artefact! Comparative Biochemistry and Physiology, Part A 132: 789-795.

Storch D, Lannig G, Pörtner H-O (2005) Temperature dependent protein synthesis capacities in stenothermal (Antarctic) and eurythermal (North Sea) fish (Zoarcidae). Journal of Experimental Biology 208. 2409-2420.

Torres J J, Somero G N (1988) Vertical distribution and metabolism in Antarctic mesopelagic fishes. Comparative Biochemistry and Physiology, B 90B(3): 521-528.

Ulleweit J (1995) Zur Ökologie zweier Standfischarten, der Aalmutter (*Zoarces viviparus*, L.) und des Butterfusches (*Pholis gunnellus*, L.) aus dem Niedersächsischen Wattenmeer. Diploma thesis, Fachbereich 2, Biologie /Chemie, Universität Bremen.

Van Dijk P L M, Tesch C, Hardewig I, Pörtner H O (1999) Physiological disturbances at

critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). J. Exp. Biol. 202(24): 3611-3621.

Walker T R (2005) Vertical organic inputs and bio-availability of carbon in an Antarctic coastal sediment. Polish Polar Research 26 , 91-106.

Wieser W (1986) Bioenergetik. Georg Thieme Verlag, Stuttgart, New York.

Zakhartsev M V, De Wachter B, Sartoris F J, Pörtner H-O, Blust R (2003) Thermal physiology of the common eelpout (*Zoarces viviparus*). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 173, 365-378.

Figure legends

Figure 1:

Experimental food intake (a), growth (b) and food conversion efficiency (c) of *Pachycara brachycephalum* (black bars and filled circles) and *Zoarces viviparus* (white bars and open circles). All values are mean values and the vertical bars depict the standard error (SEM). Food intake and growth are given in mass units (g/d). Food conversion efficiency was calculated from the measured energy equivalents (kJ) of gained weight and food. Significant differences are marked with asterisks.

Figure 2:

The hepatosomatic index (a) and the condition factor (b) of *Pachycara brachycephalum* (filled circles) and *Zoarces viviparus* (open circles) plotted over the temperature range of the experiment. Vertical bars show the standard error (SEM). Significant differences are marked with small letters, within species with "a", between species at overlapping temperatures with "b".

Figure 3a:

The respiration rate of *Pachycara brachycephalum* (black bars) and *Zoarces viviparus* (grey bars) long term acclimated at different experimental temperatures. The standard error (SEM) is expressed in vertical bars.

Figure 3b:

Arrhenius plot of individual respiration data of *Zoarces viviparus*). The solid line depicts a linear regression with the equation: $MO_2 = 10.44 - 3.93T$ (R^2=0.3923), the dotted line is the 95% confidence interval.

Figure 3c:

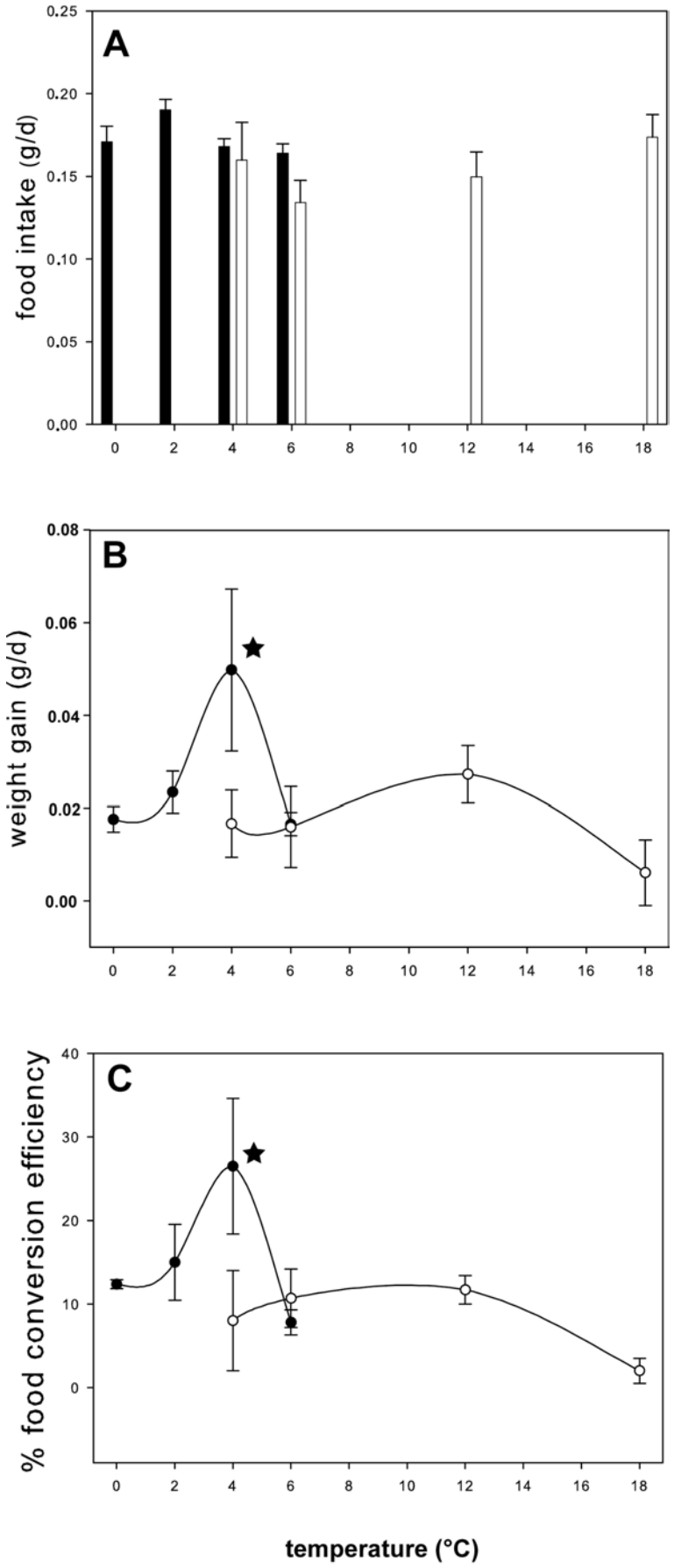
Arrhenius plot of individual respiration data of *Pachycara brachycephalum*. The solid line depicts a linear regression with the equation: $MO_2 = -2.37 - 0.49T$ (R^2=0.0033), the dotted line is the 95% confidence interval.

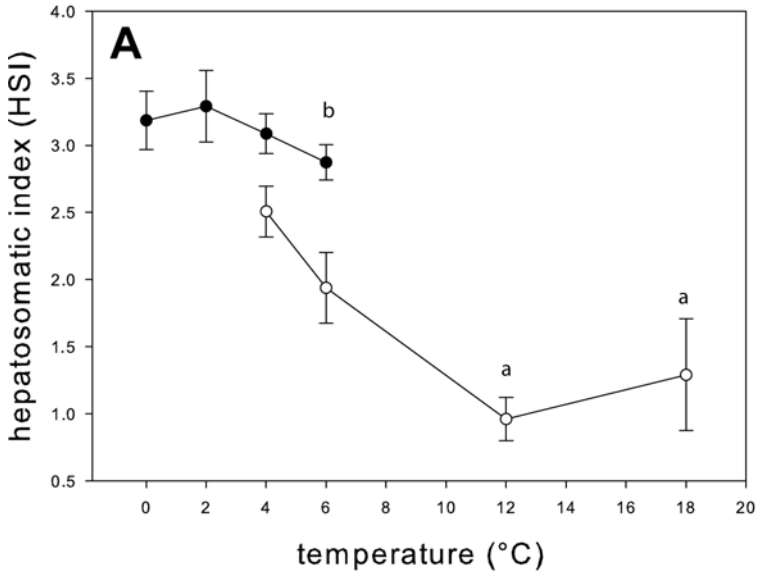
Figure 4:

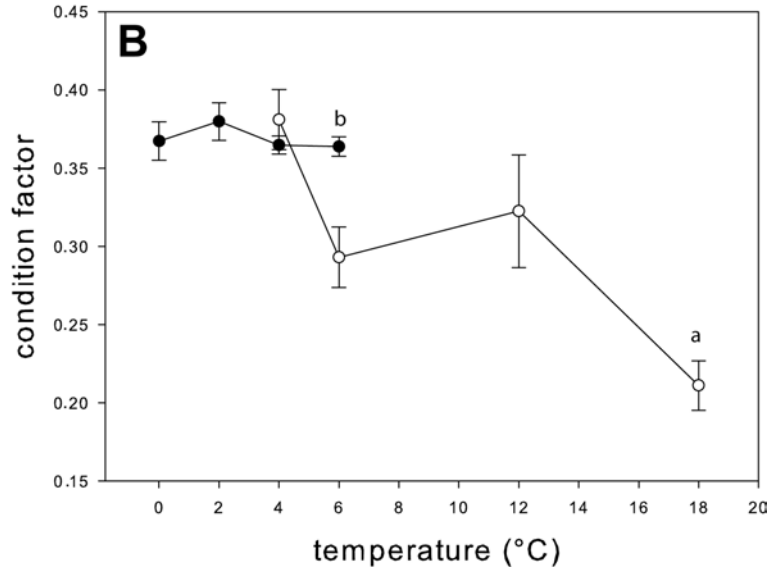
Energy budgets of *Pachycara brachycephalum* (a, c) and *Zoarces viviparus* (b, d) at different temperatures. The measured parameters were converted into energy units and

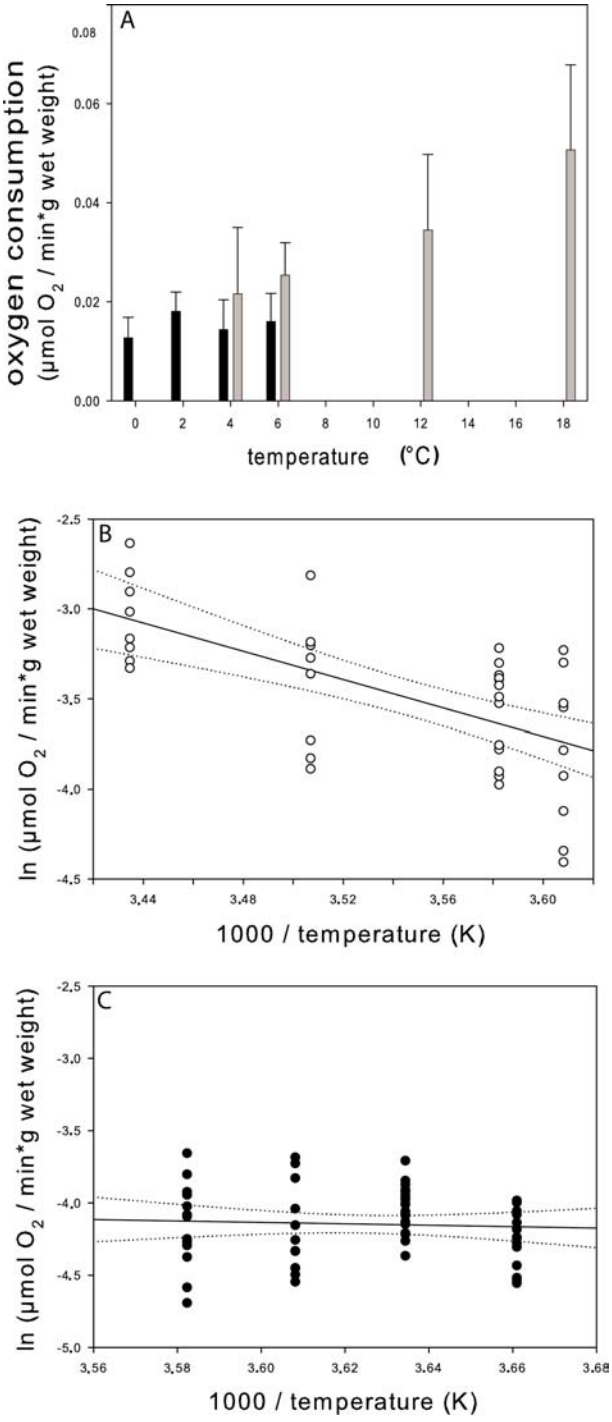
stacked as energy allocation or excretion processes. In graphs a and b the energy input (energy content of the ingested food) was set to 100% and the % fraction of the enlisted processes was plotted. Inset graphs c and d show the fractions of assimilated energy (without faecal deposition) normalized to a sum of 100%. In all graphs the standard error is expressed as vertical bars.

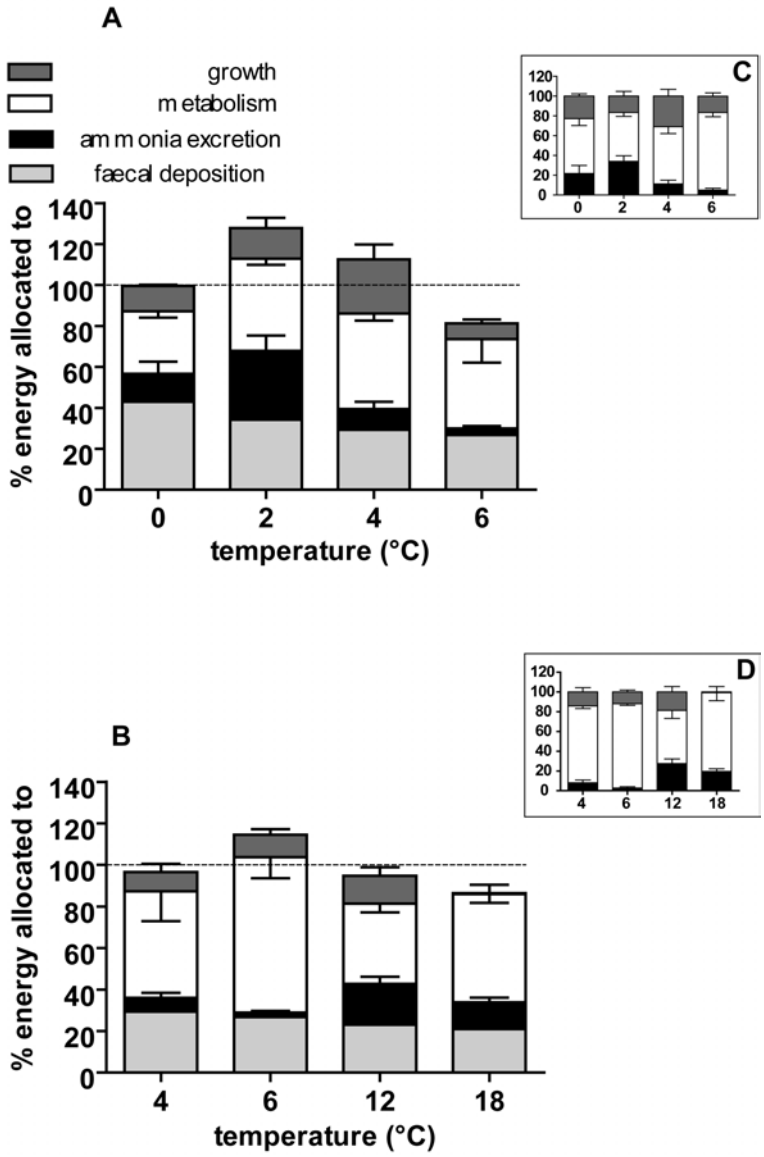
Figure 5:
The composition of *Pachycara brachycephalum* liver (a) and muscle (b) and of *Zoarces viviparus* liver (c) and muscle (d) tissue depending on acclimation temperature. Filled circles reflect protein levels, open circles reflect carbohydrate and filled triangles lipid levels. Values are mean fractions of tissue dry weight; vertical bars depict the standard error (SEM). Significant differences are marked by small letters (a= significant difference to 0 °C, b to 2 °C, c to 4 °C and d to 6 °C).


A
food intake (g/d)
0.25
0.20
0.15
0.10
0.05
0.00
0 2 4 6 8 10 12 14 16 18
B
weight gain (g/d)
0.08
0.06
0.04
0.02
0.00
0 2 4 6 8 10 12 14 16 18
C
% food conversion efficiency
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18
temperature (°C)


A
hepatosomatic index (HSI)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
b
a
a
0
2
4
6
8
10
12
14
16
18
20
temperature (°C)


B
condition factor
0.45
0.40
0.35
0.30
0.25
0.20
0.15
b
a
0
2
4
6
8
10
12
14
16
18
20
temperature (°C)


A
oxygen consumption
(µmol O2 / min*g wet weight)
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
12
14
16
18
temperature (°C)
B
ln (µmol O2 / min*g wet weight)
-2.5
-3.0
-3.5
-4.0
-4.5
3.44
3.48
3.52
3.56
3.60
1000 / temperature (K)
C
ln (µmol O2 / min*g wet weight)
-2.5
-3.0
-3.5
-4.0
-4.5
-5.0
3.56
3.58
3.60
3.62
3.64
3.66
3.68
1000 / temperature (K)


A
growth
metabolism
ammonia excretion
faecal deposition
% energy allocated to
140
120
100
80
60
40
20
0
0
2
4
6
temperature (°C)
C
120
100
80
60
40
20
0
0
2
4
6
B
% energy allocated to
140
120
100
80
60
40
20
0
4
6
12
18
temperature (°C)
D
120
100
80
60
40
20
0
4
6
12
18

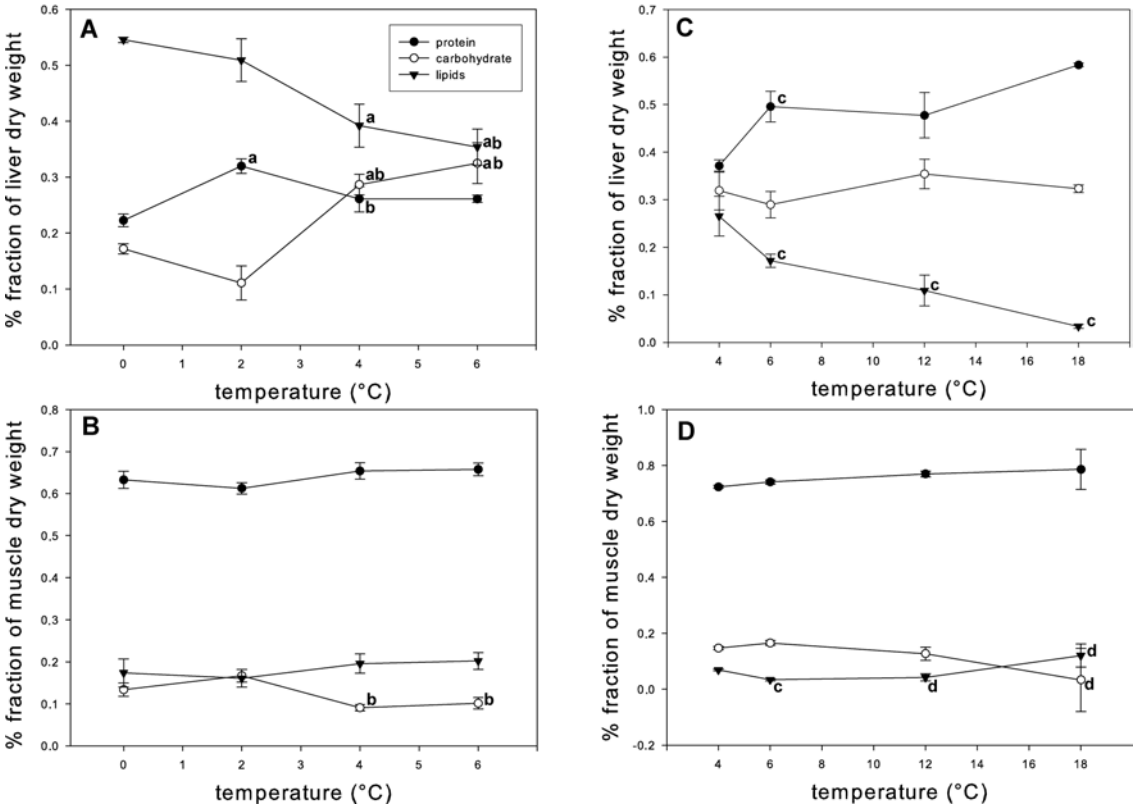

A
protein
carbohydrate
lipids
% fraction of liver dry weight
temperature (°C)
B
% fraction of muscle dry weight
temperature (°C)
C
% fraction of liver dry weight
temperature (°C)
D
% fraction of muscle dry weight
temperature (°C)

Table 1:

Growth rates in body length per year (experimental results compiled with literature and field data of selected fish species). The field growth data are calculated over specific age or size ranges to take the differences in ontogenetic growth rate into account. These age classes are indicated with asterisks (* age range 2 to 3 years and ** age range of 5 to 9 years).

species	data source	temperature [°C]	dL [cm/a]	SEM	source
Zoarces viviparus	laboratory	4	0.92	0.118	this paper
		6	1.73	0.247	
		12	1.21	0.569	
		18	1.76	0.532	
	laboratory	2	3.10	0.114	Fonds et al., 1989
		5	8.80	0.358	
		6	8.87	1.076	
		10	15.84	1.355	
		14	19.75	1.255	
		15	19.93	1.159	
		18	15.62	1.412	
		20	15.37	1.201	
		22	3.80	0.379	
	field	~ 3-24	2.78*	0.444	Brodte, 2001
	field	-	6.39*	2.738	Fonds et al., 1989
Pachycara brachycephalum	laboratory	0	1.74	0.255	this paper
		2	2.56	0.394	
		4	2.70	0.625	
		6	1.37	0.203	
	field	~ 0-0.6	1.58**	0.113	Brodte et al., 2006
Gadus morhua (North Sea)	laboratory	10	36.5	-	Fischer, 2003
	field	-	23.0	-	
Gobionotothen marionensis (Antarctic)	laboratory	-	0.47	-	North, 1998
	field	-	0.40	-	
Trematomus eulepidotus (Antarctic)	field	-1.9 - 0	1.52	-	Morales-Nin et al., 2001
Lycodes reticulatus (off Greenland)	field	> 0 - < 0	1.33	-	Dorrien, 1993
Lycodes reticulatus (Barents Sea)	field	< 0 - 6	1.18	-	Dorrien, 1993

Temperature dependent lipid levels and components in polar and temperate eelpout (Zoarcidae)

E. Brodte*[1], M. Graeve[2], U. Jacob[3], R. Knust[1] and H.-O. Pörtner[1]

*Corresponding author

Eva Brodte (corresponding author)

Alfred–Wegener–Institute for Polar- and Marine Research

Physiology of marine animals

Postfach 12 01 61

D - 27515 Bremerhaven

Germany

Telephone ++49 471 4831 2014

Fax ++49 471 4831 1048

ebrodte@awi-bremerhaven.de

Authors Affiliations:

Alfred–Wegener-Institut for Polar- and Marine Research, [1] Physiology of marine animals, [2] Chemical Ecology, and [3]Animal Ecology, D - 27515 Bremerhaven, FRG

Keywords

Antarctic; boreal; eurythermy; fatty acids; fish; fishes; lipid classes; stable isotopes; stenothermy; thermal; total lipid content

Abstract

For an analysis of the role of lipids in fishes from different thermal habitats, total lipid content, lipid classes and fatty acid composition were analysed in tissues from two eelpout species fed on the same diet, the Antarctic *Pachycara brachycephalum* and the temperate *Zoarces viviparus*. In liver of both species the lipid content increased with decreasing temperature, suggesting enhanced lipid storage in the cold. Lipid composition in liver and muscle from *P. brachycephalum* was strongly dominated by triacylglycerols at all temperatures between 0 and 6°C. Lipid class composition showed a change with temperature in the temperate species. When acclimatized to 4 and 6 °C *Z. viviparus* not only displayed a shift to lipid anabolism and pronounced lipid storage indicated by high triacylglycerol levels but also patterns of cold adaptation reflected by an increase of polyunsaturated fatty acids in the lipid extract. Unsaturated fatty acids were also abundant in the Antarctic eelpout, but when compared at the same temperatures *Z. viviparus* displayed significantly higher ratios of polyunsaturated to saturated fatty acid levels, whereas the Antarctic eelpout showed significantly higher ratios of monounsaturated to saturated fatty acid levels. High $\delta\ ^{15}N$ values of the Antarctic eelpout reflect the high trophic level of this scavenger in the Weddell Sea food web. Stable carbon values suggest that lipid enriched prey forms a major part of its diet. The strategy to accumulate storage lipids in the cold is interpreted to be adaptive at colder temperatures and during periods of irregular, pulsed food supply.

[1]Abbreviations: CHOL - cholesterol; DAGE - diacylglycerol ethers; FFA - free fatty acids; MUFA- monounsaturated fatty acids; PC – Phosphatidylcholine; PL - polar lipids; PUFA - polyunsaturated fatty acids; SFA - saturated fatty acids; TAG – triacylglycerols; UFA – unsaturated fatty acids; WE - wax esters.

Introduction

Lipids have different functions in marine organism. In fish, lipids are primarily used as storage deposits and as structural components, e.g. in cell membranes (Urich 1990). These uses are linked with requirements set by both metabolism and temperature adaptation. In general, carnivorous and omnivorous fish show an emphasis on protein and lipid metabolism as energy sources (Brett and Groves 1979). Seasonal shifts in the total lipid content of fishes reflect sexual maturation and reproduction state, but also food quantity and diet composition as well as temperature (Ackman 1989). The function of lipids is reflected in the lipid classes and fatty acids found. Neutral lipids, like triacylglycerols (TAG) and wax esters are mainly storage components. Therefore, the amount of TAGs varies during ontogeny, as seen during postlarval development of *Sebates jordani* (Norten et al. 2001). At the same time TAGs are the most responsive lipid class to changes in food uptake (Fraser et al. 1987). In contrast most polar lipids (PL) are structural components in membranes. Composition and ratios of PLs (especially of phosphatidyl ethanolamine and phosphatidyl choline) and the fatty acid composition affect the biophysical attributes of membranes (Hazel 1995).

The negative correlation between the content of unsaturated fatty acids (UFA) and environmental temperature has been confirmed in many fish species, both freshwater and marine (Ackman 1989). Adaptive changes in the fluidity of membranes through changing levels of components with temperature and ontogeny have been observed in common carp (Farkas et al. 2001).

An increased lipid accumulation from low to high latitudes is found in many species (Sidell 1991). The high lipid content of Antarctic fish might be related to a metabolic preference for lipid catabolism (Crockett and Sidell 1990) and anabolism (Pörtner 2002). A cold-induced shift to lipid anabolism at high mitochondrial densities and low metabolic rates may be the primary cause of high lipid levels in the cold especially in more active Antarctic ectotherms (cf. Pörtner 2002). Higher level functions evolved in due course, which took advantage of the high lipid levels present. These include neutral buoyancy, e.g. in the pelagic notothenioid *Pleuragramma antarcticum* (Eastman and DeVries 1982; Hubold 1985) or mere storage of lipids in support of survival during long periods of low food availability. Such trends were emphasized by a diet containing high amounts of lipids that would then accumulate in the predator (Wöhrmann 1998). High lipid levels also increase oxygen diffusion into cells and

between cell compartments (Egginton and Sidell 1989; Desaulniers et al. 1996, for review see Pörtner et al. 2005).

Comparisons of related species from various climates are suitable to elucidate the metabolic and functional background of these patterns. A recent comparison of con-familial eelpout species *Pachycara brachycephalum* (Antarctic) and *Zoarces viviparus* (boreal, temperate) demonstrated higher lipid contents in the Antarctic species (Brodte 2001). These species were chosen for further investigation of the role of temperature in setting the lipid composition of the whole organism. The present study was intended to compare the lipid composition of both species over a broad range of temperatures. The experimental temperatures reflect the thermal extremes in the habitat of the boreal eelpout and a temperature regime that includes high, potentially stressful temperatures (up to 6 °C) for the Antarctic eelpout.

Material and methods

Antarctic eelpout *P. brachycephalum* were caught with baited traps in Admiralty Bay (King George Island) near the Antarctic Peninsula between 360 and 460 m depth, with ambient water temperatures between 0 and 0.6 °C and salinity of 34.6 PSU (CTD data; Gerdes, Alfred-Wegener-Institute for Polar- and Marine Research, Bremerhaven, pers. comm.). Additionally, two individuals were caught in the Weddell Sea at 860 m depth and 0.2 °C. The North Sea eelpout *Z. viviparus* was caught with a beam trawl in the German Wadden Sea in the vicinity of the islands Spiekeroog and Langeoog at 1.5 to 5.0 m depth, ambient water temperatures between 17.5 to 19.5 °C and salinities of 30.6 to 31.4 PSU.

Individuals of both species with similar body lengths and weights were chosen to reduce the influence of allometry. The Antarctic eelpout *P. brachycephalum* displayed a mean total length of 20.8 cm (± 1.3 cm; SD) and mean body wet weight of 34.1 g (± 6.6 g; SD). The individuals of the North Sea eelpout *Z. viviparus* showed a somewhat lower mean total length (18.8 ± 3.0 cm; SD) and wet weight (22.3 ± 14.6 g; SD). The differences in length and weight were not significant. For both species males (six fishes for each of both species) as well as females (nine females of *P. brachycephalum*, eight of *Z. viviparus*, respectively) were analysed. Male fishes ranged between maturity stages I and III in both eelpout species. Female Antarctic eelpout displayed identical maturity stages I to III, whereas female North Sea eelpout were

mainly in a gonadal stage two months after release of the juveniles. Taking the different reproduction modi of the fishes into account (oviparity in the Antarctic zoarcid and viviparity in the North Sea eelpout) maturation stages were comparable. The average age of the fishes was 7 years (*P. brachycephalum*) and 2 years (*Z. viviparus*) (Brodte et al. 2006a; b).

Before starting the acclimation period animals were kept for three months at 10 °C (*Z. viviparus*) and at 0 °C (*P. brachycephalum*) to familiarize them with cockle meat (*Cerastoderma edule*) as a diet. Subsequently, specimens were kept for four months in seawater (salinity: 31.9 - 33.3 PSU) at temperatures of 0, 2, 4 and 6 °C for the Antarctic eelpout and of 4, 6, 12 and 18 °C for the North Sea eelpout. Experimental temperatures were kept constant at ± 0.4 °C and the fishes were fed ab libitum with cockle meat (*C. edule*), once every second day. To monitor the food intake the food portion of each fish as well as the unconsumed rests of the food were weighed. For lipid analyses, muscle and liver were carefully dissected, weighted, frozen in liquid nitrogen and transferred to separated glass vials filled with a mixture of dichlormethane:methanol (2:1; by vol.). These samples were deposited at –80 °C until lipid analysis. The tissue was lyophilised and, after determination of the dry weight, homogenised and extracted in dichlormethane:methanol (2:1; by vol.) after Folch et al. (1957). The total lipid content was determined gravimetrically.

Lipid classes were determined by high performance thin layer chromatography (HPTLC) densitometry (Olsen and Henderson 1989). Pre-coated HPTLC silica gel 60 plates (20x10 cm, Merck) were pre-developed in hexane:diethylether (1:1; by vol.) to remove impurities. The plates were then dried in a vacuum desiccator for 1 h and spotted with samples (duplicates) and standards. For calibration, the following standards were used in concentrations of 0.1, 1, 2, 5, 10 and 15 $\mu g*\mu l^{-1}$: phospholipids (phosphatidylcholine, PL), sterols (cholesterol, ST), free fatty acids (oleic acid, FFA), triacylglycerols (triolein, TAG), wax esters (oleic acid palmityl ester, WE) (all from Sigma) and 1-O-alkyldiacylglycerol ethers (DAGE) that were isolated from shark liver oil by preparative TLC. Five µl of sample extracts of muscle tissue, three µl of sample extract of liver tissue and five µl standard solutions were spotted on the HPTLC plates with a CAMAG-Linomat 4. The separation of lipid classes was performed in a CAMAG horizontal chamber with hexane:diethylether:acetic acid (80:20:2; by vol.). Thereafter, the plate was dried in a desiccator under vacuum for 30 min. Lipid classes

were visualised by submerging the plate in manganese (II)-chloride (4 H_2O), methanol and sulphuric acid reagent in a CAMAG immersion device for 5 sec followed by combustion at 120°C for 20 min. Quantification was carried out at 550 nm wavelength with a TLC Scanner (CAMAG 3) equipped with a wolfram lamp and combined with winCATS software.

Fatty acid composition was analysed by gas-liquid chromatography according to Kattner and Fricke (1986). Fatty acids of the total lipid extracts were converted to their methyl esters by transesterification for 4 hours in methanol containing 3% concentrated sulphuric acid at 80 °C. After extraction with hexane, fatty acid methyl esters were analysed with a Chrompack 9000 Series gas chromatograph with a DB-FFAP fused silica capillary column (30 m x 0.25 mm inner diameter, 0.25 µm film thickness) using temperature programming (160-240 °C at 4 °C min^{-1}, hold 15 min). For recording and integration, Class-VP software (4.3) (Shimadzu, Germany) was used. Fatty acids were identified with standard mixtures.

Subsequent to gravimetric analyses the total lipid content was determined by CHN analysis and stoichiometric calculations according to Gnaiger and Bitterlich (1984) using a larger number of individuals than for the gravimetric analyses. Tissue samples were lyophilised, the dry weight was determined and the samples were ground to fine powder and redried for at least three days at 60 °C in a drying closet before starting the CHN analysis with three replicates each (Euro EA 3000 series, Elemental Analyser, Euro Vector CHNS-O; Software: Callidus 1.0). The same procedure was applied to the ash samples of the tissue (burned to ash in a muffle oven at 580 °C for two hours) after redrying for at least three days.

For an analysis of food web relationships, stable isotope signatures were determined in *P. brachycephalum* and potential prey items. Samples of suspected prey items were collected along the eastern Weddell Sea shelf. The samples were stored at –30 °C, lyophilised for 24 hours and ground to a fine powder. Following the procedure by Jacob et al. (2005) the samples were acidified with 1 molar hydrochloric acid (HCl) to remove the $CaCO_3$. After redrying at 60 °C samples were ground again. Stable isotope analysis was conducted with an isotope-ratio mass spectrometer (Thermo/Finnigan Delta plus) by the GeoBioCenter in Munich.

Results were displayed as means and standard deviation of the mean (SD). For statistical analyses the results were tested using ANOVA (with a post hoc test for

unequal N) and t-tests (Statistica, Statsoft) and differences were considered significant at $p < 0.05$. The fatty acid composition of the fish tissues was analysed from individual values by principal component analysis (PCA) with the program Primer (version 5.2, PRIMER-E Ltd). The vast number of variables used in the PCA was limited to the twelve most abundant fatty acids. Therefore, the fatty acids were ranked in a cumulative way – over temperature and species. The results of the PCA were plotted using the two new coordinates (PC1 and PC2) describing the largest and the second largest fraction of the variance among samples.

Results

Bulk parameters

Water content in liver and muscle was stable over the experimental temperature range and did not differ significantly between the two species. The fraction of water was lower while the total lipid content was higher in liver than in muscle tissue (Figure 1a, b). In both tissues the mean values of the total lipid content were significantly higher for the Antarctic eelpout than for the North Sea eelpout, despite consumption of the same diet. In liver tissue the total lipid content of ash free dry weight decreased with increasing temperatures (Figure 1 a, b). The figure also shows the results from the CHN analyses. The two methods for determination of the total lipid content led to different results. In general, the stoichiometric determination via CHN analysis yielded, on average, 30% higher values than the gravimetric method.

Lipid classes

Although lipid classes were highly conserved, the Antarctic eelpout *P. brachycephalum* displayed minimum levels of PL and maximum levels of TAG and a low PL/TAG ratio in muscle tissue at 4 °C (Table 1). In liver tissue of the Antarctic eelpout the PL/TAG ratio was virtually independent of temperature with an insignificant maximum at 2 °C (Table 2). In contrast, *Z. viviparus* showed a distinct change in the PL and TAG content in both muscle and liver tissue with a drastic rise in the PL/TAG ratio at warmer temperatures (Table 1,2). Similarly, the CHOL/TAG ratio remained more or less unchanged in *P. brachycephalum* tissues whereas CHOL became more abundant than TAGs at higher temperatures in *Z. viviparus* muscle and liver associated with a drastic rise in CHOL/TAG ratios. Conversely, the highest contents of TAG were found in muscle and liver tissue of *Z. viviparus* at 4 and 6 °C (Table 1, 2).

Fatty acids

Dominant fatty acids in the muscle tissue of *P. brachycephalum* and *Z. viviparus* are shown in Table 3. Ratios of MUFA and PUFA levels over those of the saturated fatty acids were significantly different between both species at all temperatures (Figure 3). Within *P. brachycephalum* the UFA/SFA ratio displayed a significant maximum at 4 °C, but the PUFA / SFA ratio peaked at 0 °C. At 0 °C the amounts of ∑ n-3 and ∑ n-4 fatty acids displayed a significant maximum compared to the other experimental temperatures. In *Z. viviparus* the ∑ n-3 and ∑ n-7 fatty acids displayed a significant maximum at 4 °C, the ∑ n-4 fatty acids at 12 °C. When compared at same temperatures, MUFA/SFA ratios resulted lower and PUFA/SFA ratios higher for *Z. viviparus* than for *P. brachycephalum* (Figure 3a, b).

Abundant fatty acids in liver tissue for both species are listed in Table 4. In *Z. viviparus* liver tissue remarkable amounts of 22:6 (n-3) fatty acids were found at 12 and 18 °C. A trend to accumulate ∑ n-3 fatty acids upon warming was also seen in the Antarctic eelpout.

PCA

Principal component analysis of fatty acid composition was conducted separately for both tissues but each analysis included both species. In both tissues three groups were separated from each other. A difference between species was always detectable (Figure 4a, b). Two vectors explained 73 % (liver) and 78 % (muscle) of the components of the data. Therefore we were able to use both components (PC1 and PC2) to illustrate our data. The first coordinate PC1 (x-axis) separates the values due to species differences, the second (PC2, y-axis) due to temperature. In the plot of the liver tissue (Figure 4a) the most important fatty acids determined the PC1 and therefore the differences between species were characterized by 24:1(both isomers) and 16:1 (n-7) fatty acids. In the coordinate, which separates groups with different experimental temperatures (PC2) highest scores were reached by 22:5 (n-3) and 20:5 (n-3) fatty acids. The fatty acids display different levels between lower and higher temperatures for *Z. viviparus* (20:5 (n-3) and 22:5 (n-3)) and for *P. brachycephalum* (20:5 (n-3)). While the North Sea eelpout separated in two distinct groups at higher (12 and 18 °C) and "lower" (4 and 6 °C) temperatures, the Antarctic eelpout values showed no distinct group separation with respect to temperature. In liver tissue of *P. brachycephalum* just a trend is visible by

splitting the values in two fractions (4 with 6 °C, 0 with 2 °C, respectively). This reflects the results described for Tables 3 and 4.

In the PCA plot for muscle tissue (Figure 4b) three groups aggregate, but in contrast to liver values, the muscle data of the North Sea eelpout do not separate in two groups according to temperature. Nonetheless, the values for *Z. viviparus* show a slight temperature dependent shift along the y-axis. For the distinction between species along PC1 the fatty acids 16:1 (n-7) and 18:1 (n-9) are statistically the most important variables. For the temperature dependent shift in muscle composition (PC2) the two saturated fatty acids 18:0 and 16:0 are most important.

Stable isotopes

Because of missing analyses of stomach contents stable isotope analyses were used as an approximation to elucidate the position of *P. brachycephalum* in the Antarctic food web. Stable isotope ratios of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ are used as proxies for the trophic position. The stable isotope analyses of muscle tissue of *P. brachycephalum* revealed high $\delta\ ^{15}N$ values (13.8 ± 0.7 ppm; mean ± SD) and a $\delta\ ^{13}C$ of –24.0 ± 0.8 ppm (mean ± SD). The high $\delta\ ^{15}N$ value characterizes this eelpout as a scavenger and/or a consumer of scavengers. The molar C/N ratio was 6.4 ± 0.9 (mean ± SD) and considered high, indicating a high lipid content, which is in support of lipid analyses. The carbon isotope signatures of the Antarctic eelpout plotted against the C/N ratio place this species right in the middle of its potential prey items (Figure 2). The $\delta\ ^{13}C$ values and C/N-ratios also reflect a high lipid content of the potential diet (cf. Lipsky 2001).

Discussion

The quantification of the total lipid content led to 30% lower values with the gravimetric method, except for those samples with high amounts of sterols. With the gravimetric method we encountered problems during fully drying the lipids extracted from some samples. Even after unusually long drying periods under nitrogen some extracts were still viscous indicating incomplete removal of solvents. We therefore decided to also determine the total lipid content by CHN analysis. Data from CHN analyses and the subsequent stoichiometric calculations (Figure 1) are assumed to be more reliable, firstly because the technique is more specific at small tissue mass and secondly because the respective data set is based on a larger number of samples and replicates. The water content was always the same in liver and muscle tissues from

different temperature regimes and even the same between species. Therefore, the water content has no influence on the relative lipid content.

Although the temperate and the Antarctic species display similar life styles the ecological and physiological relevance of lipids and their functional roles appear to be quite different, likely due to temperature. The diet of the Antarctic eelpout is rich in lipids (Figure 2), while the diet of *Z. viviparus* in the Wadden Sea consists of fewer lipids and is more protein based with crustaceans (*Crangon crangon*) being predominant food components in summer and polychaetes in the winter months (Fonds et al. 1989; Ulleweit 1995). However, despite consumption of the same diet with the same lipid contents during laboratory maintenance, a three times larger lipid fraction was present in the Antarctic eelpout compared to the boreal eelpout (this study and Brodte 2001). The pronounced shift to lipid metabolism in many cold adapted Antarctic ectotherms is thus temperature driven and may be the result of an evolutionary shift to high mitochondrial densities and low metabolic rates in the cold (cf. Pörtner 2002). This is likely a unifying trend for many components of the food web, and thus lipid enrichment in Antarctic ectotherms is secondarily supported by high availability of a lipid-enriched diet.

In *Z. viviparus* the total lipid content of liver and muscle tissue was higher than seen in field samples from the same species by Pekkarinen (1980), indicating better food supply in our laboratory study (cf. Fonds et al. 1989; North 1998). On top of the difference between species, an increase in lipid content at lower temperatures could clearly be detected in liver of both species based on CHN analyses but not to the same extent in muscle. From a metabolic point of view muscle is a relatively inert tissue with much lower protein turnover rates than liver (Houlihan et al. 1988). Accordingly, temperature dependent shifts in lipid composition were not as pronounced as in liver tissue. In contrast, the liver displays higher turnover rates (Houlihan et al. 1988) and, usually, a high storage potential as for lipids (Larsen et al. 2001).

Patterns of lipid composition were different between investigated tissues and the two species at various acclimation temperatures. The lipid class fractionation should elucidate the functional roles of lipids (Norton et al. 2001) by separating the functional lipid groups into storage lipids (e.g. TAGs) and structural lipids (e.g. PLs). The changes in lipid composition in the boreal eelpout are in fact temperature related. The decrease of PLs and the drop of CHOL (Table 1 and 2) between 18 and 4 °C accompanied by an

increase in TAG values seem to reflect a thermally triggered metabolic change. In liver and muscle tissue of *Z. viviparus*, which are involved in storage functions (Pekkarinen 1980), we found the highest content of total lipid and of TAG at 4 and 6 °C, which might indicate a cold induced metabolic shift to the production of storage lipids. We assume that this shift reflects acclimatization to winter conditions in this eurythermal species.

The environment of the investigated Antarctic eelpout population is characterized by constant temperatures of 0.8 to –1.0 °C at the Antarctic Peninsula (CTD data; Gerdes, Alfred-Wegener-Institute for Polar- and Marine Research, Bremerhaven, pers. comm.). Elevated TAG levels in the Antarctic cold may also reflect enhanced levels of stored lipids but also lipid turnover in the cold. Capacities of enzymes involved in lipid catabolism were found higher in Antarctic fish than in temperate fishes (Crockett and Sidell 1990; Sidell and Hazel 2002). High activities of TAG lipase in Antarctic fishes (Sidell and Hazel 2002) promote the use of lipids as energy source.

The small response in the lipid class composition of the Antarctic eelpout at 2 and 4 °C might not necessarily be related to thermal compensation but rather to faster growth at these temperatures. In growing muscle tissue the cell volume might increase and result in a lower percent fraction of PL in total cell mass. Therefore, the low PL values in muscle tissue of *P. brachycephalum* (Table 1) may relate to maximum growth efficiency seen at 4 °C (Brodte et al. 2006b). In ^{13}P-NMR spectra of muscle tissue Bock et al. (2001) found high amounts of phosphomono- and –diesters in *P. brachycephalum* in contrast to *Z. viviparus*. They speculate the Antarctic eelpout might have higher FFA contents, reflecting a faster lipid turnover or greater lipase activity. We see no significant elevated FFA levels in muscle in the Antarctic eelpout compared to the boreal species (Table 1). High TAG values found in muscle tissue at 4 °C might again mirror enhanced accumulation of storage lipids supported by minimized baseline energy demand as observed at this temperature (Mark et al. 2005).

High lipid availability from the diet of Antarctic eelpout in its natural habitat is reflected by the δ^{13}C and C/N values. The relatively high δ^{15}N signatures of 13.8 ppm (Figure 2), which are similar to data for another Antarctic eelpout (*Lycodichthys dearborni*) (Burns et al. 1998), indicate a high trophic position within the Antarctic food web. The carbon isotope signatures of potential prey species as well as their molar C/N

ratios reflect the high lipid content (Figure 2). Lipids contain few nitrogen isotopes and the heavier carbon isotopes (Nyssen et al. 2002; 2005). A high C/N ratio and a low $\delta^{13}C$ value are thus used as indicators for high lipid contents.

Differences between the two fish species and their mode of life in stable and cold environments of the Antarctic versus wide annual temperature fluctuations of the Wadden Sea may also be reflected in the degree of fatty acid unsaturation. In general, unsaturated fatty acids seem to be more prominent in the Antarctic eelpout, but when compared at the same temperatures (4 and 6 °C); *Z. viviparus* displayed significantly higher PUFA to SFA ratios, whereas the Antarctic eelpout showed significantly higher MUFA to SFA ratios. *Z. viviparus* might be able to readily adapt to seasonal and fluctuating temperatures by increasing the amount of polyunsaturated and ∑ n-3 fatty acids, thereby displaying eurythermy characters (Pörtner 2004). Guderley et al. (1997) observed a seasonal difference with more polyunsaturated fatty acids in membrane lipids of trout in the fall compared to summer individuals. At "higher" temperatures of 4 and 6 °C *P. brachycephalum* emphasizes monounsaturated fatty acids instead of polyunsaturated fatty acids, possibly to support homoeviscous adaptation of membranes upon warming. Loss of PUFAs in the warmth may also serve the alleviation of oxidative stress (Jobling and Bendiksen 2003).

At first sight, the role of docosahexaenoic acid (DHA, 22:6 (n-3)) seems to also depend on the thermal environment of the fishes. However, high amounts of this fatty acid in liver and muscle of *P. brachycephalum* correlate with the temperature of highest growth (4 °C), indicating that the level of this fatty acid may be more closely influenced by growth than by temperature (Jangaard et al. 1967). Data by Farkas and co-workers (1994) and Grove and Sidell (2004) support this hypothesis by showing no participation of 22:6 (n-3) in the process of cold adaptation of phosphatidyl ethanolamine in membranes from more stenothermal fish. In eurythermal carp, however, Farkas et al. (1980) found that adaptation to cooling was paralleled by the accumulation of especially 22:6 (n-3). The eurythermal eelpout *Z. viviparus* seems to make use of this fatty acid as a modulator in membrane fluidity as well. In muscle tissue increasing amounts of this fatty acid were not found at maximum growth temperature (12 °C) (Brodte et al. 2006b), but at lower temperatures. During field investigations spring levels of 22:6 (n-3) in *Z. viviparus* liver, muscle and ovaries were found higher than the respective summer levels (Pekkarinen 1980). Guderley et al. (1997) observed an increase of 22:6

(n-3) contents in mitochondrial phospholipids contents in rainbow trout during cold acclimation, a finding that also emphasizes the importance of 22:6 (n-3) in membrane lipids in eurythermal fishes. From our data we conclude that the role of this fatty acid likely differs in eurythermal and stenothermal fishes. High levels of this lipid may contribute to the elevated cost of cold eurythermy and thus, patterns of metabolic cold adaptation seen in eurythermal ectotherms (Pörtner 2004).

Principal component analysis of investigated tissues showed different group separations between liver and muscle. For the eurythermal eelpout *Z. viviparus* the PCA analysis of the liver fatty acid profile shows distinct shifts with temperature. The temperature coordinate in the liver tissue plot is mainly determined by the fatty acids 22:5 (n-3) and 20:5 (n-3). These fatty acids change in the boreal eelpout between the two low (4 and 6 °C) and the two high (12 and 18 °C) temperatures with 22:5 (n-3) being more abundant at lower temperatures whereas the 20:5 (n-3) levels were elevated at 12 and 18 °C. This might be due to preferred synthesis of elongated fatty acids at lower temperatures. This feature is also known from fishes living in the permanent cold like the Antarctic demersal fish *Gobionotothen gibberifrons* (Grove and Sidell 2004), and might be a general mechanism of adaptation to cold. In skeletal and cardiac muscle tissue of this species Grove and Sidell (2004) also found high capacities for the oxidation of long chain fatty acids.

In the stenothermal Antarctic eelpout only the PCA of fatty acids in muscle showed temperature depended group separation. The most relevant fatty acids for this separation are16:0 and 18:0. Palmitic acid (16:0) is known to be involved in the process of cold adaptation of membranes (Farkas et al. 1994) and Grove and Sidell (2004) found very high activities and capacities for the oxidation for this fatty acid in Antarctic fish muscle

Conclusions

We conclude that in the eurythermal eelpout lower temperatures elicit higher levels of total lipids and TAGs and, therefore, an increased accumulation of lipids as storage compounds. The composition of membrane lipids shifts to enhanced use of 22:6 (n-3) fatty acids and thereby, may contribute to the cost of eurythermal cold adaptation. In contrast, temperature hardly influences the high lipid content and high TAGs values typifying the tissue of the stenothermal Antarctic eelpout. This polar fish has already

optimised its metabolism to colder temperatures and lipid enriched diet by emphasizing the use of monounsaturated over polyunsaturated fatty acids and by possessing low levels of polar lipids and cholesterol. For further conclusions future studies should separate lipids by origin (membrane or cytosol) to develop a clearer picture of changes in membrane and cytosolic functions.

Acknowledgements

The authors would like to thank Marco Böer for his support in the lipid class and fatty acid analyses and Dr. Ulrich Struck (GeoBioCenter, München) for analysing the stable isotope samples.

References

Ackman, R. G. 1989. Marine Biogenic Lipids, Vol. 1. CRC Press, Boca Raton, USA.

Bock, C., Sartoris, F., Wittig, R.-M., and Pörtner, H.-O. 2001. Temperature-dependent pH regulation in stenothermal Antarctic and eurythermal temperate eelpout (Zoarcidae): an in-vivo NMR study. Polar Biology 24: 869-874.

Brett, J. R. and Groves, T. D. 1979. Physiological energetics. Fish Physiology 8: 279-352.

Brodte, E. 2001. Wachstum und Fruchtbarkeit der Aalmutterarten *Zoarces viviparus* (Linne) und *Pachycara brachycephalum* (Pappenheim) aus unterschiedlichen klimatischen Regionen. diploma thesis in Biology, Universität Bremen, Fachbereich 2.

Brodte, E., Knust, R., Pörtner, H.O. and Arntz, W.E. 2006a. The biology of the Antarctic eelpout *Pachycara brachycephalum*. Deep-Sea Research II, in press.

Brodte, E., Knust, R., and Pörtner, H.-O. 2006b. Temperature dependent energy allocation to growth in Antarctic and boreal eelpout (Zoarcidae), Polar Biology, submitted.

Burns, J. M., Trumble, S. J., Castellini, M. A., and Testa, J. W. 1998. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biology 19(4): 272-282.

Crockett, E. L. and Sidell, B. D. 1990. Some pathways of energy metabolism are cold adapted in Antarctic fishes. Physiological Zoology 63(3): 472-488.

Desaulniers, N., Moerland, T. S., and Sidell, B. D. 1996. High lipid content enhances rate of oxygen diffusion through fish skeletal muscle. American Journal of Physiology 271: R42-R47.

Eastman, J. T. and DeVries, A. L. 1982. Buoyancy studies of notothenioid fishes in McMurdo Sound, Antarctica. Copeia 2: 385-393.

Egginton, S. and Sidell, B. D. 1989. Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. American Journal of Physiology 256(1): R1-R9.

Farkas, T., Csengeri, I., Majoros, F. and Olah, J. 1980. Metabolism of fatty acids in fish 3. combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linnaeus 1758. Aquaculture 20: 29-40.

Farkas, T., Dey, I., Buda, C. and Halver, J. E. 1994. Role of phospholipid molecular species in maintaining lipid membrane structure in response to temperature. Biophys. Chem 50: 147-155.

Farkas, T., Fodor, E., Kitajka, K., and Halver, J. E. 2001. Response of fish membranes to environmental temperature. Aquaculture Research 32(8): 645-655.

Folch, J., Lees, M., and Stanley, G. H. S. 1957. A simple method for isolation and purification of total lipid from animal tissue. Journal of Biological Chemistry 266: 467-509.

Fonds, M., Jaworski, A., Iedema, A., and Puyl, P. V. D. 1989. Metabolism, food consumption, growth and food conversion of shorthorn sculpin (*Myoxocephalus scorpius*) and eelpout (*Zoarces viviparus*). ICES council meeting papers:G:31, 19 pp.

Fraser, A. J., Sargent, J. R., Gamble, J. C., and MacLachlan, P. 1987. Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. AM. FISH. SOC. SYMP. SER. 2: 129-143.

Gnaiger, E. and Bitterlich, G. 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62: 289-298.

Grove, T. J. and Sidell, B. D. 2004. Fatty acyl CoA synthetase from Antarctic notothenioid fishes may influence substrate specificity of fat oxidation. Comparative Biochemistry and Physiology , Part B 139: 53-63.

Guderley, H., St.Pierre, J., Couture, P. and Hulbert, A. J. 1997. Plasticity of properties of mitochondria from rainbow trout red muscle with seasonal acclimatization. Fish Physiology and Biochemistry 16: 531-541.

Hazel, J. R. 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annual Reviews of Physiology 57: 19-42.

Houlihan, D. F., Hall, S. J., Gray, C., and Noble, B. S. 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences 45(6): 951-964.

Hubold, G. 1985. Krill and fishes in the Antarctic ecosystems. J. IOUSP. 3(8-11): 2-4.

Jacob, U., Mintenbeck, K., Brey, T., Knust, R. and Beyer, K. 2005. Stable isotope food web studies: a case for standardized sample treatment. Marine Ecology-Progress Series 287: 251-253.

Jangaard, P. M., Ackman, R. G., and Sipos, J. C. 1967. Seasonal changes in fatty acid composition of cod liver, flesh, roe, and milt lipids. Journal of Fisheries Research Board of Canada 24: 613-627.

Jobling, M. and Bendiksen, A. A. 2003. Dietary lipids and temperature interact to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr. Aquaculture Research 34: 1423-1441.

Kattner, G. and Fricke, H. S. G. 1986. Simple gas-liquid chromatographic method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. Journal of Chromatographie 361: 263-268.

Larsen, D. A., Beckman, B. R., and Dickhoff, W. W. 2001. The effect of low temperature and fasting during the winter on growth and smoltification of Coho Salmon. North American Journal of Aquaculture 63(1): 1-10.

Lipski, J. 2001. AMLR 2000/2001 Field season report - Objectives , accomplishment and tentative conclusions. 187pp. NOAA-TM-NMFS-SWFSC-314, Southwest Fisheries Science Center - Antarctic Ecosystems Research Division.

Mark, F. C., Hirse, T., and Pörtner, H.-O. 2005. Thermal sensitivity of cellular energy budgets in Antarctic fish hepatocytes. Polar Biology 28: 805-814.

North, A. W. 1998. Growth of young fish during winter and summer at South Georgia. Polar Biology 19: 198-205.

Norton, E. C., Macfarlane, R. B., and Mohr, M. S. 2001. Lipid class dynamics during development in early life stages of shortbelly rockfish and their application to condition assessment. Journal of Fish Biology 58(4): 1010-1024.

Nyssen, F., Brey, T., Dauby, P. and Graeve, M. 2005. Trophic position of Antarctic amphipods - enhanced analysis by a 2- dimensional biomarker assay. Marine Ecology Progress Series 300: 135-145.

Nyssen, F., Brey, T., Lepoint, G., Bouquegneau, J. M., DeBroyer, C. and Dauby, P. 2002. A stable isotope approach to the eastern Weddell Sea trophic web: focus on benthic amphipods. Polar Biology 25: 280-287.

Olsen, R. E. and Henderson, R. J. 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. Journal of Experimental Marine Biology and Ecology 129(2): 189-197.

Pekkarinen, M. 1980. Seasonal variations in lipid content and fatty acids in the liver, muscle and gonads of the eel-pout, *Zoarces viviparus* (Teleostei) in brackish water. Ann. Zool. Fenn. 17(4): 249-254.

Pörtner, H. O. 2002. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. Journal of Experimental Biology 205(15): 2217-2230.

Pörtner, H.O. 2004. Climate variability and the energetic pathways of evolution: the origin of endothermy in mammals and birds. Physiological and Biochemical Zoology 77, 959-981.

Pörtner H.O., Lucassen M., Storch D. 2005. Metabolic biochemistry: its role in thermal tolerance and in the capacities of physiological and ecological function. In *The Physiology of Polar Fishes* (Guest editors: J. F. Steffensen and A. P. Farrell) Fish Physiology Volume 22 - Series editors D.R. Randall and A.P. Farrell.

Sidell, B. D. 1991. In: Biology of Antarctic Fish. pp. 220-231. Edited by Di Prisco, G., Maresca, B. and Tota, B. Springer Verlag. Berlin, Heidelberg, New York.

Sidell, B. D. and Hazel, J. R. 2002. Triacylglycerol lipase activities in tissue of Antarctic fishes. Polar Biology 25: 517-522.

Ulleweit, J. 1995. Zur Ökologie zweier Standfischarten, der Aalmutter (*Zoarces viviparus*, L.) und des Butterfusches (*Pholis gunnellus*, L.) aus dem Niedersächsischen Wattenmeer. Diploma thesis biology, Fachbereich 2*,* Universität Bremen*.*

Urich, K. 1990. Vergleichende Biochemie der Tiere. Gustav Fischer Verlag. Stuttgart, New York.

Wöhrmann, A. P. A. 1998. In: Fishes of Antarctica - A Biological Overview. pp. 119-128. Edited by Di Prisco, G., Pisano, E. and Clarke, A. Sprinter. Milano, Italia.

Figure legends

Figure 1:

Total lipid content in liver (a) and muscle (b) tissue of the Antarctic (*P. brachycephalum*, black bars) and boreal eelpout (*Z. viviparus*, light grey bars). The total lipid content was determined by CHN analyses with subsequent stochiometric calculations. Values are means and standard deviation shown as vertical bars (15 replicates per temperature and species).

Figure 2

Stable carbon isotope signatures of *P. brachycephalum* (⬢) and of suspected prey (open symbols) are plotted against the respective molar C/N ratios. High C/N ratios indicate high lipid content of the investigated species. The position of the eelpout as a predator or scavenger is reflected in the quantities of lipid enriched prey species in its diet (data for prey species: U. Jacob and K. Mintenbeck, Alfred-Wegener-Institute for Polar- and Marine Research, Bremerhaven, unpublished data).

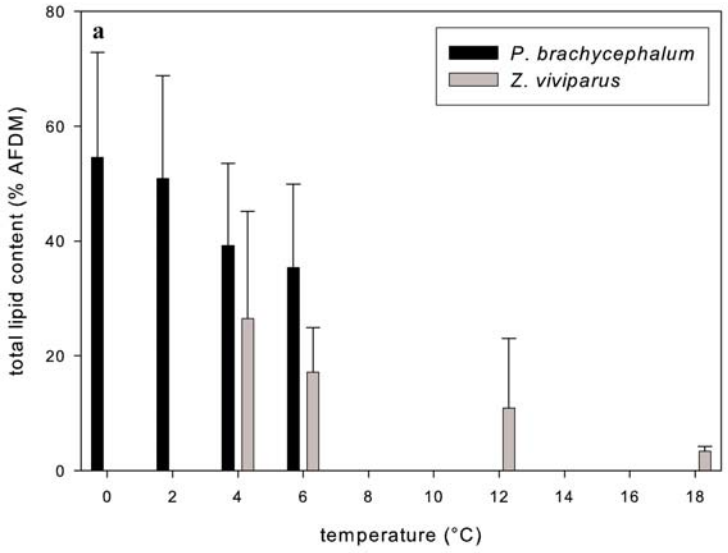
Figure 3

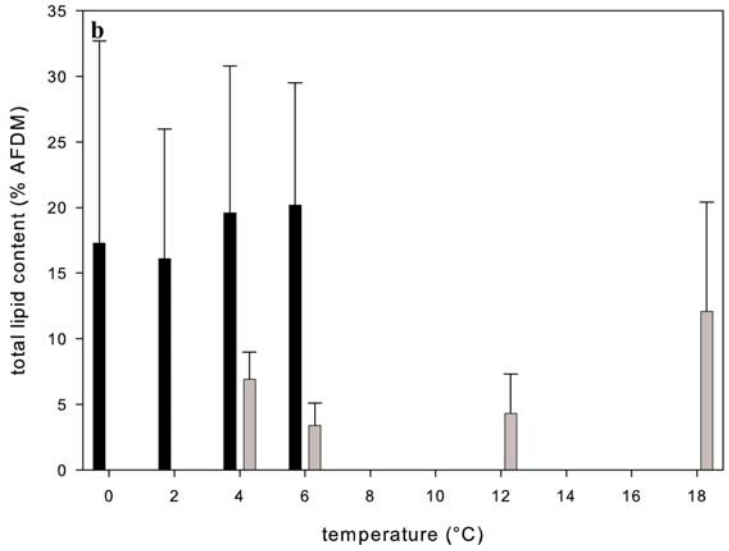
The ratios of unsaturated over saturated fatty acid levels in muscle tissue of *P. brachycephalum* (2a) and *Z. viviparus* (2b). Asterisks indicate significant differences in *Z. viviparus*, when data at higher (12 and 18 °C) and lower (4 and 6 °C) experimental temperatures were grouped and compared. Mean values and standard deviations are displayed.

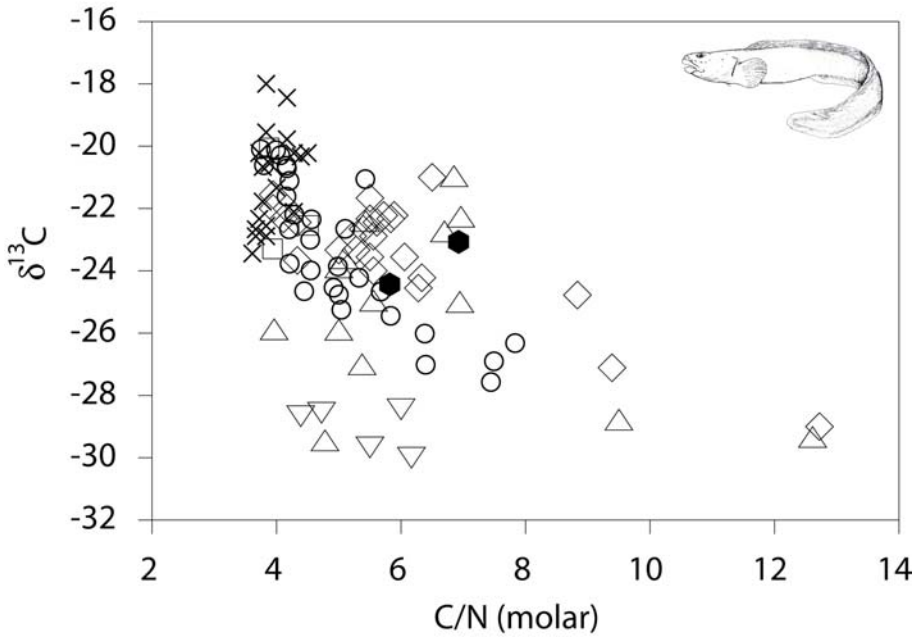
Figure 4

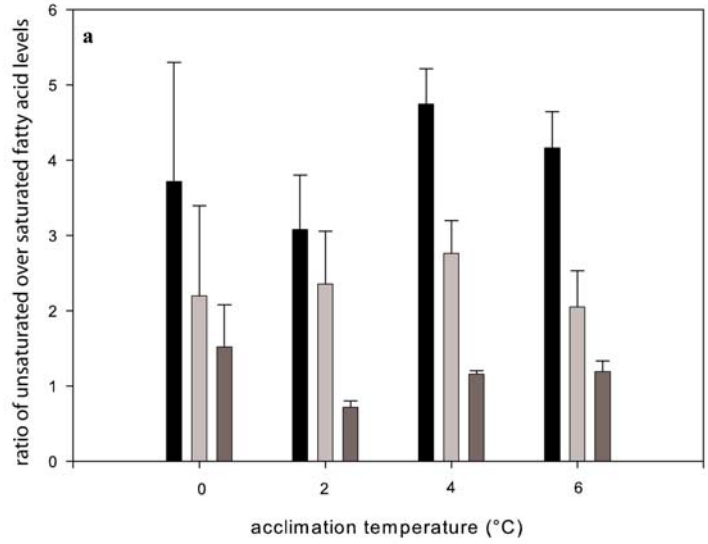
Principal component analysis (PCA) of the most important fatty acids in liver tissue (a) and in muscle tissue (b) of both eelpout species at experimental temperatures. The PCA was conducted with individual values for each of the twelve most important fatty acids (see material and methods). The triangles mark values from the North Sea eelpout (*Z. viviparus*), the rectangles those from the Antarctic eelpout (*P. brachycephalum*). Closed symbols represent the temperatures of 4 and 6 °C, while the open symbols stand for the higher temperatures (12 and 18 °C) in the North Sea eelpout and the lower temperatures (0 and 2 °C) in the Antarctic eelpout. In both figures (a and b) the principal component PC1 separates the values into species-specific groups. For liver tissue of the North Sea

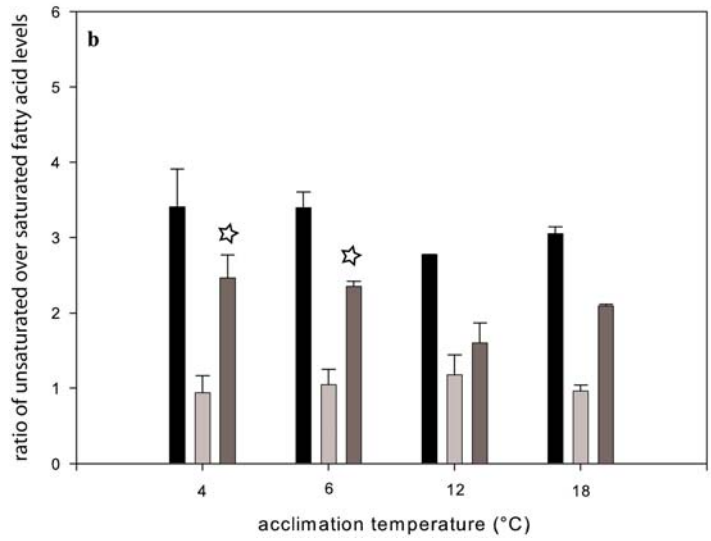
eelpout the temperature effect represented by the second principal component (PC2) discerns one group at lower experimental temperatures and another one at higher temperatures, due to the fatty acids 22:5 (n-3) and 20:5 (n-3). In figure 3b, PC 2 also represents the temperature dependent difference in the samples, but a clear group separation is detectable only in the Antarctic eelpout (for further explanation see text).


a
P. brachycephalum
Z. viviparus
total lipid content (% AFDM)
temperature (°C)
0
20
40
60
80
0
2
4
6
8
10
12
14
16
18


b
total lipid content (% AFDM)
temperature (°C)
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
14
16
18


-16
-18
-20
-22
-24
-26
-28
-30
-32
δ13C
2
4
6
8
10
12
14
C/N (molar)


a
ratio of unsaturated over saturated fatty acid levels
0
1
2
3
4
5
6
0
2
4
6
acclimation temperature (°C)


b
ratio of unsaturated over saturated fatty acid levels
0
1
2
3
4
5
6
4
6
12
18
acclimation temperature (°C)

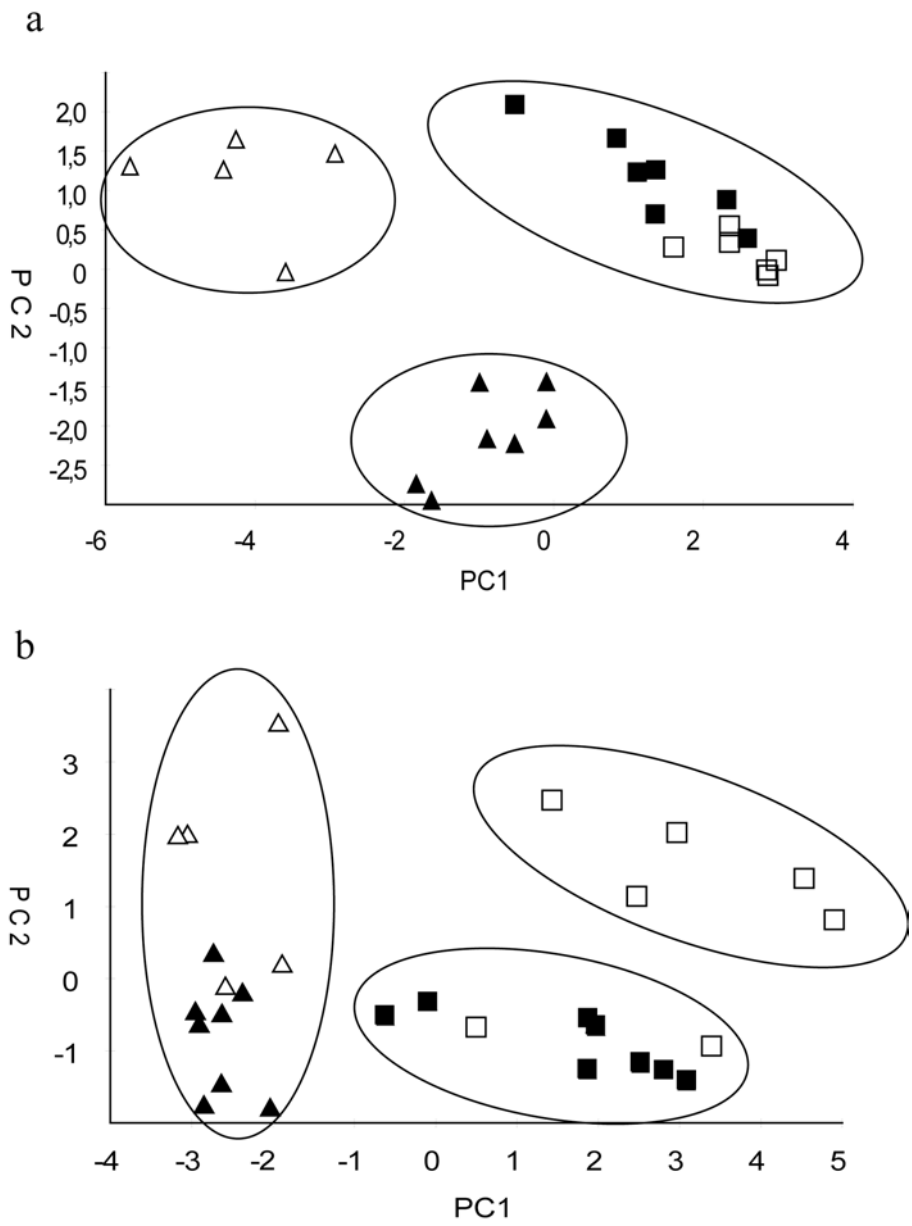

a
2,0
1,5
1,0
0,5
0
-0,5
-1,0
-1,5
-2,0
-2,5
PC 2
-6
-4
-2
0
2
4
PC1
b
3
2
1
0
-1
PC 2
-4
-3
-2
-1
0
1
2
3
4
5
PC1

Table 1
Lipid class composition of the muscle tissue of *P. brachycephalum* and *Z. viviparus* as well as of the diet (*Cerastoderma edule,* mean±S.D.). (PL = polar lipids, CHOL = cholesterol, FFA = free fatty acids, TAG = triacylglycerols, DAGE = diacylglycerol ethers, WE = wax esters). Asterisks indicate intra-specific significant differences between temperatures.

Species	***Cerastoderma edule* (diet)**	***Pachycara brachycephalum***				***Zoarces viviparus***			
Temperature	**-**	**0 °C**	**2 °C**	**4 °C**	**6 °C**	**4 °C**	**6 °C**	**12 °C**	**18 °C**
Lipid class	**n = 4**	**n = 4**	**n = 4**	**n = 4**	**n = 4**	**n = 4**	**n = 4**	**n = 3**	**n = 4**
PL	12.9 ± 3.3	30.5 ± 12.3	13.6 ± 8.1	**7.9 ± 2.2***	22.1 ± 9.7	45.6 ± 14.5	**65.1 ± 2.9***	54.6 ± 4.1	52.9 ± 1.8
CHOL	5.4 ± 1.0	4.4 ± 3.5	**1.5 ± 1.0***	**2.2 ± 0.3***	5.5 ± 2.3	**9.5 ± 2.1***	18.3 ± 3.9	16.7 ± 0.8	**24.9 ± 5.1***
FFA	0.9 ± 0.2	3.8 ± 2.1	0. 7 ± 0.7	0.2 ± 0.0	0.5 ± 0.4	0.3 ± 0.3	0.1 ± 0.2	0.7 ± 1.0	2.9 ± 5.7
TAG	79.1 ± 4.6	60.3 ± 16.2	84.1 ± 9.0	86.7 ± 3.3	67.0 ± 13.3	**37.8 ± 14.4***	10.3 ± 5.0	0.0 ± 0.0	1.3 ± 1.9
DAGE	0.7 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
WE /Sterols	1.0 ± 0.1	1.0 ± 0.5	0.2 ± 0.2	2.7 ± 1.0	4.6 ± 1.1	6.8 ± 4.5	6.2 ± 3.7	27.9 ± 2.3	18.3 ± 4.8
PL:TAG ratio	0.2 ± 0.1	0.68 ± 0.4	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	**1.5 ± 0.9***	**7.5 ± 3.3***	13.3 ± 2.6	197.6 ± 191.7
CHOL:TAG ratio	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	**0.3 ± 0.2***	**2.3 ± 1.4***	4.3 ± 1.0	102.7 ± 101.7

Table 2
The lipid class composition of liver tissue of the Antarctic eelpout, *Pachycara brachycephalum*, and the boreal eelpout, *Zoarces viviparus*. Values are means with standard deviation. The following abbreviations were used: PL = polar lipids, CHOL = cholesterol, FFA = free fatty acids, TAG = triacylglycerols, DAGE = diacylglycerol ethers and WE = wax esters. Asterisks indicate intra-specific significant differences between temperatures.

Species	Pachycara brachycephalum				Zoarces viviparus			
Temperature	**0 °C**	**2 °C**	**4 °C**	**6 °C**	**4 °C**	**6 °C**	**12 °C**	**18 °C**
Lipid class	**n = 4**	**n = 4**	**n = 4**	**n = 5**	**n = 5**	**n = 4**	**n = 3**	**n = 3**
PL	7.8 ± 3.3	14.6 ± 8.2	10.1 ± 3.9	9.7 ± 2.2	26.7 ± 7.5	20.8 ± 16.8	33.05 ± 20.4	**63.8 ± 12.1***
CHOL	1.7 ± 0.8	1.6 ± 1.6	3.6 ± 1.5	3.1 ± 0.8	4.9 ± 0.8	3.5 ± 1.3	7.0 ± 3.9	9.5 ± 5.1
FFA	7.0 ± 1.4	12.5 ± 6.5	0.1 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	2. 7 ± 2.3	2.4 ± 1.4
TAG	82.5 ± 4.3	70.7 ± 15.9	83.0 ± 6.0	83.9 ± 3.9	63.3 ± 8.1	63.4 ± 14.6	**5.6 ± 9.7***	**2.0 ± 1.7***
DAGE	0.8 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.7	0.0 ± 0.0
WE /Sterols	0.2 ± 0.3	0.6 ± 0.5	3.2 ± 1.7	3.1 ± 1.7	5.5 ± 2.4	12.3 ± 16.3	51.3 ± 16.1	22.4 ± 15.8
PL:TAG ratio	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.4 ± 0.3	0.6 ± 0.1	**55.8 ± 52.5***
CHOL:TAG ratio	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	**10.3 ± 12.4***

Table 3
Fatty acid composition of the diet and of the muscle tissue in *Pachycara brachycephalum* and *Zoarces viviparus* at different experimental temperatures (mass% of fatty acids (mean± S.D.)) Asterisks indicate intra-specific significant differences between temperatures. Numbers in *italics* indicate differences between species at same temperatures.

Species	***Cerastoderma edule (diet)***	***Pachycara brachycephalum***				***Zoarces viviparus***			
Temperature	**-**	**0 °C**	**2 °C**	**4 °C**	**6 °C**	**4 °C**	**6 °C**	**12 °C**	**18 °C**
Fatty acid	**n=3**	**n = 4**	**n = 4**	**n = 4**	**n = 5**	**n = 4**	**n = 4**	**n = 3**	**n = 4**
14:0	2.8 ± 0.2	3.1 ± 1.0	3.8 ± 0.3	2.8 ± 0.3	2.0 ± 0.6	1.0 ± 0.3	0.7 ± 0.1	1.0 ± 0.3	0.7 ± 0.1
15:0	0.6 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
16:0	18.2 ± 1.0	14.8 ± 4.4	16.2 ± 2.8	11.6 ± 0.9	13.6 ± 1.5	16.0 ± 1.8	15.1 ± 1.4	17.8 ± 0.2	17.0 ± 0.5
16:1(n-7)	7.5 ± 01.0	12.2 ± 3.8	14.5 ± 2.1	13.9 ± 2.4	9.4 ± 2.8	4.0 ± 0.9	3.6 ± 0.6	3.7 ± 0.9	2.3 ± 0.3
16:1(n-5)	-	0.7 ± 0.3	0.2 ± 0.0	-	-	-	-	0.7 ± 0.2	0.7 ± 0.2
16:2(n-4)	1.3 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.2
16:3(n-4)	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	1.8 ± 1.6	1.5 ± 0.2
18:0	6.8 ± 0.4	4.5 ± 2.0	3.8 ± 1.2	2.6 ± 0.4	3.5 ± 0.7	4.4 ± 0.5	5.4 ± 0.3	6.3 ± 1.2	6.4 ± 0.2
18:1(n-9)	2.7 ± 0.6	22.1 ± 7.4	31.6 ± 5.6	24.4 ± 4.5	21.2 ± 3.1	10.6 ± 1.4	11.6 ± 2.1	15.4 ± 5.2	11.8 ± 0.5
18:1(n-7)	5.8 ± 0.6	5.7 ± 0.5	6.1 ± 0.4	6.5 ± 0.5	6.0 ± 0.5	5.1 ± 0.6	5.4 ± 0.7	4.5 ± 1.2	4.3 ± 0.7
18:2(n-6)	0.4 ± 0.1	0.6 ± 0.1	1.0 ± 0.2	1.4 ± 0.3	0.9 ± 0.3	0.6 ± 0.1	1.0 ± 0.5	0.7 ± 0.3	1.0 ± 0.5
18:2(n-4)	1.9 ± 0.3	0.8 ± 0.3	0.6 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
18:3(n-4)	1.3 ± 0.5	0.6 ± 0.2	0.5 ± 0.1	-	-	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.2 ± 0.0
18:4(n-3)	3.2 ± 0.3	1.1 ± 0.2	0.7 ± 0.0	1.2 ± 0.3	0.8 ± 0.3	0.0 ± 0.0	-	0.3 ± 0.2	0.1 ± 0.1
20:1(n-9)	2.5 ± 0.3	2.0 ± 0.5	2.6 ± 0.2	0.2 ± 0.1	1.7 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	1.3 ± 0.1	1.0 ± 0.2
20:1(n-7)	2.3 ± 0.2	1.0 ± 0.4	1.0 ± 0.1	0.5 ± 0.5	-	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.3	0.2 ± 0.1
20:4(n-6)	2.0 ± 0.4	3.0 ± 1.8	1.4 ± 0.6	2.8 ± 0.5	4.8 ± 1.6	6.0 ± 0.9	8.5 ± 3.3	2.8 ± 4.0	9.8 ± 1.7
20:4(n-3)	1.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.7	0.7 ± 0.5
20:5(n-3)	30.2 ± 4.0	13.8 ± 2.8	6.8 ± 1.7	14.4 ± 1.5	17.9 ± 2.8	21.3 ± 1.6	20.0 ± 2.7	18.9 ± 1.3	18.6 ± 1.0
22:5(n-3)	2.9 ± 0.5	1.6 ± 0.2	0.9 ± 0.3	1.8 ± 0.2	2.1 ± 0.1	2.8 ± 0.7	3.1 ± 1.1	2.6 ± 0.9	2.3 ± 1.1
24:1(n-?)		0.3 ± 0.3	0.3 ± 0.3	0.5 ± 0.1	1.0 ± 0.3	0.7 ± 0.8	2.5 ± 1.2	4.3 ± 4.0	2.8 ± 2.4
22:6(n-3)	8.3 ± 1.3	7.0 ± 2.8	3.0 ± 0.7	8.8 ± 0.8	11.4 ± 1.7	22.1 ± 4.2	18.0 ± 4.0	12.1 ± 3.6	15.6 ± 2.9
n-3 group	46.3 ± 5.6	24.6 ± 5.7	12.2 ± 2.6	12.8 ± 0.8	14.9 ± 1.4	47.2 ± 3.0	41.9 ± 6.5	35.2 ± 4.8	37.7 ± 3.0
n-4 group	5.6 ± 0.3	3.1 ± 0.71	2.5 ± 0.3	2.4 ± 0.4	2.0 ± 0.4	2.0 ± 0.4	1.8 ± 0.2	2.9 ± 1.7	2.6 ± 0.1
n-6 group	2.8 ± 0.9	4.3 ± 1.8	3.1 ± 0.6	4.7 ± 0.2	6.2 ± 1.4	6.8 ± 0.9	9.6 ± 3.7	4.3 ± 4.0	11.4 ± 2.1
n-7 group	15.5 ± 1.7	18.9 ± 3.3	21.6 ± 1.6	20.8 ± 3.3	15.4 ± 3.0	9.5 ± 1.2	9.3 ± 1.2	8.6 ± 2.4	6.7 ± 0.8
SFA	28.7 ± 1.9	23.0 ± 7.4	25.0 ± 4.3	17.5 ± 1.4	19.5 ± 1.7	22.9 ± 2.5	22.8 ± 1.1	26.5 ± 0.0	24.7 ± 0.5
UFA	19.8 ± 1.2	77.0 ± 7.4	75.0 ± 4.3	82.5 ± 1.4	80.5 ± 1.7	77.1 ± 2.5	77.2 ± 1.1	73.3 ± 0.0	75.3 ± 0.5
PUFA	54.8 ± 6.7	32.4 ± 7.1	18.0± 3.4	20.3 ± 0.9	23.2 ± 2.3	55.9 ± 1.9	53.5 ± 2.7	42.1 ± 7.0	51.6 ± 1.0
UFA:SFA	0.7 ± 0.0	3.7 ± 1.6	3.1 ± 0.7	4.7 ± 0.5	4.2 ± 0.5	3.4 ± 0.5	3.4 ± 0.2	2.8 ± 0.0	3.1 ± 0.1
PUFA:SFA	1.8 ± 0.3	1.5 ± 0.6	0.7 ± 0.1	*1.2 ± 0.0*	*1.2 ± 0.1*	*2.5 ± 0.3*	*2.4 ± 0.1*	**1.6 ± 0.0***	**2.1 ± 0.0***

(-)= not detected or traces

Table 4
Fatty acid composition of the liver tissue of *P. brachycephalum* and *Z. viviparus* at different experimental temperatures. (mass% of fatty acids (mean± S.D.)) Asterisks indicate intra-specific significant differences between temperatures.

Species	***Pachycara brachycephalum***			***Zoarces viviparus***				
Temperature	**0 °C**	**2 °C**	**4 °C**	**6 °C**	**4 °C**	**6 °C**	**12 °C**	**18 °C**
Fatty acid	**n = 4**	**n = 4**	**n = 4**	**n = 5**	**n = 4**	**n = 4**	**n = 3**	**n = 4**
14:0	1.5 ± 0.3	1.8 ± 0.2	2.1 ± 0.6	2.3 ± 0.6	0.8 ± 0.5	1.5 ± 0.4	0.3 ± 0.4	0.9 ± 0.3
14:1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.2	0.5 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	-	-
16:0	8.9 ± 2.6	10.3 ± 2.5	9.6 ± 2.4	13.4 ± 0.9	13.5 ± 1.5	14.1 ± 2.2	17.8 ± 0.8	17.1 ± 4.5
16:1(n-7)	17.9 ± 1.9	14.9± 2.7	14.1 ± 4.0	20.9 ± 3.9	8.1 ± 3.5	11.5 ± 3.4	1.4 ± 0.3	4.8 ± 0.8
16:1(n-5)	0.2 ± 0.2	0.2 ± 0.2	-	-	-	-	0.8 ± 0.0	1.2 ± 0.3
16:2(n-4)	0.3 ± 0.0	0.3 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	2.0 ± 0.1	2.0 ± 0.6	4.6 ± 0.8	4.9 ± 2.7
16:3(n-4)	0.4 ± 0.1	0.4 ± 0.1	1.1 ± 0.3	1.0 ± 0.0	0.9 ± 0.2	0.9 ± 0.4	0.6 ± 0.2	0.7 ± 0.1
18:0	2.8 ± 0.5	3.5 ± 1.1	4.1 ± 2.7	4.2 ± 0.2	4.7 ± 0.2	4.2 ± 0.6	7.3 ± 0.2	7.3 ± 2.3
18:1(n-9)	62.4 ± 6.1	60.7 ± 2.1	34.0 ± 7.7	27.9 ± 6.4	15.2 ± 2.4	24.4 ± 3.5	7.7 ± 0.2	17.0 ± 0.5
18:1(n-7)	0.2 ± 0.0	0.2 ± 0.2	7.1 ± 1.0	6.1 ± 0.5	6.7 ± 1.0	6.1 ± 1.2	2.4 ± 0.2	4.1 ± 1.0
18:2(n-6)	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	1.0 ± 0.9	0.5 ± 0.1	0.6 ± 0.2	0.9 ± 0.3	0.6 ± 0.3
18:2(n-4)	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.2	0.6 ± 0.0	0.8 ± 0.3	0.7 ± 0.4	0.1 ± 0.1	0.1 ± 0.2
18:3(n-4)	0.2 ± 0.0	0.2 ± 0.1	0.5 ± 0.2	0.3 ± 0.4	0.4 ± 0.4	0.6 ± 0.2	-	2.3 ± 3.8
18:4(n-3)	0.2 ± 0.0	0.2 ± 0.1	0.9 ± 0.3	0.7 ± 0.0	0.7 ± 0.4	0.7 ± 0.5	0.4 ± 0.6	0.2 ± 0.4
20:1(n-9)	0.7 ± 0.2	0.7 ± 0.1	1.2 ± 0.3	1.2 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	0.6 ± 0.5	1.4 ± 0.3
20:1(n-7)	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	-	0.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.5	0.1 ± 0.1
20:2(n-6)	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.2	0.3 ± 0.1	0.9 ± 0.2	3.2 ± 3.3
20:4(n-6)	0.3 ± 0.2	0.6 ± 0.3	1.6 ± 0.5	1.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.4
20:3(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0	3.8 ± 1.1	4.0 ± 2.4	8.8 ± 4.1	5.2 ± 4.9
20:4(n-3)	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.0		0.1 ± 0.1	3.0 ± 1.7	0
20:5(n-3)	1.7 ± 0.7	2.9 ± 1.5	13.1 ± 4.2	11.4 ± 0.6	0.5 ± 0.2	0.6 ± 0.2	13.8 ± 0.9	9.9 ± 3.2
22:5(n-3)	0.2 ± 0.1	0.3 ± 0.1	1.2 ± 0.5	1.0 ± 0.1	21.7 ± 2.1	13.4 ± 4.2	1.3 ± 0.3	1.3 ± 0.2
24:1(n-?)	0.2 ± 0.2	0.4 ± 0.4	0.6 ± 0.1	0.5 ± 0.1	2.2 ± 0.8	1.7 ± 0.5	4.7 ± 1.0	3.6 ± 0.7
22:6(n-3)	0.5 ± 0.3	1.0 ± 0.4	5.4 ± 2.4	4.1 ± 0.1	1.4 ± 0.2	1.6 ± 1.2	13.2 ± 3.0	10.3 ± 1.9
∑ n-3	2.9 ± 1.1	4.6 ± 2.1	**21.3 ± 7.6***	**17.6 ± 0.7***	28.2 ± 2.7	20.6 ± 3.9	**40.4 ± 6.0***	27.0 ± 2.9
∑ n-4	1.1 ± 0.2	1.2 ± 0.3	3.0 ± 0.5	1.7 ± 1.5	4.2 ± 0.7	4.2 ± 0.6	5.4 ± 0.7	8.0 ± 6.9
∑ n-6	0.6 ± 0.3	1.0 ± 0.1	2.4 ± 0.7	2.7 ± 1.0	1.1 ± 0.3	1.2 ± 0.1	2.0 ± 0.3	4.1 ± 3.1
∑ n-7	18.3 ± 1.8	15.3 ± 2.8	21.4 ± 3.8	27.0 ± 4.4	15.4 ± 4.0	17.8 ± 4.9	4.1 ± 0.6	9.0 ± 1.9
SFA	13.3 ± 3.4	15.7 ± 3.6	15.9 ± 1.9	20.1 ± 1.0	19.3 ± 2.0	20.2 ± 2.0	26.5 ± 2.6	25.8 ± 6.7
UFA	86.7 ± 3.4	84.3 ± 3.6	83.1 ± 0.6	79.9 ± 1.0	67.6 ± 2.4	71.4 ± 2.0	68.0 ± 7.3	71.9 ± 7.4
PUFA	4.6 ± 1.6	6.8 ± 2.4	26.8 ± 8.7	22.9 ± 0.7	33.5 ± 2.3	26.0 ± 3.3	47.7 ± 4.9	39.2 ± 5.5
UFA:SFA	6.9 ± 1.9	5.6 ± 1.4	**3.7 ± 2.5***	**4.0 ± 0.2***	3.5 ± 0.3	3.6 ± 0.4	**2.6 ± 0.5***	3.0 ± 1.1
PUFA:SFA	0.4 ± 0.1	0.5 ± 0.2	**1.7 ± 0.4***	**1.1 ± 0.0***	1.8 ± 0.3	1.3 ± 0.3	1.8 ± 0.4	1.6 ± 0.6

(-)= not detected or traces

4. Discussion

As laid out in the introductory chapter this thesis investigates temperature specific growth and energy allocation patterns of a eurythermal and a stenothermal perciform fish species. The following chapter will provide an integrate view of the main results and conclusions of publications I – III and complement these with some additional data. An integrative discussion of the findings and literature data will be consolidated to answer the core questions of this thesis (chapter 1.6). The results will be discussed with regards to implications for the distribution of the species and the ecosystem.

4.1 Energy budgets

Studies on growth of fish have been more concerned with the nutritional adequacy of the diet (Alvarez-Gonzalez et al., 2001; Anwar and Jafri, 2001; Catacutan et al., 2001; El-Dahhar, 2001; Gibson-Gaylord and Catlin, 2001; Alam et al., 2002; Grisdale-Helland et al., 2002) than the provision of an energy component suited to the life stage, environmental conditions and routine activity of the species. Energy fluxes in living systems are difficult to measure and interpret, even on an individual level. By accepting some approximations, the energy allocation – resolved in an energy budget - can be a powerful tool in understanding traits in the life histories of fish (Scofiani and Hawkins, 1985). In a balanced equation ($ingestion = metabolism + growth + excretion$) all energy ingested by a fish must turn up in one form or another. After determination of "bulk" parameters like respiration as an approximation for metabolism, like growth and like ammonia excretion, the allocation of adsorbed energy can be traced back to these processes. The strategies of energetic allocation differ between the species and temperatures at which they were determined. Figure 10 illustrates the energy allocation of *Pachycara brachycephalum* and *Zoarces viviparus* (as averaged over the whole experimental temperature range) in comparison with a budget of a North Sea flatfish (Fonds et al., 1992) and with a generalized budget of a young carnivorous "standard" fish (Brett and Groves, 1979). The budget compiled by Fonds et al. (1992) refers to the absorbed energy (without faecal deposition), the three other budgets deal with ingested energy amounts.

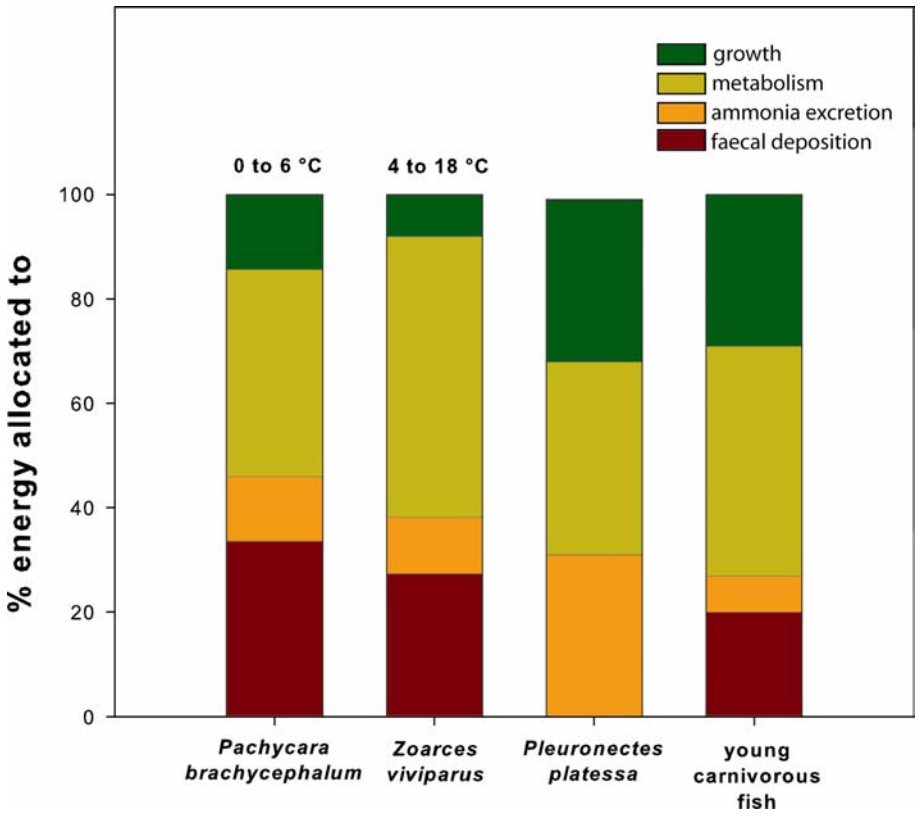


Figure 10:
Energy budgets of both eelpout species (averaged over the experimental temperature range), the plaice (Fonds et al., 1992) and a standardized well fed fish (Brett and Groves, 1979). The energy budget of the plaice excludes faecal deposition.

The energy allocation patterns of both investigated eelpout species were compared to those of the other fish. The amounts of energy remaining for growth processes are higher in the "young standard fish" and the flatfish than in both eelpout species. Both eelpout spend a relatively small fraction of the energy budget for growth. Nonetheless, a comparison of both eelpout species shows that the energy invested into growth is also the main difference between both budgets. The boreal eelpout (averaged for all experimental temperatures) spends less energy for growth than the Antarctic species.

In both eelpout species metabolism and excretion are the most energy demanding processes. The allocation patterns of the "standard" fish resemble this pattern (Brett and Groves, 1979). Winberg (1956) specified for carnivores that a general

fraction of 20% of the food intake is assigned to excretion. Consequently, a simplified form of the balanced energy budget equation:

$$0.8 * ingestion = metabolism + growth$$

is accepted as applicable in many cases. Cui and Lui (1990b,c) determined the portioning of assimilated energy between metabolism and growth for six different fish species. While noting the uniqueness of species and their responses to different diets and environmental conditions, 60 ± 16% of the assimilated energy were, on average, used for metabolism and 40 ± 16% for growth (Cui and Lui, 1990a-d). The high variability in energy allocation is a result of the different factors influencing metabolic costs and therefore energy surplus for growth. In a comparison of energy allocation for metabolism and growth in 18 fish species (Fonds et al., 1989; Cui and Lui, 1990a-d; Cui et al. 1996; Zhu et al., 2000; Brodte, 2001; Maciejewska et al. 2001), I found a trend – though not statistically significant (one-way ANOVA)– to higher energetic demands of routine metabolism from freshwater via brackish waters to the marine environments (Figure 11A) as well as from demersal via benthopelagic to pelagic lifestyles (Figure 11C). Sampaio and Bianchini (2002) related the diminished growth of the euryhaline flounder, *Paralichthys orbignyanus,* in freshwater to higher energy costs for the accelerated activity of the Na^+/K^+-ATPase in the gills. Energy allocation to growth processes seems to be highly diverse between species and can be seen as a result of different metabolic demands. Among the literature data a temperature effect on energy budgets under different thermal regimes (Figure 11B) could not be detected due to different lifestyles and activity levels of the investigated fishes. Therefore, only a direct comparison of similar ecotypes, as carried out in this thesis, is capable of detecting differences in energy allocation due to temperature regimes.

[1] *Cyprinus carpio*, *Oreochromis mossambicus*, *Pelteobagrus fulvidraco*, *Carassius auratus auratus*, *Mystus chinensis*, *P. parva*, *Cichlasoma bimaculatum*, *Gadus morhua*, *Salmo trutta*, *Oncorhynchus mykiss*, *Ophiocephalus striatus*, *Rutilus rutilus*, *Phoxinus phoxinus*, *Silurus meridionalis*, *Carassius auratus gibelio*, *Acipenser transmontanus*

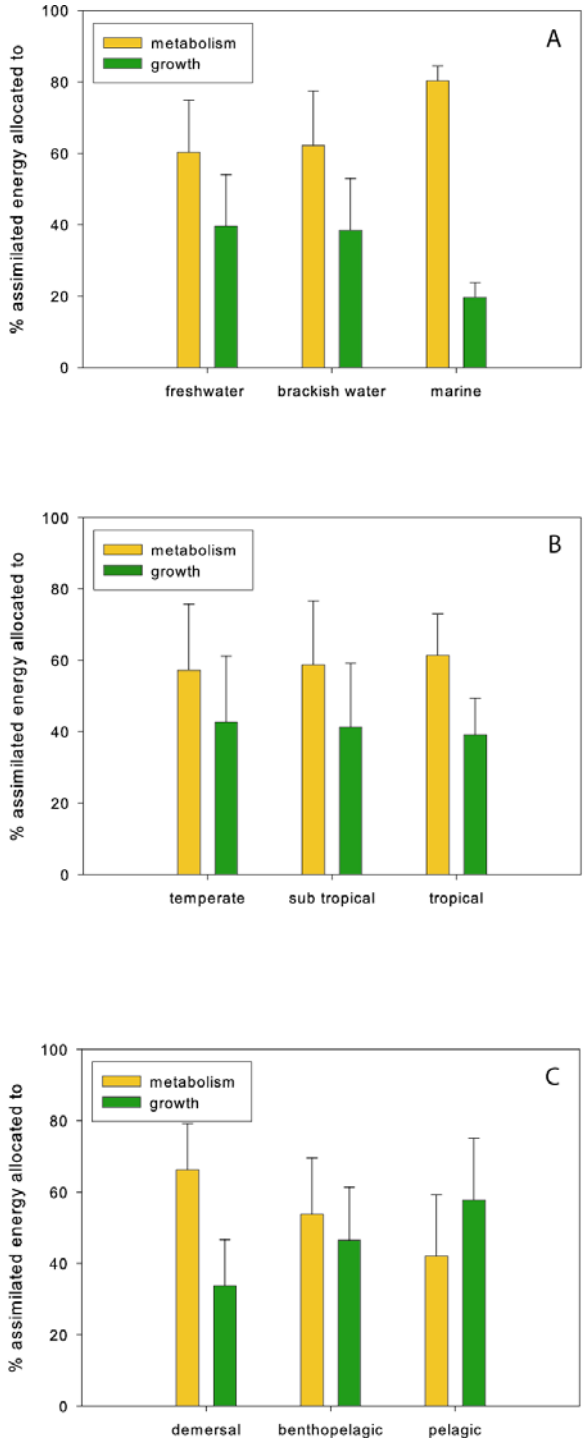


Figure 11:
Energy budgets compiled from literature data (Fonds et al., 1989; Cui and Lui, 1990a-d; Cui et al. 1996; Zhu et al., 2000; Brodte, 2001; Maciejewska et al. 2001). These budgets reflect the allocation of assimilated energy to metabolism and growth between different habitat salinities (A), different thermal regimes (B) and different activity levels (C). For fish species see footnote on previous page[1]

4.1.1 Influence of temperature on energy allocation

Since temperature acts as a controlling factor (Brett, 1979) by pacing the metabolic requirements for food and governing the rate of processes involved in food processing, responses of various parameters, like absorption efficieny (Elliott, 1976a) and enzymatic activities, can be anticipated. The organismic budget changes with temperature after long-term acclimation, differently from the cellular energy budgets, which seem to be robust against acute thermal changes (Mark et al., 2005).

The relative contribution of energy demanding processes, namely metabolic maintenance, growth and excretion to the energy budget of both species is compiled in publication II (Figure 4). Some energy is lost in faecal deposition. This loss is higher at low temperatures in both species. Surplus energy fractions allocated to growth shift in accordance with temperature dependent baseline metabolic requirements for maintenance and species-specific growth optima. The optimum temperature for maximum energy invested into growth is dependent on the ration size (Brett, 1979). At maximum ration almost all species show a rapid increase towards a peak (the optimum temperature). In *Zoarces viviparus* maintenance costs are much higher below and above the average habitat temperature, thereby leading to an optimum at about 12 °C, due to enhanced energy efficiency in baseline metabolism.

In the Antarctic eelpout the amount of energy invested into metabolic maintenance is more or less unchanged over the experimental temperature range, as reflected in the Arrhenius plot of oxygen consumption rates. This species shows almost full temperature compensation in the oxygen demand during long-term acclimation (publication II). The energy invested into growth is highest at 4 °C without visible changes in the whole animal metabolic rates. The origin of the energy fraction put into growth in *Pachycara brachycephalum* at 4 °C is most likely based on high (cellular) efficiency (Mark et al., 2005) and a high food conversion adsorption capacity (see below). The high food conversion efficiency found in *Pachycara brachycephalum* is a useful ability in environments where food supply is unstable and pulsed. In the Antarctic environment the spring bloom (Scott et al., 2000) also influences deeper water layers and the benthos (Arntz et al., 1994; 1997). This pulsed food availability and the scavenging feeding mode – e.g. reacting to large food falls – favours the ability to digest large amounts of diet in a limited time period. Usually the absorption efficiency decreases with increasing ration in fish (Elliott, 1976a,b; Jobling, 1994). At maximum

food availability, however, the conversion efficiency of the Antarctic eelpout is still high (publication II). Although the dependence of gastric evacuation rates on energy content and particle size of the diet (Jobling, 1986, 1987; Andersen, 2001) and therefore the rates of food adsorption and amount of faecal deposition is still discussed, the positive correlation of faecal deposition and temperature is accepted (Elliot, 1976a).

However, low temperature conditions in the Antarctic might increase the ability of the Antarctic eelpout to convert food at high assimilation rates, similar to findings in northern cod populations (Nicieza et al., 1994; Purchase and Brown, 2000). This argument is supported by the large capacity of the stomach of *Pachycara brachycephalum*. The ratio of stomach weight to the gutted wet weight is significantly higher in the Antarctic eelpout than the boreal *Zoarces viviparus* (Figure 12). This ratio was calculated from field catches and is an approximation of the surface area of the gut available for absorption. Little physiological information is published on normally fed fish (Carter et al., 2001), the mechanism of enhanced efficiency is not known. The adsorption of the diet depends certainly on the surface area of the guts and on time spent in the digestive organs. High food conversion efficiency and a cold compensated capacity for protein synthesis in the Antarctic when compared to the temperate species (Storch et al., 2005) might then explain the higher growth rates of *Pachycara brachycephalum* observed in the cold, at same rate of food uptake (publication II).

Because of the missing quantified stomach analyses data stable isotope analyses were used as an approximation to enlighten the position of *Pachycara brachycephalum* in the Antarctic food web. Stable isotope ratios of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ are used as proxies for the trophic position in a food web. The analyses yielded high $\delta\ ^{15}N$ values (13.8 ± 0.7 ppm; mean ± SD) and a $\delta\ ^{13}C$ of –24.0 ± 0.8 ppm (mean ± SD) and placed this species at an upscale trophic level (publication III). This species is a scavenger and also feeds on scavengers. Data from *Lycodichthys deaborni*, a related Antarctic eelpout from the Ross Sea, placed it in a comparable trophic position (Burns et al., 1998).

The energetic structure of the diet is equally important as the amount of food. In the present experiments *Zoarces viviparus* displayed lower growth rates than in former experiment carried out by Fonds and co-workers (1989).

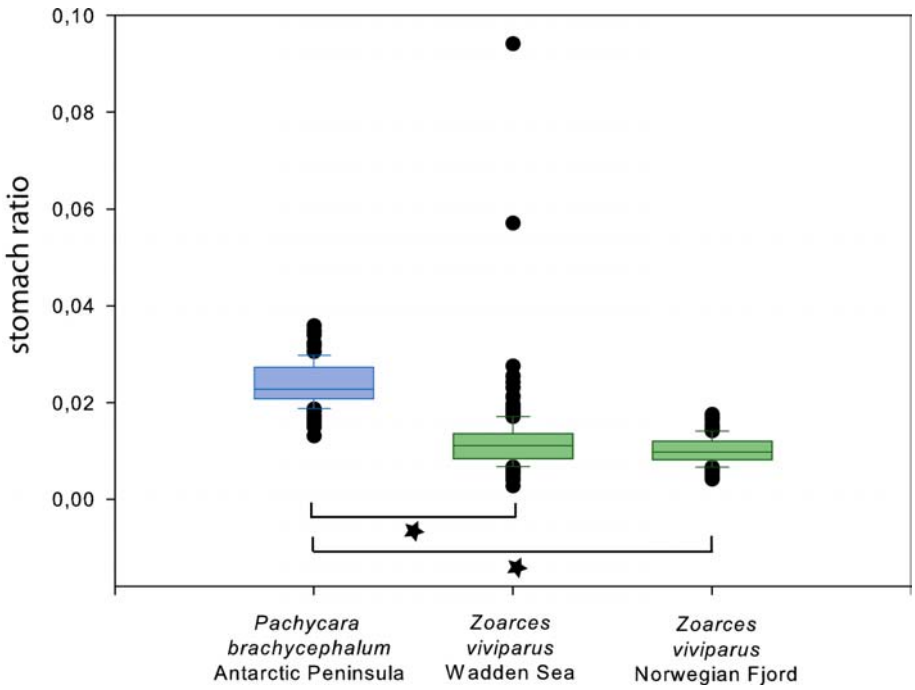


Figure 12:
Ratio of empty stomach weight to gutted weight was determined as an approximation of the morphologic adsorption capacity of different eelpout populations or species, respectively. The relative stomach weight was determined only on fish without long periods of aquarium maintenance. Asterisks mark significant differences (number of individuals used: *Pachycara brachycephalum* 109; *Zoarces viviparus* from the Wadden Sea 253; *Zoarces viviparus* from Norway 177).

The major difference between both experiments was in the diet provided. In growth experiments with the boreal eelpout fresh *Mytilus* meat or *Crangon crangon* is the optimal energy source (M. Fonds, personal communication). It is likely that lower food quality in my trial lowered the daily length increments by about an order of magnitude. It is known that high similarity concerning composition of prey and predator is energetically convenient (Kooijman, 2000) and thus reflects a better diet. When analysed, the diet I used was found to contain in dry weight 62 % proteins, 18% carbohydrates and 12 % lipids. The diet used by Fonds et al. (1989) consisted of more proteins and less lipids.

The cockle flesh used as a diet for the Antarctic eelpout in this thesis might be more suitable for this species than a diet with fewer lipids. In contrast to the Antarctic

eelpout, *Zoarces viviparus* might be specialized on food with fewer lipids and higher protein contents. These assumptions seem to be supported by the difference in tissue and lipid composition (see below). The high availability of lipids in diet for the Antarctic eelpout in the natural habitat is indicated by the stable isotope analyses. The measured carbon isotope signatures of potential prey species (cf. Lipsky, 2001) in correlation to their respective molar C/N ratio show an eelpout diet with a high lipid content (publication III). The ratio of C/N to the $\delta^{13}C$ signature has been used in Antarctic species to approximate the lipid content and diet source. A high C/N and low $\delta^{13}C$ value is used as an indicator for high lipid content. Lipids are poor of both, nitrogen isotopes and the heavier carbon isotope (Nyssen et al., 2002; 2005). High activities of TAG lipase in Antarctic fishes (Sidell and Hazel, 2002) promote the use of lipids as energy source. The pronounced lipid metabolism in this Antarctic eelpout may be the result of evolutionary processes of high mitochondrial densities and low metabolic rates, as known for active Antarctic ectotherms (cf. Pörtner, 2002) and the high availability of a lipid-enriched diet.

4.1.2 Bias in energy budgets

The energy budgets are compiled from measured (consumption, growth, respiration and ammonia excretion) and estimated (faecal excretion) parameters. Compiling parameters and using summed values where individual components were determined different ways, is susceptible to methodical bias. Neither the energy demanding processes for *Pachycara brachycephalum* at 2 and 4 °C, nor for *Zoarces viviparus* at 6 °C were met by the nutritional energy uptake (Figure 13).

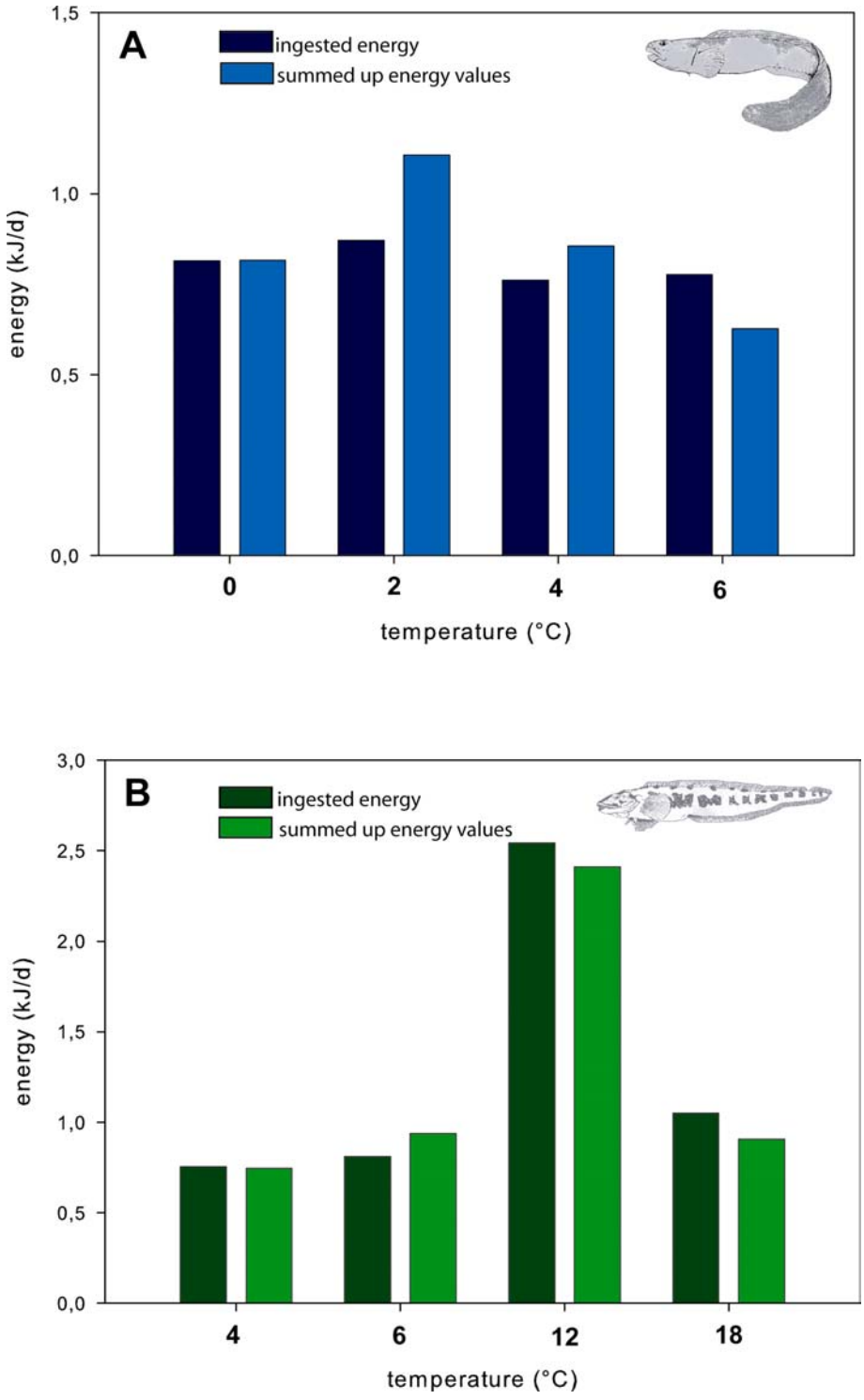


Figure 13:
In both *Pachycara brachycephalum* (A) and *Zoarces viviparus* (B) differences were found between total ingested energy determined by food consumption on the one hand and the sum of all measured processes on the other hand. The darker bars illustrate the amount of energy ingested with the diet, the brighter bars depict the energy summed up by the enlisted processes (see Material and Methods).

Metabolic demands were calculated by using the oxycaloric coefficient. The stoichiometric calculation of this coefficient neglects structural atomic parameters, which modify the energy content of molecules (Wieser, 1986). This leads to overestimations of the heat of combustion by about 2-5% for shorter fatty acids and to underestimation by 6% for glycogen (Wieser, 1986). The measured higher amount of lipids than carbon hydrates in the diet as well as a major importance of fatty acids and lipids in carnivorous fish (Brett and Groves, 1979) may contribute probably to an overestimation of the energy fraction allocated to metabolism, which is approximated by the oxycaloric coefficient.

4.1.3 Synopsis of the bioenergetic strategies of both eelpout species

In conclusion, energy allocation changes in both species with temperature. Although the underlying mechanisms need to be further investigated, energy allocation to growth at one optimal temperature seems to be driven differently in the stenothermal Antarctic eelpout and the eurythermal boreal eelpout, although the principles are the same. The bioenergetic strategy of the Antarctic species enables optimised growth at 4 °C by low cellular costs and high food conversion efficiency.

In *Zoarces viviparus* the energy allocated to growth at the optimum temperature of 12 °C is supported by reduced baseline metabolism, visible at the whole organism level. The higher acclimated baseline cost seen at 6 °C indicates cold compensation at the expense of low growth. At 18 °C the high metabolic costs of maintenance lead to an abated growth rate. The bioenergetic strategy of *Zoarces viviparus* comprises enhanced growth and a high energy efficiency in summer supported by amount and high quality of food and temperatures adequate for positive growth.

Van der Veer et al. (2003) analysed the difference between species in a latitudinal cline with respect to food conditions, the “maximum surface area– specific assimilation” rate (see Dynamic Energy Budget (DEB) theory) and the fraction of energy spent on maintenance and growth. In applying the general DEB-theory Van der Veer and co-workers (2003) predicted an increase in food conditions and maximum body size within and among species at increasing latitude. The absolute quantity of food availability is likely to diminish in the tropics (Van der Veer et al., 2003). At higher latitudes temperature oscillations divide the yearly cycle into very high and very low production months with winter starvation periods, which weaken the populations. More

constant food densities (food availability per individual) at lower latitudes select for small body volumes, since a small volume aids survival due to its lower maintenance costs (Van der Veer et al., 2003). Fluctuating food densities at high latitudes select for larger body volume, because a large volume enables better survival over prolonged periods of starvation (Van der Veer et al., 2003).

Energy allocation to growth changes with temperature in both eelpout species
In the Antarctic eelpout surplus energy for growth is gained by high food absorption and low cellular respiration
In the temperate eelpout surplus energy for growth is gained by lower routine metabolism

4.2 Growth and temperature

Organismic growth of fish has been the subject of many studies (Weatherley and Gill, 1987). This central process in the life of fish is signified by a change in magnitude of length, weight, mass or volume and it can be related to protein, lipid or caloric content, to the whole body or to component tissues. While young fish grow predominantly in length, growth in weight becomes more pronounced when fish reach adulthood (Ricker, 1979). Structural synthesis and accumulation of lipids and proteins are the underlying processes (Lambert and Dutil, 1997), which are influenced by temperature. Biochemical reactions are thermodynamically accelerated with rising temperature (Wieser, 1986). Relevant processes in organisms like protein biosynthesis are known to be thermally compensated (Hochachka and Somero, 1984; Clarke, 1991; Storch et al, 2005). The growth patterns of individuals –already a complex process – affects the growth of the population, which is composed of organisms as individuals interacting and reacting to biotic and abiotic parameters, such as temperature. A combination of temperature effects will thus affect growth uni-directionally towards higher or lower rates across levels of biological organisation (Pitcher and Hart, 1982). The growth patterns of both investigated eelpout species are different, as indicated in the results of the field studies and in the experimental data set. A comparison of both species in the field shows that

the growth rate of *Pachycara brachycephalum* is lower than the growth rate of *Zoarces viviparus*. The growth rate of *Zoarces viviparus* differs between populations in a latitudinal cline. Comparing growth rates, the *Zoarces viviparus* population from the White Sea displays the greatest similarities with the Antarctic species (Table 3; publication I; Brodte et al., in prep.).

4.2.1 Growth patterns in the laboratory

Growth investigations in laboratories are valuable in estimating the impact of indivdual factors on growth but will usually not yield the same growth rates as in the field. In the following paragraphs I will concentrate on weight gain during the experiments. Length increments are described in more detail in publication II. In laboratory growth experiments gain in length and weight is not always simultaneous. For length increments structural synthesis, building up functional molecules and cell differentiation are underlying processes. The gain in weight is a process of accumulating lipids and proteins (Lambert and Dutil, 1997). In contrast to field observations the growth of the Antarctic eelpout under laboratory conditions was not lower than in the boreal species.

Ad libitum food consumption in both species was the same over the whole temperature range. The average weight gain for *Pachycara brachycephalum* was 0.03 g wet weight /d, slightly higher than in the boreal eelpout (0.02 g wet weight /d; publication II). There was no difference in growth rate between the age classes of the Antarctic eelpout (five to nine years); this would indicate an infinitesimal slope in a growth curve at maximum food supply from five years onward. The Antarctic species reaches first maturity at the age of five years and has to split energy between somatic growth and reproductive growth (publication I). An age depended difference in growths patterns is more likely found between the first five years and later life (cf. Pitcher and Hart, 1982).

The experimental growth performance of *Zoarces viviparus* was low compared to laboratory investigations by Fonds et al. (1989) and lower than the measured rates of field growth. Experimental growth rates of fish are often accelerated (Fonds et al., 1989; North, 1998; Fischer, 2003) due to optimal food conditions and minimized foraging effort. Food quality in the present experiment was lower than of the diet used by Fonds et al. (1989). At the same food consumption rates, growth rates were reduced indicating low growth efficiency for *Zoarces viviparus* with the provided diet. The muscle tissue

of *Zoarces viviparus* displays on average a low lipid to protein ratio and might have found too little protein in the cockle diet for optimal growth (publication II). In contrast the cockle flesh might be more suitable for the Antarctic eelpout *Pachycara brachycephalum* than a diet with lower lipid levels (publication II; see chapter 4.1.1).

Moreover, the difference in condition factor (higher in the data of Fonds et al. (1989a)) and population parameters like maximum age, length and weight (Iedema, 1989) suggest that the fish used by Fonds and co-workers originated from a different population which is characterized by faster field growth than the population we used (Figure 14; Iedema, 1989; Brodte et al., in prep.). However, despite of the lower growth rate in the eelpout population from the Wadden Sea, the optimal temperature of 15 °C determined by Fonds and co-workers is quite similar to that found in the present study.

Temperature modulates the experimental growth rate of both fish species towards a clear optimum. A temperature optimum for growth has been found in other fish species, such as sockeye salmon (Brett et al., 1969), plaice and flounder (Fonds et al., 1992) or sculpin (Fonds et al., 1989) and can be related to the ontogeny of fishes. These optima often shift to lower temperatures with adulthood (plaice: Fonds et al., 1992; cod: Björnsson and Steinarsson, 2002) and are the same in North Sea and Norwegian coastal cod despite different habitat temperatures (Pörtner et al., 2001). If we apply the known thermal limits of both species and assume the investigated temperature range as ecologically tolerable, because growth was still measurable, we find a lop-sided bell shaped growth curve (publication II, Figure 1A). This curve reflects a narrow window in the stenothermal Antarctic eelpout *Pachycara brachycephalum* (publication II). Surprisingly, this optimum temperature found at 4 °C is elevated above habitat temperature. Analyses of respiration in isolated hepatocytes support this temperature to be optimal for growth due to minimized metabolic costs at 3 °C (Mark et al., 2005).

The flattened growth curve of *Zoarces viviparus* reflects the maintenance of growth over a wider range of temperatures. In this growth patterns there seems to be a trade–off against highly efficient capacities at one specific temperature. Clarke and Fraser (2004) elaborate that the higher basal metabolism of eurythermal species (Pörtner te al., 2000; 2001) is an advantage. In particular factorial aerobic scope tends to be broadly constant across the range of physiological temperatures and therefore enables broader growth patterns (Clarke and Fraser, 2004). In terms of the temperature tolerance

model (Figure 1), described in the introductory chapter, the fish live right at the upper thermal distribution limit in the Wadden Sea. Van Dijk et al. (1999) found an upper critical temperature between 21-24 °C. Temperature in the sampled area ranges from 3-23 °C and therefore periodically reaches the critical domain.

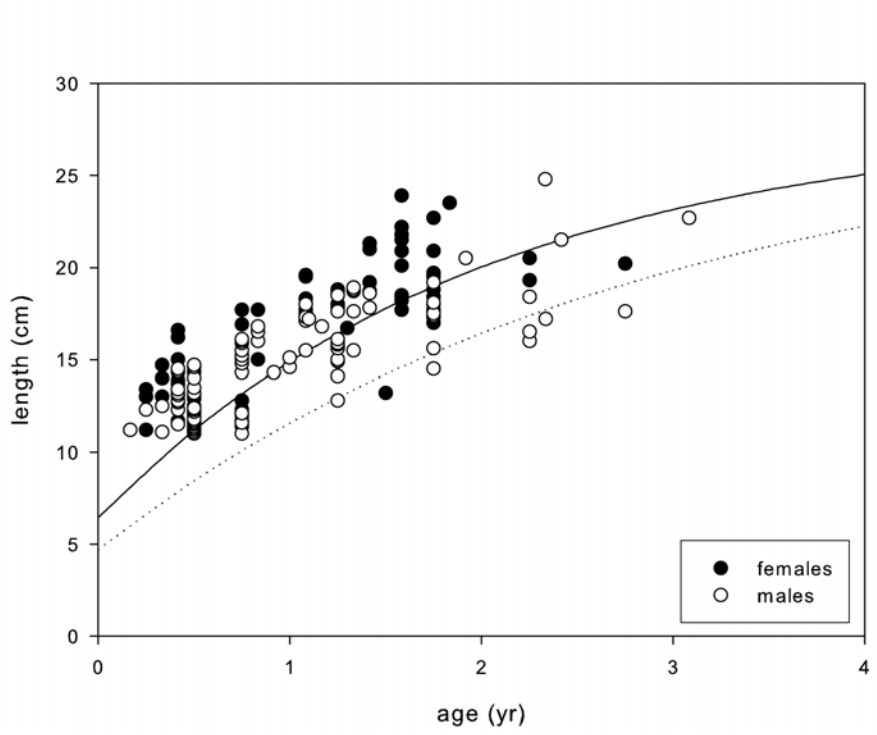


Figure 14:
Von Bertalanffy growth curves of a *Zoarces viviparus* population from the German Waddensea (solid line: females; dotted line: males)

Exposure to these stressful temperatures might increase metabolic energy costs. Before reaching the critical temperatures, in the *pejus* ranges high metabolic demands and oxygen debts due to decreased aerobic scope occur in the organisms. The energetic demands of the metabolism in the *pejus* range are most likely high, and accompanied with low efficiency. A high metabolic energy demand and a low condition factor for these animals were found even in long-term acclimation at 18 °C, which confirms this assumption (publication II; see chapter 4.2.4).

4.2.2 Field growth patterns

On the basis of individual values of length, weight, age, sex etc. the Von Bertalanffy growth model is applied to the data of this thesis to depict different structures of the eelpout populations or species. Applying growth models one has to consider the status of the fish population. The fishery impact on a species affects age and growth parameters (Brander, 1994). Here I assume no fishery impact for both species, because even in the frequently exploited North Sea and Wadden Sea area *Zoarces viviparus* is a non-target species and not commercially exploited (Knijn et al., 1993) and showing a low mortality due to discards (Beermann – Schleiff, 1988; Berghahn et al., 1992). Moreover, it inhabits areas with locally low fishery intensity due to ground morphology. In the Antarctic only a few fish species are commercially exploited and the eelpout is not one of them.

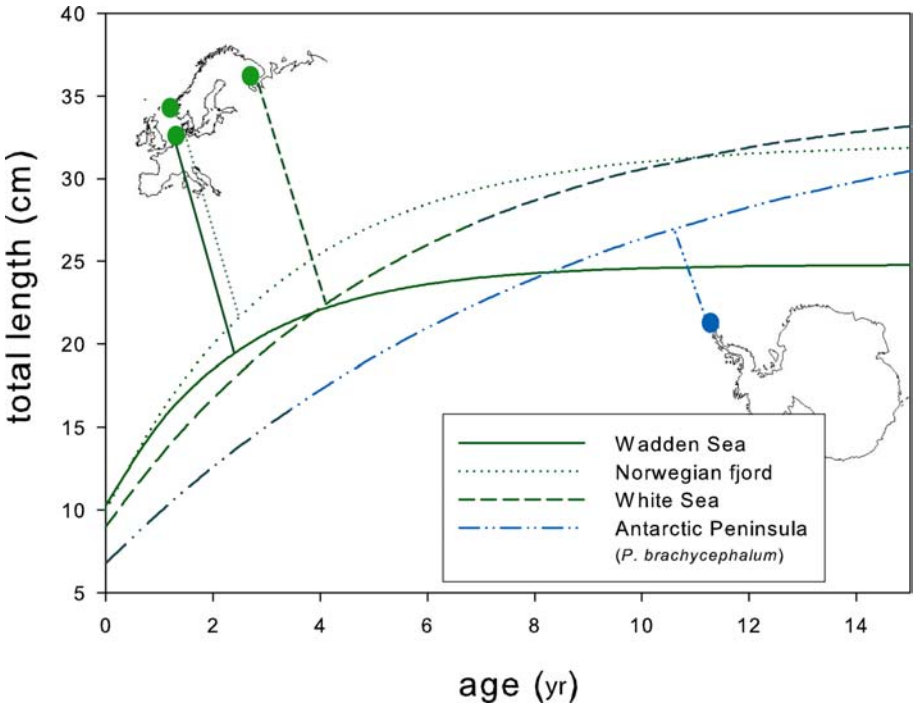


Figure 15:
Von Bertalanffy growth models of *Zoarces viviparus* populations (green lines) inhabiting different areas of the North Sea and the White Sea and of the *Pachycara brachycephalum* population (blue line) from the Antarctic Peninsula.

In field studies of the Peninsula population of the Antarctic eelpout, *Pachycara brachycephalum*, a maximum total length of 36 cm (females) and 35 cm (males) was determined with a corresponding maximum age of 14 and 10 years, respectively (publication I). In *Pachycara brachycephalum* I found no sexual dimorphism in the growth curves, neither in length nor weight. There were no other studies published on this species. However, these values are matching the data available for other southern zoarcids, located at the upper range of maximum body size concerning other Antarctic eelpout species (Anderson, 1990).

Table 2:
The Von Bertalanffy "k" as indicator for growth rate of different eelpout populations in latitudinal comparison. The upper and lower confidence interval shows significant differences between sexes and populations (marked by asterisks).

population	Wadden Sea			Norway			White Sea		
	females	males	both	females	males	both	females	males	both
K	0.49	0.35	0.52*	0.28	0.22	0.26*	0.21	0.18	0.21*
Lower confidence interval 95 %	0.43	0.29	0.47	0.26	0.20	0.24	0.16	0.10	0.16
Upper confidence interval 95 %	0.56	0.40	0.56	0.30	0.24	0.27	0.26	0.25	0.25

In the European eelpout *Zoarces viviparus* growth rate and growth performance after Pauly (1979) (Table 4) differ between populations (Brodte et al., in prep.). For this boreal eelpout salinity and temperature seem to be the main abiotic factors influencing growth in the field. In general salinities below marine values accelerate the growth rate of *Zoarces viviparus* (Wiecaszek, 1998). After the Oosterschelde was separated from the North Sea by a dike system in 1985, the abundance of eelpout increased (Figure 16; Jol et al., 2001). This was most likely a positive effect of the reduced salinity monitored by Nienhuis et al. (1994) after the building of this dike. A separation of different salinities is crucial to detect temperature-based effects on growth.

The comparison of marine conditions in different regions shows an increase of maximum age, maximum total length and body weight towards higher latitudes and decreasing average habitat temperatures. This coincides with a simultaneous decrease in growth rate (k-value, growth velocity) (Figure 15; Table 2). The same trend is found in a comparison of the brackish waters of the Kiel Bight population and other eelpout

populations in the Baltic Sea (Kristoffersson and Oikari, 1975; Brodte et al., in prep.) (Figure 18).

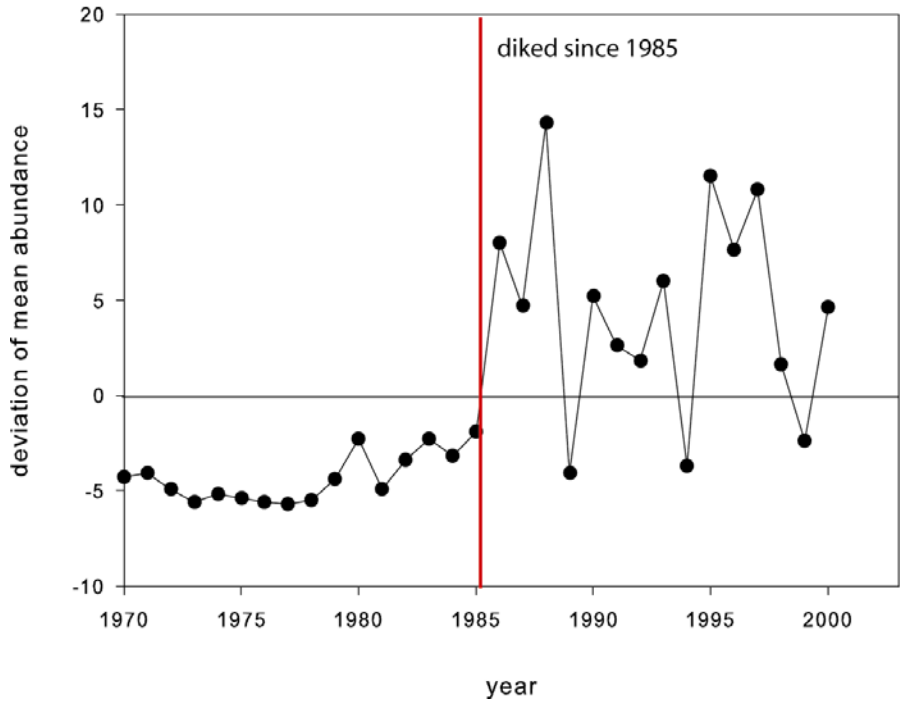


Figure 16:
Abundance of *Zoarces viviparus* in the Oosterschelde. The annual deviation of abundance to the mean abundance of this species over thirty years is posively correlated with the building of the dike (red line) and the succeeding reduction in salinity. Data are recalculated from Jol et al. (2001).

Over its distribution range the boreal eelpout displayed a higher plasticity concerning growth. For *Zoarces viviparus* eurythermy is evident in different populations inhabiting regions with different habitat temperature averages, resulting in a wide distribution area (encompassing about 14 degrees of latitude). This attribute of thermal flexibility is also evident in single populations (White Sea and Wadden Sea populations) inhabiting environments with high annual temperature amplitudes. Plasticity is stretched at the high temperature limit (combined with a large yearly temperature amplitude) in the Wadden Sea. A very low average maximum age in this population is combined with a young maturation age (Brodte et al., in prep.).

These population patterns of the Wadden Sea eelpout are most likely caused by high turnover rates as seen in overfished species, e.g. Atlantic cod (Brander, 1994). The

early reproductive effort (age at first maturity already after the first year) weakens the fish physically and hence makes them more susceptible to environmental stress (Roff, 1982). This might result in a reduced life span of the organisms and a reduced maximal size by trade offs in early maturation (Gunderson, 1997).

Table 3:
Short overview of the mean salinity and surface water temperatures in sampling areas of *Zoarces viviparus.*

area	Salinity (PSU)	Surface temperature (°C)	source
Norway, Hafrsfjord	34	3- 5 (spring)* 14 – 15 (summer)*	Own observation * Böhncke and Dietrich 1951
Wadden Sea	27 (spring) 31 (summer)	6 -7 (spring) 17 - 18 (summer)	Own observation
Kiel Bight	14	2 – 4 (spring) * 13 (summer)	Fischer et al., 1992 * Böhncke and Dietrich 1951
White Sea	19 – 24* *	4 (annual mean) 0 (spring) 10 (summer) 5 (autumn) -1 (winter)	**pers. comm. D. Lajus (Zoological Institute St. Petersburg) Babkov 1982

The White Sea population of *Zoarces viviparus* lives at the lower edge of temperature dependent distribution and experiences a large yearly amplitude in habitat temperature like the Wadden Sea population (Table 3). Growth rates are reduced with a delay in maturation, a high maximum age, a high maximum length and half the growth rate of the Wadden Sea population (Brodte et al., in prep.). The annual thermal plasticity required by these fish results in a high energy cost (Pörtner et al., 2000; 2001). These costs have to be covered by food consumption in the field. Both areas, where the populations face large annual swings in temperature, are highly productive shelf regions (Beverton, 1992; Beukema and Cadeé, 1997). Beverton (1992) found in a comparison of different gadoids, Clupeiformes and Pleuronectiformes a geographical trend with increased maximal length (as well as age) and length (and age) at first maturity in more northerly habitats. The difference with respect to maximal age and growth rate was most expressed at the northerly and southerly ends of the distribution ranges (Beverton, 1992).

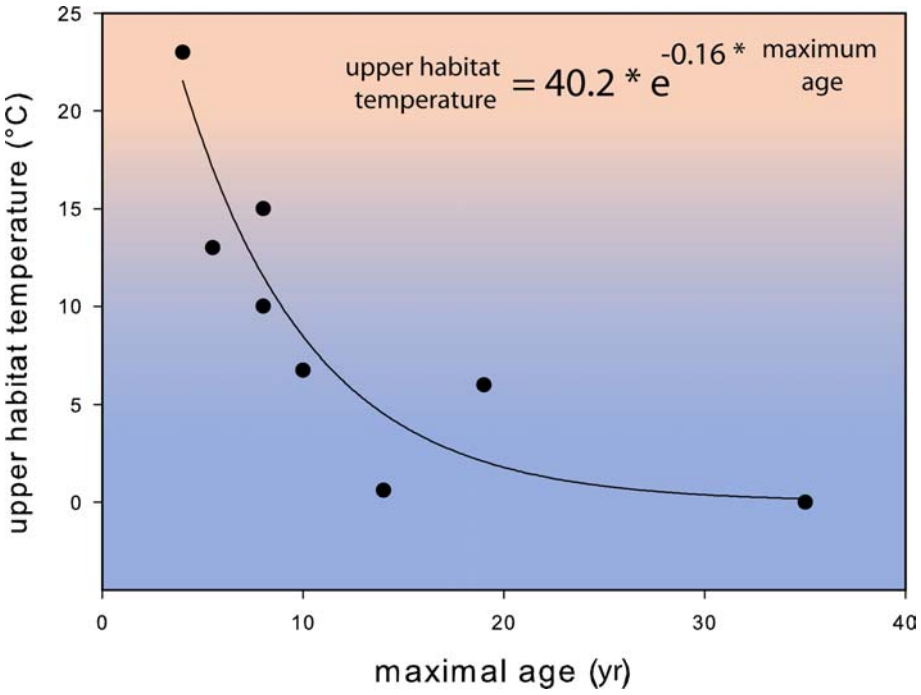


Figure 17:
Maximum age of selected eelpout populations of *Lycodes reticulatus* (Arctic eelpout), *Zoarces viviparus* (boreal eelpout) and *Pachycara brachycephalum* (Antarctic eelpout) in correlation to the maximal upper habitat temperature.

A thermal impact of habitat temperature on the maximum age of zoarcids can be expressed as an exponential decline of maximum age with increased temperatures (based on data of the Arctic species *Lycodes reticulatus* (Dorrien, 1993), the boreal species *Zoarces viviparus* and the Antarctic species *Pachycara brachycephalum*) (Figure 17). In a comparison of growth patterns of *Pachycara brachycephalum* with the *Zoarces viviparus* population from the White Sea the similarities are associated with maximum length, the Von Bertalanffy "k" and age at first maturation.

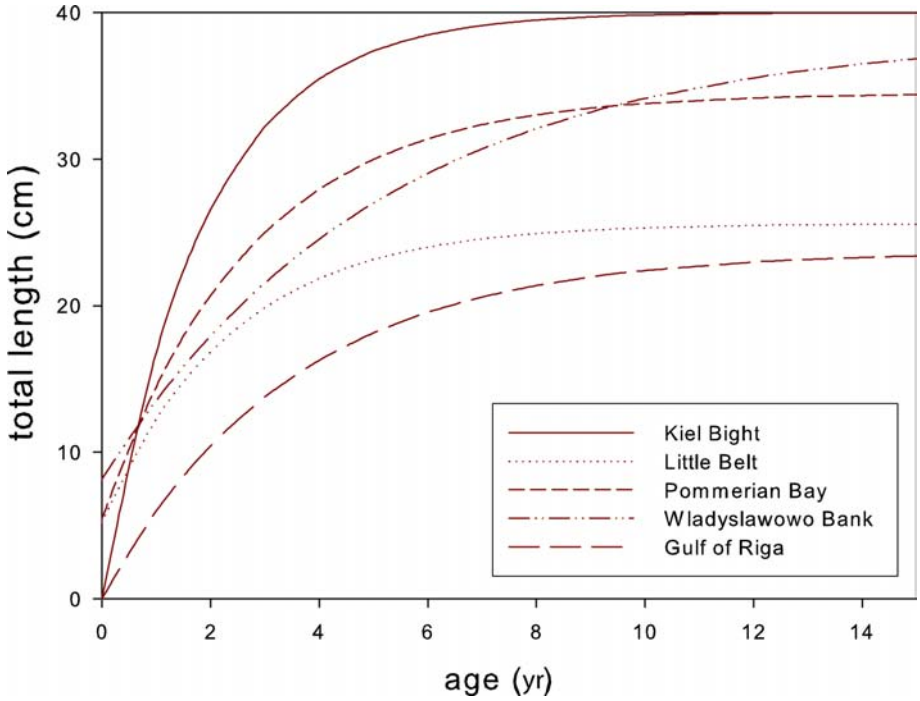


Figure 18:
Von Bertalanffy growth models of *Zoarces viviparus* populations inhabiting different areas in the Baltic Sea. (Data sources: Kiel Bight: this thesis; Pommerian Bay, Wladyslawowo Bank and Little Belt: Wiecaszek, 1998 and parameters for the Gulf of Riga population by Ojaveer and Lärv, 2003).

Table 4: Indices of growth performance ("P-values"; Pauly, 1979) in different fish species from different geographical regions. *Zoarces viviparus* populations are marked in green, polar eelpouts are marked in blue.

species	sex	area	Pauly index of growth performance	source
Lycodes reticulatus	both sexes	Northeast Greenland	1.55	Dorrien, 1993
Lycodes reticulatus	both sexes	Barents Sea, Arctic	1.04	Dorrien, 1993
Zoarces viviparus	males	White Sea	1.57	Brodte et al., in prep.
Zoarces viviparus	females	White Sea	1.65	Brodte et al., in prep
Zoarces viviparus	males	South West Norway	1.75	Brodte et al., in prep
Zoarces viviparus	females	South West Norway	1.95	Brodte et al., in prep
Zoarces viviparus	both sexes	Bay of Finland		Kristoffersson and Oikari, 1975
Zoarces viviparus	males	Kiel Bight	1.48	Brodte et al., in prep
Zoarces viviparus	females	Kiel Bight	1.77	Brodte et al., in prep
Zoarces viviparus	males	Wadden Sea	1.67	Brodte et al., in prep
Zoarces viviparus	females	Wadden Sea	1.63	Brodte et al., in prep
Pachycara brachycephalum	males	Antarctic Peninsula	1.27	Publication I
Pachycara brachycephalum	females	Antarctic Peninsula	1.26	Publication I
Trematomus lepidorhinus	males	Antarctica	1.36	Ekau, 1988
Trematomus lepidorhinus	females	Antarctica	1.69	Ekau, 1988
Trematomus bernacchii	both sexes	Ross Sea, Antarctica	1.70	La Mesa and Vacchi, 2001
Trematomus bernacchii	both sexes	Adelie Island, Antarctica	2.03	La Mesa and Vacchi, 2001
Trematomus bernacchii	both sexes	Weddell Sea, Antarctica	1.72	La Mesa and Vacchi, 2001
Pleuragramma antarcticum	both sexes	Antarctic Peninsula	1.01	La Mesa and Vacchi, 2001
Pleuragramma antarcticum	both sexes	Weddell Sea, Antarctica	1.29	La Mesa and Vacchi, 2001
Pleuragramma antarcticum	both sexes	Mawson Sea, Antarctica	1.54	La Mesa and Vacchi, 2001
Pleuragramma antarcticum	both sexes	Cosmonaut Sea, Antarctica	1.42	La Mesa and Vacchi, 2001
Pleuragramma antarcticum	both sexes	Prydz Bay, Antarctica	1.63	La Mesa and Vacchi, 2001

4.2.3 Interaction of reproduction and growth in the field

Growth in size is not the only kind that fish have to cope with. Gonads require energy input for their development and compete with somatic growth for resources. An elevated energy input mitigates this competition. Therefore reproduction as non-somatic growth has a high effect on the energy allocation and the bioenergetic strategy of fish. For example, in female turbot the feeding regime and ratio size has a significant positive effect on maturation and fecundity (Bromley et al., 2000).

The genus *Zoarces* is the only genus in the eelpout family that displays viviparity. This reproduction mode has been proven in *Zoarces gillii* (Anderson, 1984), *Zoarces elongatus* (Koya et al., 1994) and the boreal eelpout *Zoarces viviparus*. The viviparity of *Zoarces viviparus* has been known since the Middle Ages (Anderson, 1984). Viviparity could be addressed as extreme parental care and often corresponds with low fecundity and high reproductive cost per individual. I found a maximum of 158 larvae per ovary in *Zoarces viviparus* from Kiel Bight. This effort leads to an optimised survival of the offspring (Wourms, 1991; Gunderson, 1997). The Antarctic eelpout *Pachycara brachycephalum* is oviparous (Anderson, 1984). Nonetheless the reproduction of both species is quite comparable (Figure 19). In the Antarctic eelpout the maximum fecundity, with 79 eggs per ovary is in the same range as for the boreal one and a high reproductive effort is spent on oocytes of 9 mm diameter (publication I). The fish start to produce ripe eggs at five years, which corresponds with a body weight of 41 g. The weight is - like in some other fish species (Roff, 1982; Beverton, 1992; Bromley et al., 2000) - a more exact indicator of maturation than age.

In the boreal eelpout the maximum fecundity with age (relative fecundity) as well as the age of maturation differs between the populations with latitude (Brodte et al., in prep.). The age of maturation increases with increasing latitude, which consolidates the observations of somatic field growth. One reason is the linkage of body weight (and thus growth rate) with maturation and relative fecundity (Roff, 1982). This latitudinal trend is found as well in other fish species. Arctic charr have a lower age at first maturation at 70 °N than at 80 °N (Dansgard et al., 1999).

Although viviparous species usually have delayed maturity, a lower gonadosomatic index (GSI) and a lower natural mortality than their oviparous counterparts (Gunderson, 1997), the age at first reproduction is the same comparing the boreal *Zoarces viviparus* population from the White Sea with the Antarctic eelpout

Pachycara brachycephalum (Figure 19). This might be due to competition of ecological and evolutionary advantages of high growth rate, high fecundity and early maturation. Fecundity increases with body size and hence optimising age at first reproduction means trading of future increased egg production associated with growth (assuming that energy available for growth and reproduction is limiting) against the advantages of early maturity (Roff, 1982). Both eelpout species display an energetically expensive, benthic reproduction mode, which emphasises survival of few at the expense of a high number of offspring and wide dispersal of larvae. Benefits of this reproduction mode are relatively large, fully developed larvae. Large larvae have an advantage under conditions of limited or variable food supply (Bagarino and Chua, 1996). Moreover, large larvae have larger mouths and are thus capable of ingesting high-caloric prey. The caught early juvenile of *Pachycara brachycephalum* was relatively large (4.4 cm total length) and capable of feeding. It occurred during a planktonic bloom in the Antarctic (publication I) and hence even if not feeding directly on the plankton, a coupling of hatching with the productive season is suggested.

Parental care or viviparity on the basis of lecithotrophy is exalted in "extreme" environments such as the deep sea (*Sebastes* species: Wourms, 1991), tidal ponds (*Austrolycus depressiceps*: Matallanas et al., 1990; *Hypsurus caryi*, *Embiotoca jacksoni*: Behrens, 1977; *Myoxocephalus scorpius*: Knijn et al., 1993) or the Arctic (*Lycodes* species: Anderson, 1994). The crucial matching of hatching events with periods of high food availability for the reproductive success of a species (match – mismatch hypothesis, Cushing, 1975; 1990) is mitigated by this reproduction mode which thus appears beneficial in highly seasonal environments.

The fecundity and number of offspring in both species seems to be resource limited by either limitation of total available food energy or by restricted energy surplus (after satisfying hierarchically higher energy demanding processes). Especially in the *Zoarces viviparus* population from the Wadden Sea the "reproductive effort - adult survival" trade off is a secondary result of the trade off between reproductive effort and growth. If most surplus energy goes into reproduction after sexual maturation, early maturation implies reduced body size (Gunderson, 1997).

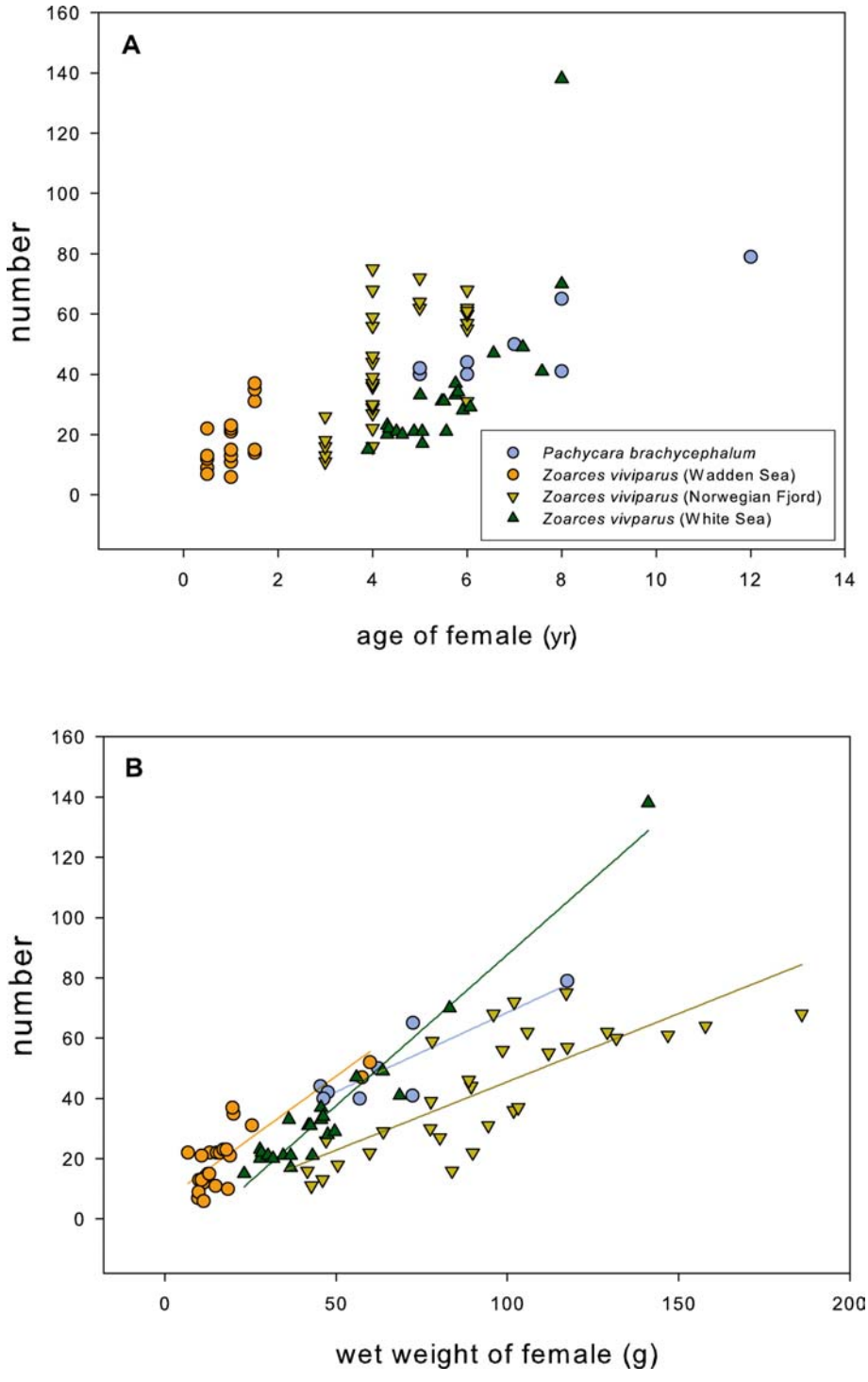


Figure 19:
A **Number of eggs (*Pachycara brachycephalum*) or larvae (*Zoarces viviparus*) in the ovary of ripe females plotted against age.**
B **Number of eggs (*Pachycara brachycephalum*) or larvae (*Zoarces viviparus*) in the ovary of ripe females plotted against weight of the adult.**

4.2.4 Synthesis: Field and experimental growth in relation to the thermal optimum

Comparing the measured field growth parameters and the experimental growth rates I found differences and similarities between the two. Some were established in the experimental set-up. The slow field growth of the Antarctic eelpout compared to the temperate species is contrary to their similar growth in the laboratory. One major difference between field and lab conditions was food availability.

The determination of experimental growth optima excludes effects of food limitation. The optimal temperature for growth will shift to lower temperatures once food becomes limited and is thus most likely influenced by the feeding regime (Brett, 1979). For young sockeye salmon at optimised ration (the amount of food needed for optimal growth usually smaller than the maximum ration) the progression towards a temperature optimum is much steeper than with maximum food supply (Brett et al., 1969). Like oxygen, food is in terms of growth a limiting factor (Brett and Groves, 1979). Temperature as a controlling factor (Brett and Groves, 1979; Pitcher and Hart, 1982) accelerates growth until resources, oxygen or food, become limited on the relevant functional level.

The thermal range where positive growth occurs corresponds with the tolerance window set by oxygen and capacity limitations according to a model developed by Frederich and Pörtner (2000). According to this model (Figure 1), growth is supported by the availability of excess aerobic scope within the thermal window of a species. An acclimated window results for the Antarctic eelpout between 0 and 6 °C with a clear optimum at 4 °C. *Pejus* temperatures delineate the onset of a loss in aerobic scope. The growth optima of both species are shifted to the upper thermal limits of each species. The *pejus* temperature for the Antarctic eelpout *Pachycara brachycephalum* was determined at 7°C (Mark et al., 2002). The upper critical limit for the boreal eelpout was suggested to be between 21 and 24 °C by different authors (Fonds et al., 1989; Van Dijk et al., 1999) with a *pejus* level at between 13 and 15 °C (Zakhartsev et al., 2003). Accordingly, the eurythermal boreal eelpout *Zoarces viviparus* displays a thermal window of ecological tolerance from at least 4 to 18 °C with a growth maximum at 12 °C, again close to upper *pejus* levels.

The observed maximum growth would therefore occur where thermodynamically enhanced aerobic scope is still matched by oxygen supply to tissues. A maximal aerobic scope enhances growth capacity up to a point were resources of

oxygen and possibly, energy (diet) became limited. Differences in aerobic scope may also relate to optimal growth temperatures of two flatfish species where Fonds et al. (1992) found a two-degree higher growth optimum for flounder than for plaice. The flounder possesses higher respiratory capacities such as higher hematocrit and higher hemoglobin levels, a higher oxygen affinity of the respiratory pigment and a larger gill surface (Fonds et al., 1992). This enables sufficient oxygen supply to tissues at higher temperatures and therefore optimal growth at 22 °C. Thermal limits can be widened with sufficient oxygen and medium supply. This was shown by Mark et al. (2005) who found suspended cells of the Antarctic eelpout, *Pachycara brachycephalum*, to be functional up to 15 °C.

In evolutionary terms, a close matching of the optimal growth temperature and the mean habitat temperature is useful. For the eelpout population from the Wadden Sea the optimal growth temperature was 12 °C, which matches the annual mean in the natural habitat. This would imply close to optimal growth in the field depending on sufficient food availability. The growth optimum of 4°C for the Antarctic eelpout might be a relict of the deep-sea origin of the genus *Pachycara* (Anderson, 1989), which invaded the Antarctic after cooling probably via the Scotia Ridge (publication I). This thermal preference has implications for their distribution in the field (publication I). *Pachycara brachycephalum* occurs predominantly in water layers above or around 0 °C.

In conclusion I assume that the low growth rate of the Antarctic eelpout compared to the boreal eelpout in the field may be related to low food availability or higher costs of prey collection. However, the field growth rate of *Pachycara brachycephalum* was determined in a habitat with ambient temperatures below the experimental growth optimum (publication II). Growth performance in the field is then restricted firstly by habitat temperatures below the thermal optimum and only secondarily by reduced energy availability from the diet or by higher field metabolic rates.

The thermal growth optimum of *Zoarces viviparus* matches the mean habitat temperature in the Wadden Sea
The slower growth rate of the Antarctic eelpout compared to the temperate species is due to habitat temperatures below the thermal optimum and food limitation in the field

4.3 Lipids in fish

As major storage components in fish, lipids are important when dealing with growth and energy budgets. Most storage materials have the dual function as a reserve for both energy and elementary compounds. Being (temporarily) immobilized energy and part of the metabolic "machinery" the role and function of lipids in fish is closely linked to the energy budget. Lipid content and composition is flexible in relation to the acclimation temperature (Sidell, 1991; Farkas et al., 2001) (publications II and III).

Investigating lipids means dealing with different hierarchies of consideration. Determination of the total lipid content of tissues or animals serves as a bulk parameter. The amount of lipid says something about the importance of lipids in the tissue or in animals. The determination of lipid classes allows differentiation between storage lipids and structural lipids. These lipid classes are groups of similar structure with similar chemical properties (Urich, 1990).

Fatty acids are components of lipid classes or parts of cell structures, e.g. of membranes (Stryer, 1989; Urich, 1990). One distinctive feature of fatty acids is the degree of saturation. Ratio, saturation and type of fatty acid influence the biophysical properties of lipids (e.g. polar lipids) and membranes (Hazel, 1995). The proportion of saturated fatty acids is known to increase with colder temperatures in most fish (Ackman, 1989). This adaptation elevates the fluidity of membranes and guarantees the proper function of membranes even in colder temperatures (homeoviscous adaptation).

In this thesis lipid content and lipid classes were determined in liver and muscle tissue. In contrast to other studies, which analysed fatty acid profiles of individual lipid classes, the fatty acid composition of the total lipid extract was determined due to the time frame and focus of this thesis. This limitation makes the interpretation of the results in some respect more difficult than the fatty acid determination of a certain lipid group.

4.3.1 Lipids as storage material

Carnivorous and omnivorous fish show an emphasis on lipid and protein metabolism (Brett and Groves, 1979). Triacylglycerols (TAG) are one lipid class which is used as storage material in fish. The high amount of this class in liver tissue corresponds with the storage function of the liver (Greene, 1990). In both eelpout species the total lipid content increased in liver tissue with decreasing temperature (publication II). There is

apparently an enhanced importance of lipids in cold water as well in the Antarctic eelpout as in the boreal eelpout. Whereas the measured lipid values for *Zoarces viviparus* are higher than the field data given by Pekkarinen (1980) for the same species, the lipid content is still lower than in the Antarctic eelpout. Both species consume the same diet; therefore a nutritional effect (different sources of lipids) can be excluded. This result is in line with previous investigations, which found a three times larger lipid fraction in the Antarctic eelpout compared to the boreal eelpout fed on C*rangon crangon* (Brodte 2001). In *Pachycara brachycephalum* the predominant lipid class in muscle and liver tissue over the whole temperature range is TAG (publication III) (Figure 20a). Antarctic fish have evolved an efficient use of lipids as an energy source. Capacities of enzymes of Antarctic fish involved in lipid catabolism are larger than those of temperate fish (Crockett and Sidell, 1990; Sidell and Hazel, 2002).

The increased importance of storage lipids while cooling in these eelpout species is apparent in the boreal eelpout in the reversed change of the ratio of polar lipids (more structural lipids) to neutral lipids like TAGs. The ratio of PL/TAG fell between 6 and 18 °C and the ratio CHOL/TAG seems to indicate a temperature triggered metabolic change (Figure 20b; publication III). This change to TAG lipids is conducted by the boreal eelpout as a eurythermal fish species, which encounters annual temperatures between 3 and 24 °C in its habitat. In *Pachycara brachycephalum* adapted to permanent cold this lipid class is already predominant (Figure 20).

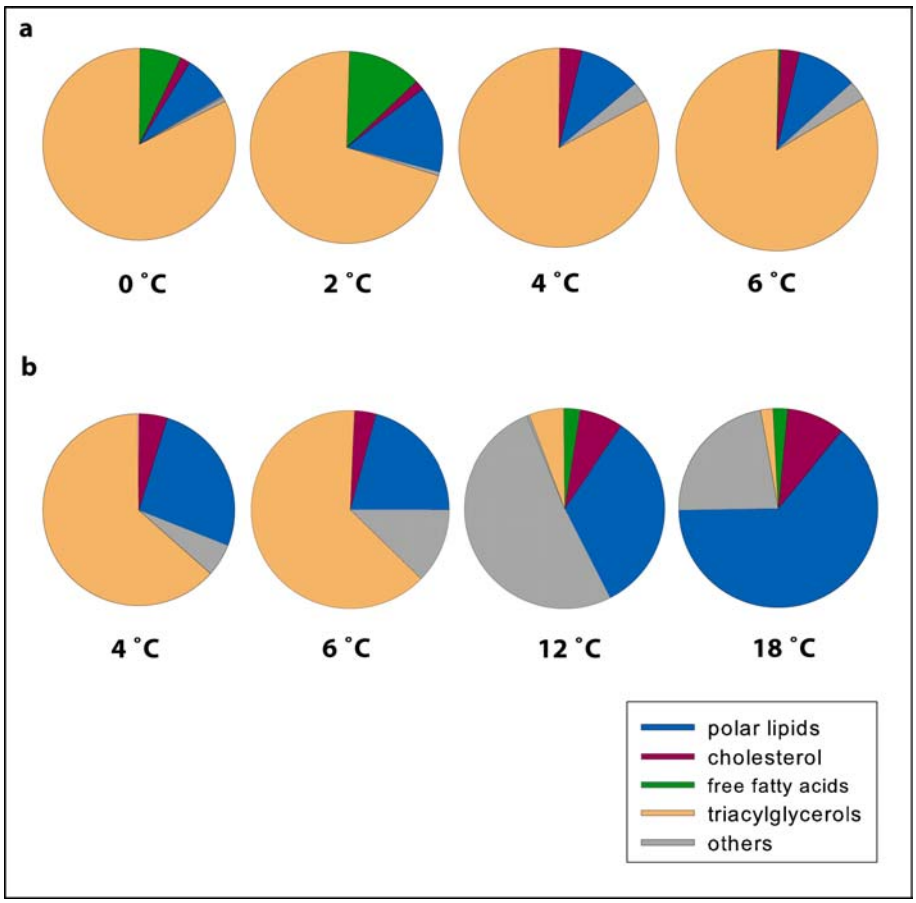


Figure 20:
The lipid class composition of the liver tissue of the Antarctic eelpout, *Pachycara brachycephalum* (a), and the boreal eelpout, *Zoarces viviparus* (b). In the boreal eelpout the ratio of polar lipids over storage lipids (triacylglycerids) changes completely at decreasing temperatures.

Another lipid class used for storage is the group of wax esters, but according to the analyses this group was not important (publication III). The low level of wax esters seems reasonable in the light of the morphology of the digestive tract and tissues of eelpout species in general. There is evidence that fish species with few or no pyloric caeca, like eelpout, cannot retain dietary wax esters in the intestine long enough to induce any appreciable assimilation (Greene, 1990).

4.3.2 Fatty acids as structural parts of membranes

Membranes consist of bipolar layers of mainly polar lipids (Stryer, 1994). Fatty acids are embedded in these lipids and directly in the layer structure. Among others, the

degree of saturation of the fatty acids influences the biophysical attributes of membranes (Hazel, 1995). In this thesis I did not analyse the fatty acid composition of the neutral and polar lipids separately. Therefore I cannot present results on the fatty acid composition of one single lipid class, but the results are interesting enough to serve as a preliminary approximation and basis for further research.

The degree of saturation of the fatty acids differs between both eelpout species. In general unsaturated fatty acids seem to be prominent in the Antarctic eelpout, but when compared at the same temperatures (4 and 6 °C) *Zoarces viviparus* displayed significantly higher PUFA to SFA ratios, whereas the Antarctic eelpout showed significantly higher MUFA to SFA ratios. *Zoarces viviparus* might be able to readily adapt to seasonal and fluctuating temperatures by increasing the amounts of polyunsaturated fatty acids, thereby displaying eurythermy characteristics. Guderley et al. (1997) observed a seasonal difference with more polyunsaturated fatty acids in membrane lipids of trout in the autumn compared to summer individuals. At "higher" temperatures of 4 and 6 °C *Pachycara brachycephalum* had more monounsaturated fatty acids than polyunsaturated fatty acids, possibly to support homoeviscous adaptation of membranes upon warming. Loss of PUFAs in the warmth may be due to ongoing oxidative stress (Jobling and Bendiksen, 2003).

Even the role of the docosahexaenoic acid (22:6 (n-3)) in the organisms seems to depend on the thermal amplitude in the natural habitat of the fish. In *Pachycara brachycephalum* high amounts of this fatty acid in liver and muscle correlate with the temperature of highest growth (4 °C), indicating that the level of this fatty acid may be more closely related to growth than to temperature (Jangaard et al., 1967). Data by Farkas and co-workers (1994) and Grove and Sidell (2004) support this hypothesis by showing no participation of 22:6 (n-3) in the process of cold adaptation of phosphatidyl ethanolamine in membranes from more stenothermal fish.

In eurythermal carp, however, Farkas et al. (1980) found that adaptation to cooling was parallelled by the accumulation of especially 22:6 (n-3). The eurythermal eelpout *Zoarces viviparus* also seems to make use of this fatty acid as a modulator of membrane fluidity. This fatty acid was not found to accumulate in muscle depending on maximum growth, but especially at low temperatures. During field investigations spring values of 22:6 (n-3) in *Zoarces viviparus* liver, muscle and ovaries were found higher than the respective summer values (Pekkarinen, 1980). Guderley et al. (1997)

emphasized the adaptational relevance of 22:6 (n-3) in membrane lipids of eurythermal fish by finding an increase of 22:6 (n-3) in mitochondrial phospholipids of rainbow trout during cold acclimation. From our data we conclude that the role of this fatty acid differs between eurythermal and stenothermal fish.

4.3.3 The role of lipid metabolism

The Antarctic species *Pachycara brachycephalum* displayed an emphasis on lipid and especially TAG metabolism between 0 and 6 °C. The pronounced lipid metabolism in this Antarctic eelpout may be the result of evolutionary processes of high mitochondrial densities and low metabolic rates, as is known for active Antarctic ectotherms (cf. Pörtner, 2002) and may be emphasized by the high availability of a lipid enriched diet.

The boreal eelpout *Zoarces viviparus* shows a strong change in lipid classes with temperature to increased levels of storage lipids (TAG) in the cold (publication III). This eurythermal switch in metabolic preferences allows the increased use of lipids at colder (winter) temperatures and benefits from cold induced storage of lipid bound energy.

The differences in lipid metabolism and the thermal responses between both species already discussed based on a single lipid class or on the levels of fatty acids, are visible in the PCA plots of muscle and liver data for both species. The statistical method PCA was used to condense the complex and multivariate information on the changes in fatty acids composition in muscle and liver tissue with temperature (Figure 21).

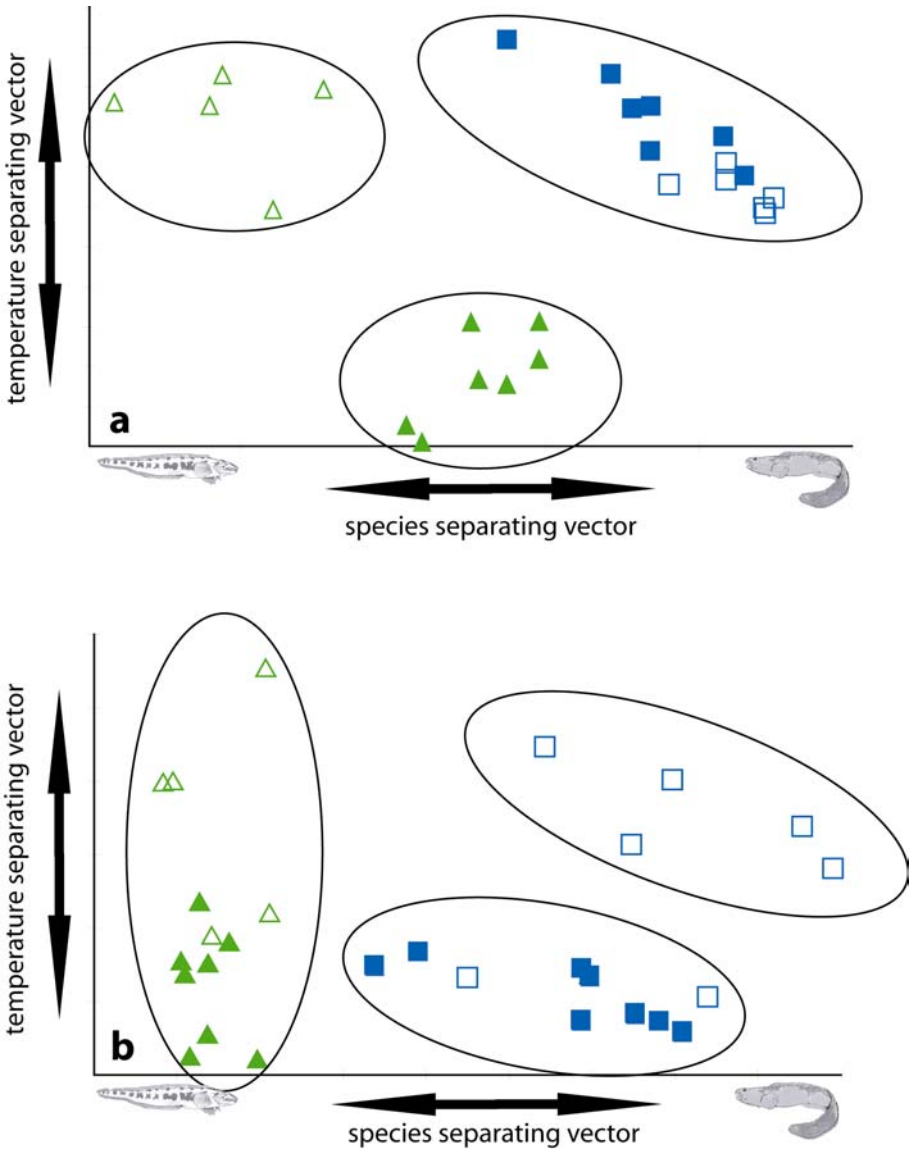


Figure 21:
PCA plot of the fatty acid composition of both eelpout species at all experimental temperatures. Samples of the boreal eelpout are marked as triangles; rectangles illustrate the values of the Antarctic eelpout. The filled symbols are always samples at experimental temperatures of 4 and 6 °C, the open triangles are the values of the boreal eelpout at 12 and 18 °C and the open rectangles are the values of the Antarctic eelpout at 0 and 2 °C. Figure (a) depicts the situation in the liver tissue, Figure (b) in the muscle tissue.

For the eurythermal eelpout *Zoarces viviparus* the PCA analysis of the liver fatty acid profile shows distinct shifts with temperature. The temperature coordinate in the liver tissue plot is mainly determined by the fatty acids 22:5 (n-3) and 20:5 (n-3). This

might be due to preferred synthesis of elongated fatty acids at lower temperatures in *Zoarces viviparus* by increasing importance of rather 22:3 (n-3) than 20:5 (n-3). This feature is known from fish living in the permanent cold (Grove and Sidell 2004), like the Antarctic demersal fish *Gobionotothen gibberifrons*, and might be an adaptation to colder temperatures.

I conclude that in the eurythermal eelpout lower temperatures affect tissues towards higher total lipid content and higher TAG values and therefore an increased importance of the storage purpose of lipids. Temperature moderately influences the high lipid content in liver in the Antarctic eelpout. The tissue of the stenothermal Antarctic eelpout displays high TAG values and the ratio of PL/TAG is not affected by moderate warming to 6 °C. This polar fish has permanently optimised its metabolism to colder temperatures and lipid enriched diet. The fatty acid 22:6 (n-3) may modulate membrane flexibility only in eurythermal fish species. Nonetheless, for further studies it is advisable to separate lipids by origin (membrane or cytosol) to avoid ambiguous interpretation of the results.

Overall, temperature affects lipid metabolism of both species by increasing total lipid content at colder temperatures using the lipids as energy storage. However, the lipid composition changes with temperature only in the eurythermal species.

In both eelpout species lipids become more important with decreasing temperature
The lipid metabolism of the Antarctic eelpout is adapted to lipid-enriched diet and an high amount of storage lipids
The lipid composition of the temperate eelpout changes with cooling to an high amount of storage lipids

4.4 Synopsis of interactions of energy budgets, growth and lipid composition

Unlike cellular energy budgets, which seem to be robust against acute thermal changes (Mark et al., 2005), the organismic budget changes with temperature after long-term acclimation, thereby defining energy availability for growth. The direct comparison of the energy budget of two confamilial, similar species from Antarctic and temperate

zones detected variable energetic demands of the whole animal at different temperatures in the boreal eelpout, but not in the Antarctic eelpout.

These unchanged energetic demands of *Pachycara brachycephalum* are reflected in lipid metabolism, which is optimised to colder temperatures and lipid enriched diet. The decrease in total lipid content with rising temperature is not accompanied by a functional change in the lipid classes. Under laboratory conditions with maximum food supply the Antarctic eelpout shows a distinct growth optimum above the ambient habitat temperature. Therefore, growth performance in the field is restricted firstly by habitat temperatures below the thermal optimum and secondly by reduced energy availability from the diet or by shifts in energy budget due to cold compensated field metabolic rates.

In the boreal eelpout the changing metabolic demands with temperature are reflected by the increase of total lipid content with colder temperatures and the associated shift to storage lipids. The thermal plasticity of metabolism includes a wider window of growth. The ability to grow over a wide range of temperatures leads to a large distributional range of the different eelpout populations in the field.

The eurythermal eelpout *Zoarces viviparus* has a wider thermal window of growth and conducts a change in lipid composition and fatty acids when exposed to colder temperatures. These abilities demand higher metabolic costs.

The more stenothermal eelpout *Pachycara brachycephalum* is highly efficient in absorption of food and has a storage pronounced lipid metabolism which remains unchanged between 0 and 6 °C.

5. Conclusion and future perspectives

This section will provide an integrative synopsis of the results followed by an outlook suggesting further research topics. The aim of this thesis was to evaluate thermal effects on the energy budget and the implications on growth and lipid composition. In comparison of the two related fish species a temperature effect on the energy budget was detected, which shows different patterns between the two eelpout species. This finding is accompanied by similar thermal patterns in growth and lipid composition.

5.1 Energetic differences between eurythermal and stenothermal fish

The eurythermal boreal eelpout displays high metabolic demands at all experimental temperatures. However, growth and reproduction can only occur when surplus energy is available (Pitcher and Hart, 1982; Wieser, 1986). Boutilier (1998) stated that surplus energy is channelled to growth rather than locomotion, if food availability as well as uptake rates are high. Therefore, when temperature influences baseline metabolism, it affects growth. Enhanced growth at the thermal optimum is due to lowered metabolic costs in both species. The potential of temperate species to grow over a larger temperature range is also known from other species like the Pit sculpin, *Cottius pitensis*, (Brown, 1989), the boreal shorthorn sculpin (Fonds et al., 1989), flatfishes (Fonds et al., 1992) or the brown bullhead, *Ictalurus nebulosus* (Jobling, 1994). In the eelpout this eurythermal feature is reflected in the distribution of populations from the White Sea to the Wadden Sea and within populations in habitats with a high yearly temperature amplitude. The differences in growth rate and terminal length between these *Zoarces viviparus* populations are in line with the latitudinal predictions of Van der Veer and co-workers (2003). More constant food densities (food availability per individual) at lower latitude select for small body volumes, since a small volume aids survival due to its lower maintenance costs (Van der Veer et al., 2003). Fluctuating food densities at high latitudes select for larger body volumes, because a large volume enables better survival over prolonged periods of starvation (Van der Veer et al., 2003). In evolutionary selection for body size the oxygen availability in the environment has to be taken into account as well. Small individuals have higher diffusion capacities than larger sized fish, because of a higher surface to volume ratio (Kooijmann, 2000). Therefore in colder water where the oxygen saturation is higher than in tropical areas, existence of large

animals is possible, as has been shown for squids (Pörtner, 2002b) and amphipods (Chapelle and Peck, 2004).

The more stenothermal *Pachycara brachycephalum* shows a distinct optimum in the experimental growth rate. The energy allocated to growth is surplus due to higher cellular efficiency, i.e. lower cellular costs at this temperature (Mark et al., 2005). At the whole animal level we did not see a decrease in metabolic demands, but a higher food adsorption efficiency, leaving even more energy for growth. The thermal range is not as restricted as in extremely stenothermal Antarctic fishes, for example in ice fish species, however, it is still narrower than in the temperate species. This stenothermal adaptation leads to high efficiencies and energy saving in a narrow temperature range at cellular levels (Lannig et al., 2003; Mark et al., 2005) and in food adsorption at the whole animal level, at the expense of narrow thermal windows. For eurythermal adaptation higher metabolic demands over a wider temperature spectrum are accepted and support a wider geographical distribution. The energetic demands for this strategy require availability of rich energy resources from the habitat.

Despite the differences in energetic strategies both species share the same pattern of growth optima, which is shifted to the upper thermal limits of each species (see conceptual figure, Figure 1). Maximal aerobic scope enhances growth capacities up to a point where energy resources (diet) and oxygen become limited. Until tissue oxygenation becomes limiting, growth is thermally stimulated with rising temperature. Fish with a higher respiratory capacity display a higher temperature of maximal growth than others with lower capacities (Fonds et al., 1992).

5.2 Implications on the ecosystem and geographical distribution

There is evidence of ecological impacts of recent climate change from polar terrestrial to tropical marine environments (Walther et al., 2002). However, consequences of global change on the distribution and survival of the two eelpout species in their actual habitat can only be roughly estimated. The impact of temperature change on the whole trophic network has to be taken into account as well.

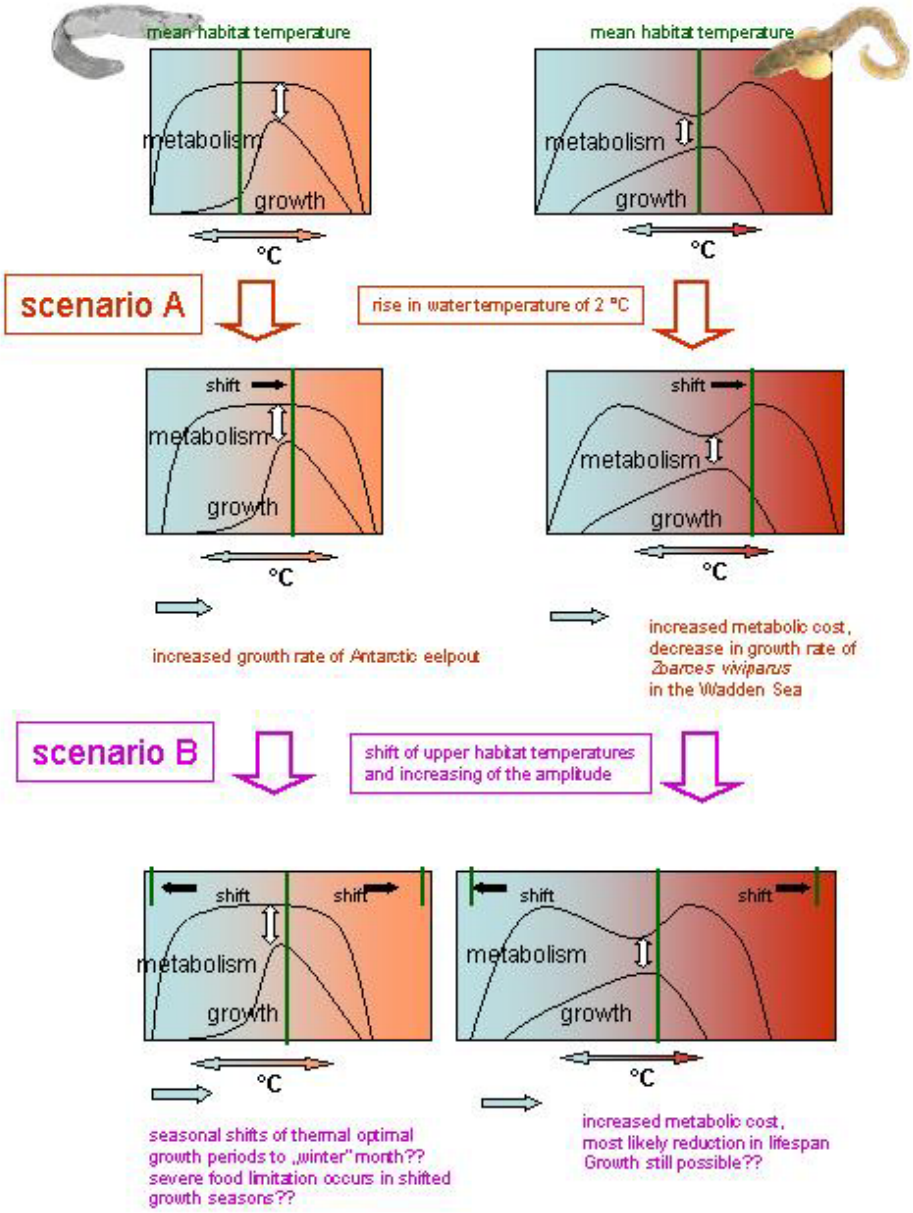


Figure 22:
Possible consequences of two scenarios of climate change on the growth performance and metabolic demands of *Pachycara brachycephalum* (left) and *Zoarces viviparus* (right). Scenario A depicts a temperature rise of 2 °C and the followed shift in habitat temperature. Scenario B depicts the increasing amplitude of climate events and temperatures.

On the ecological scale it is growth (and reproduction), which define survival of species in habitat and environment (Brett and Groves, 1979). Distribution and survival of a species under changing climate conditions depends among other things on the magnitude, velocity and time scale of the thermal change and the energy available in the ecosystem. If the magnitude and velocity of thermal change are moderate, acclimation to higher temperature seems to be possible for the Antarctic eelpout. Although the metabolic energy demands of *Pachycara brachycephalum* are stable during long-term acclimation, this acclimated accelerated growth is most likely combined with a higher gross energy demand (publication II). Sufficient supply with food in a region with already pulsed food availability will become a major problem. Models predict average global sea temperatures to rise by at least 2°C and in more severe scenarios an increase of the average global temperature by 5.8 °C by 2100 (IPCC, 2001; Houghton et al., 2001). The assumed rise in temperature would shift the habitat temperature at the Antarctic Peninsula towards or beyond the optimal growth temperature of *Pachycara brachycephalum* and therefore accelerated growth and higher population densities would be expected (Figure 22). An increase above 2 °C sea temperature or locally amplified increases, like the threefold higher increase in warming of Greenland compared to the global average (IPCC, 2001), would exceed the thermal window of this species. At the Antarctic Peninsula higher air temperatures have already been observed (Quayle et al., 2002). Even a change in temperature of a few degrees will affect predator – prey relationships in the marine food web (Jacob, 2005) and the energy resource will most likely change in quantity and quality. Recent investigations by Jacob (2005) depict the Antarctic marine food web as opportunistic within one trophic position, given the high dietary overlap, the generalist feeding nature of most of the species and capacity of diet switching. However, environmental change induces more than single species loss and already starts to affect Antarctic krill, *Euphausia superba* (Walther et al., 2002).

The boreal eelpout *Zoarces viviparus* in the Wadden Sea lives already at the thermal distribution limit (Van Dijk et al., 1999) and further rise in temperature will most likely exceed the acclimatization capacity of this population (Figure 22). Therefore the assumed temperature rise will most likely cause the extinction of the *Zoarces viviparus* Wadden Sea population. Additionally, the geographic position of the Wadden Sea makes this area very vulnerable to changes in the Gulf Stream, which have been reported and prognosticated (Bryden et al., 2005; Quadfasel, 2005) and which have a

great impact on all northern coastal areas. The predicted increased climate extremes, like storms (Houghton et al., 2001) will affect the distribution of mussel beds in the coastal areas, will reduce the abundances of old mussel bed communities and therefore will reduce a preferred habitat of *Zoarces viviparus*. Expected larger climate fluctuations (Houghton et al., 2001) will cause higher metabolic costs for this eurythermal fish species in other areas. An increase in temperature fluctuations may reduce the growth of the White Sea population. A shift in the growth pattern and an increased energy demand of the population has to be borne by the resources of the habitat.

In general, organisms have three mechanisms for coping with change: they can use physiological flexibility, evolve new adaptations, and migrate to appropriate sites (Peck, 2005). For both eelpout species climatic induced migration is unlikely on short time scales because of their lifestyle and the small-scale reproductive dispersal of the juveniles. Therefore if energy for growth is no longer available, it will cause extinction of at least local races and populations.

5.3 Further research

With regard to future research in comparative ecology and physiology I want to put forward some considerations and suggestions.

To get a clearer impression on thermal impact on the energy allocation and energy budget in the field, energy budgets at different temperatures with reduced ration and therefore limited energy supply should be investigated. Despite the experimental difficulties in the work with early life stages the changes in energy allocation during ontogeny would provide as well insights into the dynamics in the field.

In a similar set-up the fatty acid composition of single lipid classes, on the one hand membrane lipids, on the other storage lipids would be interesting to investigate. This separation of lipids by origin (membrane or cytosol) will enable to validate or falsify the assumptions in this thesis. Concerning the lipid metabolism in fish there are still gaps of knowledge especially in non-commercial fish species. The high amount of lipids in the diet and the tissue of Antarctic fish might indicate special metabolic pathways and enzyme adaptations, like those found recently in bacteria communities on whale falls in the deep-sea (Craig Smith, University of Hawaii, Manoa, Honolulu, pers. comm.).

A very general but important research area is the quantification of energy available in ecosystems in terms of food availability, especially concerning the quantity and the quality of the diet for predators.

A last topic is the application of the Dynamic Energy Budget (DEB) theory (Kooijmann, 2000) to the data of this thesis. This modelling approach combined with my data holds a high potential for further knowledge and reaction of fish of this ecotype to changing environmental requirements.

A moderate global warming would most likely not reduce the growth capacity of the Antarctic eelpout by a direct thermal effect. However, due to assumed changes in the food resources (e.g. in prey species such as amphipods) the already food limited situation will become more severe.

The distribution of the temperate eelpout *Zoarces viviparus* will most likely shift northwards like already found in other boreal fish species in the North Sea (Perry et al., 2005).

6. References

A

Ackman, R.G.e. (1989) *Marine biogenic lipids, Vol. 1*. CRC Press, Boca Raton USA.

Alam, M. S., Teshima, S. *, Yaniharto, D., Koshio, S., and Ishikawa, M. (2002) Influence of different dietary amino acid patterns on growth and body composition of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture, 210[1-4], 359-369.

Alvarez-Gonzalez, C. A. *., Civera-Cerecedo, R., Ortiz-Galindo, J. L., Dumas, S., Moreno-Legorreta, M., and Grayeb-Del Alamo, T. (2001) Effect of dietary protein level on growth and body composition of juvenile spotted sand bass, Paralabrax maculatofasciatus, fed practical diets. Aquaculture, 194[1-2], 151-159.

Andersen, N. G. (2001) A gastric evacuation model for three predatory gadoids and implications of using pooled field data of stomach contents to estimate food rations. Journal of Fish Biology, 59[5], 1198-1217.

Anderson, M.E. (1984) *Zoarcidae: development and relationship*. Special publications. American Society of Ichthyologists and Herpetologists, 17, 578-582.

Anderson. M. E. (1989) Review of the eelpout genus Pachycara Zugmayer, 1911 *(Teleostei: Zoarcidae)* with descriptions of six new species. Proceedings of the California Academy of Science, 46, 221-242.

Anderson, M.E. (1990) Zoarcidae. *Fishes of the Southern Ocean* (Gon, O. and Heemstra, P. C.), p. 462pp. 12pls. J. L. B. Smith Institute of Ichthyology, Grahamstown , South Africa.

Anderson, M. E. (1991) Studies on the Zoarcidae (Teleostei: Perciformes) of the Southern Hemisphere. 5. Two new species from the Weddell Sea, Antarctica. Cybium. Paris 15[2], 151-158.

Anderson, M. E. (1994) Systematics and osteology of the Zoarcidae (Teleostei: Perciformes). Ichthyological Bulletin, no. 60, 120 pp.

Anwar, M. F. and Jafri, A. K. (2001) The influence of dietary lipid levels on growth, feed conversion and carcass composition of fingerling Indian major carp *Labeo rohita*. Aquaculture 2001: Book of Abstracts, World Aquaculture Society, 143 J.M Parker Coliseum Louisiana State University Baton Rouge LA 70803 USA, 2001, 22.

Arntz, W. and Brey, T. (2001) *The Expedition ANTARKTIS XVII/3 (EASIZ III) of RV "Polarstern" in 2000*. Reports on Polar and Marine Research , 402.

Arntz, W.E. and Brey, T. (2005) *The Expedition ANTARKTIS XXI / 2 (BENDEX) of RV "Polarstern" in 2003 / 2004*. Reports on Polar and Marine Research, 503.

Arntz, W.E., Brey, T. and Gallardo, V.A. (1994) *Antarctic Marine Zoobenthos*. Oceanography and Marine Biology Annual Reviews, 32, 241-304.

Arntz, W.E. and Gutt, J. (1999) *The Expedition ANTARKTIS XV/3 (EASIZ II)*. Report on Polar and Maine Research, 301.

Arntz, W.E., Gutt, J. and Klages, M. (1997) Antarctic Marine Biodiversity: an overview. *Antarctic Communities. Species, Structure and Survival* (Battaglia, B., Valencia, J., and Walton, D. W. H.), pp. 3-14. Cambridge University Press, Cambridge.

Arntz, W.E. and Tarazona, J. (1990) Effects of El Niño 1982-83 on benthos, fish and fisheries off the South American Pacific Coast, (P W Glynn). *Global ecological consequences of the 1982-83 El Niño-southern oscillation* (Glynn, P. W.), pp. 323-360 . Elsevier oceanography series 52, Amsterdam.

B

Babkov, A.I. (1982) A short hydrological characteristic of Chupa Inlet in the White Sea . *Ecological investigations of species perspective for aquaculture in the White Sea* (Kulakowski, E. E.), pp. 94-98. Zoological Institute Leningrad, Leningrad.

Bagarinao, T. and Chua, T.-E. (1986) Egg size and larval size among teleosts: implications to survival potential. *Proceedings of the First Asian Fisheries Forum*, 651-656.

Barrera-Oro, E. (2003) Analysis of dietary overlap in Antarctic fish (Notothenioidei) from the South Shetland Islands:no evidence of food competition. *Polar Biology*, 26, 631-637.

Beamish, R.J. (1995) Climate change and Northern Fish Populations. *Canadian Special Publication of Fisheries and Aquatic Science*, 121, 739ff.

Beermann-Schleiff, S. (1988) Ein Beitrag zur Biologie der Aalmutter (Zoarces viviparus Linneaus 1758) aus dem Dithmarscher Wattenmeer. Diplomarbeit an der Universität Hamburg.

Behrens, D.W. (1977) Fecundity and reproduction of the viviparous perches Hypsurus caryi (Agassiz) and Embriotoca jacksoni Agassiz. California Fish and Game, 63, 234-252.

Berghahn, R., Waltemath, M., and Rijnsdorp, A. D. (1992) Mortality of fish from the by-catch of shrimp vessels in the North Sea. Journal of applied ichthyology/Zeitschrift fur angewandte Ichthyologie. Hamburg, Berlin, Vol. 8, no. 1-4, pp. 293-306.

Bernardi, G. and DeVries, A. L. (1994) Cytochrome b gene sequences from two eelpouts (Perciformes, Zoarcidae) from McMurdo Sound (Antarctica): Implications on the antifreeze gene structure. Antarctic Journal of the United States, 29[5], 159-161.

Bertalanffy, L.v. (1938) A quantitative theory of organic growth (inquiries on growth laws. II). Human Biology, 10, 181-213.

Bertalanffy, L.v. (1934) Untersuchungen über die Gesetzlichkeit des Wachstums, I. Teil : Allgemeine Grundlagen der Theorie; Mathematische und physiologische Gesetzlichkeiten des Wachstums bei Wassertieren. Wilhem Roux' Archiv für Entwicklungsmechanik der Organismen, 131, 613-652.

Bertalanffy, L.v., Beier, W. and Laue, R. (1977) *Biophysik des Fließgleichgewichts*. Akademie-Verlag, Berlin.

Bertalanffy, v.L. (1951) *Theoretische Biologie: Stoffwechsel und Wachstum*. Francke AG Verlag, Bern.

Beukema, J.J. and Cadee, G.C. (1997) Local differences in macrozoobenthic response to enhanced food supply caused by mild eutrophication,. Limnology and Oceanography, 42, 1424-1435.

Beverton, R.J.H. (1992) Patterns of reproductive strategy parameters in some marine teleost fishes. Journal of Fish Biology, 41, 137-160.

Bjornsson, B. and Steinarsson, A. (2002) The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences. Vol. 59, no. 3, pp. 494-502.

Bleil, M. and Oeberst, R. (1993) On the accuracy of cod fecundity estimations. ICES COUNCIL MEETING PAPERS, ICES, COPENHAGEN (DENMARK) CM D48, 1-14.

Boutilier, R.G. (…) Physiological ecology in cold ocean .sheries: a case study in Atlantic cod. *Cold Ocean Physiology* (Pörtner, H. O. and Playle, R.), p. 463–489. Cambridge

University Press, London.

Brander, K. (1994) Spawning and life history information for North Atlantic cod stocks. ICES cooperative research report, 205, 64-97.

Brett, J.R. (1979) Environmental factors and growth. *Fish Physiology Volume 8: Bioenergetics and growth* (Hoar, W. S., Randall, D. J., and Brett, J. R.), pp. 599-675. Academic Press, New York.

Brett, J.R. and Groves, T.D. (1979) Physiological energetics. *Fish Physiology* , 8, 279-352.

Brett, J.R., Shelbourn, J.E. and Shoop, C.T. (1969) Growth rate and body composition of fingerling sockeye salmon, *Onchrhynchus nerka*, in relation to temperature and ration size. Journal of Fisheries Research Board Canada, 26, 2363-2394.

Brockington, S. (2001) The seasonal energetics of the Antarctic bivalve *Laternula elliptica* (King and Broderip) at Rothera Piont, Adelaide Island. Polar Biology, 24, 523-530.

Brodte, E. (2001) Wachstum und Fruchtbarkeit der Aalmutterarten Zoarces viviparus (Linne) und Pachycara brachycephalum (Pappenheim) aus unterschiedlichen klimatischen Regionen. Diplom thesis im Fach Biologie, Universität Bremen, Fachbereich *2*.

Brodte, E., Knust, R., Ulleweit, J., Fonds, M. and Arntz, W.E. *Zoarces viviparus* - aspects of growth in latitudinal and temporal scale. in preparation.

Bromley, P. J., Ravier, C., and Witthames, P. R. The influence of feeding regime on sexual maturation, fecundity and atresia in first-time spawning turbot. Journal of Fish Biology. 56[2], 264-278. 2000.

Brown, L.R. (1989) Temperature preferences and oxygen consumption of three species of sculpin (*Cottus*) from the Pit River drainage, California. Environmental Biology of Fishes, 26, 223-236.

Bryden , H.L., Longworth, H.R. and Cunningham, S.A. (2005) Slowing of the Atlantic meridional overturning circulation at 25°N. Nature, 438, 655-657.

Burns, J. M. , Trumble, S. J., Castellini, M. A., and Testa, J. W. (1998) The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biology 19[4], 272-282.

Böhncke, G. and Dietrich, G. (1951) Monatskarten der Oberflächentemperaturen für die Nord - und Ostsee und die angrenzenden Gewässer. Deutsches Hydrographisches Institut, Hamburg.

C

Carter, C., Houlhan, D., Kiessling, A., Medale, F., and Jobling, M. (2001) *Physiological effects on feeding. Food Intake in Fish*, Iowa State University Press, 2121 S. State Avenue Ames IA 50014 USA.

Catacutan, M. R. *., Pagador, G. E., and Teshima, S. (2001) Effect of dietary protein and lipid levels and protein to energy ratios on growth, survival and body composition of the mangrove red snapper, *Lutjanus argentimaculatus* (Forsskal 1775). Aquaculture Research 32[10], 811-818.

Cavalli, L., Chappaz, R., Bouchard, P., and Brun, G. *. (1997) Food availability and growth of the brook trout, *Salvelinus fontinalis* (Mitchill), in a French Alpine lake. Fisheries Management and Ecology 4[3], 167-177.

Chapelle, G. and Peck, L.S. (2004) Amphipod crustacean size spectra: new insights in the relationship between size and oxygen. OIKOS, 106, 167-175.

Christiansen, F. B., Nielsen, V. H., and Simonsen, V. (1988) Genetics of *Zoarces* populations. 15. Genetic and morphological variation in Mariager Fjord. Hereditas 109[1], 99-112.

Clarke, A. (1983) Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr Mar Biol Annu Rev, 21, 341-453.

Clarke, A. (1991) What is cold adaptation and how should we measure it? American Zoologist, 31, 81-92.

Clarke, A. and Crame, J.A. (1989) The origin of the southern Ocean marine fauna. *Origins and Evolution of the Antarctic Biota* (Crame, J. A.), pp. 253-268. The Geological Society, London.

Clarke, A. and Fraser, K.P.P. (2004) Why does metabolism scale with temperature? Functional Ecology, 18, 243ff.

Clarke, A. and Johnston, N.M. (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology, 68, 893-905.

Clarke, A. and North, A.W. (1991) Is growth of polar fish limited by temperature? *Biology of Antarctic Fish* (Di Prisco, G., MAresca, B., and Tota, B.), pp. 54-69. Springer Verlag, Berlin.

Coggan, R. (1997a) Growth:Ration relationships in the antarctic fish *Notothenia corriceps* Richardson maintained under different conditions of temperature and photoperiod. Journal of Experimental Marine Biology and Ecology 210[1], 23-35.

Coggan, R. (1997b) Seasonal and annual growth rates in the Antarctic fish *Notothenia corriceps* Richardson. Journal of Experimental Marine Biology and Ecology 213[2], 215-229.

Crockett, E. L. and Sidell, B. D. * (1990) Some pathways of energy metabolism are cold adapted in Antarctic fishes. Physiological Zoology 63[3], 472-488.

Cui, Y., Hung SSO*, and Zhu, X. (1996) Effect of ration and body size on the energy budget of juvenile white sturgeon. Journal of Fish Biology 49[5], 863-876.

Cui Yibo and Liu Jiankang. (1990a) Comparison of energy budget among six teleosts -- 1. Food consumption, faecal production and nitrogenous excretion. Comparative Biochemistry and Physiology, A, vol. 96A, no. 1, pp. 163-171.

Cui Yibo and Liu Jiankang. (1990b) Comparison of energy budget among six teleosts - 2. Metabolic rates. Comparative Biochemistry and Physiology, A, vol. 97A, no. 2, pp. 169-174.

Cui Yibo and Liu Jiankang. (1990c) Comparison of energy budget among six teleosts. 3. Growth rate and energy budget. Comparative Biochemistry and Physiology, A , vol. 97A, no. 3, pp. 381-384.

Cui Yibo and Liu Jiankang. (1990d) Comparison of energy budget among six teleosts - 4. Individual differences in growth and energy budget. Comparative Biochemistry and Physiology, A, vol. 97A, no. 4, pp. 551-554.

Cui Yibo and Wootton, R. J. (1990) Components of the energy budget in the European minnow, *Phoxinus phoxinus* (L.) in relation to ration, body weight and temperature. Acta hydrobiologica sinica/Shuisheng Shengwu Xuebao. Wuhan, 14[3], 193-204.

Cushing, D.H. (1982) *Climate and fisheries*. Academic Press, Lomdon, New York.

Cushing, D.H. (1975) *Marine ecology and fisheries*. Cambridge University Press, Cambridge.

Cushing, D.H. (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Adv. Mar. Biol. 26, 249–293.

D

Dansgard, B., Arnesen, A. M., and Jobling, M. (1999) Seasonal patterns of feed intake and growth of Hammerfest and Svalbard Arctic charr maturing at different ages. Aquaculture 171[1-2], 149-160.

Dippner, J.W. and Ottersen, G. (2001) Cod and climate variability in the Barents Sea. Climate research, 17, 73-82.

Dorrien C.F.,von. (1993) Ecology and respiration of selected Arctic benthic fish species. Berichte zur Polarforschung/Reports on polar research. Bremerhaven, no. 125, 104 pp.

E

Ekau, W. (1988) Ecomorphology of nototheniid fish from the Weddell Sea, Antarctica. Berichte zur Polarforschung/Reports on polar research. Bremerhaven,51, 140pp.

El-Dahhar, A. A. (2001) Protein and energy requirements of striped mullet *Mugil cephalus* larvae. Aquaculture 2001: Book of Abstracts, World Aquaculture Society, 143 J.M Parker Coliseum Louisiana State University Baton Rouge LA 70803 USA, 2001, 204.

Elliott, J. M. (1976a) Energy losses in the waste products of brown trout (*Salmo trutta* L.). The Journal of Animal Ecology. 45[2], 561-580.

Elliott, J. M. (1979b) Body composition of brown trout (*Salmo trutta* L.) in relation to temperature and ration size. The Journal of Animal Ecology. 45[1], 273-289.

Elliot, J.M. (1976c) The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to Body weight, water temperature and ration size. The Journal of Animal Ecology, 45, 923-948.

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

Everson, I. (1977) The living ressources of the Southern Ocean. FAO Southern Ocean Fisheries Survey Program, Rome, GLO/SO/77/1, 156 pp.

F

Farkas, T., Csengeri, I., Majoros, F. and Olah, J. (1980) Metabolism of fatty acids in fish 3. combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linnaeus 1758. Aquaculture, 20, 29-40.

Farkas, T., Dey, I., Buda, C. and Halver, J.E. (1994) Role of phospholipid molecular species in maintaining lipid membrane structure in response to temperature. Biophys. Chem, 50, 147-155.

Farkas, T. *., Fodor, E., Kitajka, K., and Halver, J. E. (2001) Response of fish membranes to environmental temperature. Aquaculture Research 32[8], 645-655.

Fischer, P., Kils, U., and Walker, U. (1990) In-situ investigations on the behaviour and respiration of the three-spined stickleback (*Gasterosteus aculeatus* L.) and the eelpout (*Zoarces viviparus* L.). ICES COUNCIL MEETING 1990 (COLLECTED PAPERS)., ICES, COPENHAGEN (DENMARK) , 10 pp.

Fischer, T. (2003) The effects of climate induced temperature changes on cod (*Gadus morhua* L.): Linking ecological and physiological investigations. Berichte zur Polar- und Meeresforschung, 453.

Folch, J., Lees, M. and Stanley, G.H.S. (1957) A simple method for isolation and purification of total lipid from animal tissue. Journal of Biological Chemistry, 266, 467-509.

Fonds, M., Cronie, R., Vethaak, A.D. 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. Netherlands Journal of Sea Research, 29, 127-143.

Fonds, M., Drinkwaard, B., Resink, J.W., Eysink, G.G.J. and Toet, W. (1989) Measurements of metabolism, food intake and growth of *Solea solea* (L.) fed with mussel meat or with dry food. *Aquaculture- A biotechnology in progress*.

Fonds, M., Jaworski, A., Iedema, A., and Puyl, P. V. D. (1989) Metabolism, food consumption, growth and food conversion of shorthorn sculpin (*Myoxocephalus scorpius*) and eelpout (*Zoarces viviparus*). ICES COUNCIL MEETING 1989 (COLLECTED PAPERS)., ICES, COPENHAGEN (DENMARK), 19 pp.

Fraser, A. J., Sargent, J. R., Gamble, J. C., and MacLachlan, (1987) P. Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. ,AM. FISH. SOC. SYMP. SER. 2, 129-143.

Frederich, M. and Pörtner, H.-O. (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, Maja squinado . Am J Physiol Regul Integr Comp Physiol, 279, R1531-R1538.

Fry, F.E.J. (1971) The effect of environmental factors on the physiology of fish. Fish Physiology, 6, 1-98.

G

Gibson Gaylord, T. and Gatlin, D. M. (2001). Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). Aquaculture 194[3-4], 337-348.

Gnaiger, E. and Bitterlich, G. (1984) Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia, 62, 289-298.

Goetting, K. J. (1976) Reproduction and oocyte-development in *Zoarces viviparus* (Pisces, Osteichth.). Helgol. Wiss. Meeresunters. 28[1], 71-89.

Greene, D. H. (1990) Lipid metabolism in fish. *Fish Oils in Nutrion*, pp. 226-246.

Grisdale-Helland, B. *., Ruyter, B., Rosenlund, G., Obach, A., Helland, S. J., Sandberg, M. G., Standal, H., and Roesjoe, C. (2002) Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. Aquaculture 207[3-4], 311-329.

Grove, T.J. and Sidell, B.D. (2004) Fatty acyl CoA synthetase from Antarctic notothenioid fishes may influence substrate specificity of fat oxidation. Comparative Biochemistry and Physiology , Part B, 139, 53-63.

Guderley, H. , St.Pierre, J., Couture, P. and Hulbert, A.J. (1997) Plasticity of properties of mitochondria from rainbow trout red muscle with seasonal acclimatization. Fish Physiology and Biochemistry, 16, 531-541.

Gunderson, D.R. (1997) Trade-off between reproductive effort and adult survival in oviparous and viviparous fishes. Can. J. Fish. Aquat. Sci., 54, 990-998.

H

Hardewig, I., Van Dijk, P.L.M., Moyes, C.D. and Pörtner, H.O. (1999) Temperature-Dependent Expression of Cytochrome-C Oxidase in Antarctic and Temperate Fish. Am J Physiol, 277, R508-R516.

Hardewig, I., Van Dijk, P.L.M. and Pörtner, H.O. (1998) High-Energy Turnover at Low Temperatures: Recovery From Exhaustive Exercise in Antarctic and Temperate Eelpouts. Am J Physiol, 43, R1789-R1796.

Hazel, J.R. (1995) Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol., 57, 19-42.

Hochachka, P. W. (1988) Channels and pumps--determinants of metabolic cold adaptation strategies. Comparative Biochemistry and Physiology, B, MARINE BIOLOGY OF ANTARCTIC ORGANISMS. vol. 90B, [no. 3], pp. 515-519.

Hochachka, P.W. and Somero, G.N. (1984) *Biochemical Adaptation*. Princeton University Press .

Houde, E. D. (1989) Comparative growth, mortality, and energetics of marine fish larvae: Temperature and implied latitudinal effects. Fishery Bulletin 87[3], 471-495.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K. and Johnson, C.A. (2001) *Climate Change 2001: The Scientific Basis*. University Press, New York.

Houlihan, D.F. (1991) Protein turnover in ectotherms and its relationships to energetics. *Advances in Comparative and Environmental Physiology* (Gilles, R.), pp. 1-43. Springer-Verlag , Berlin Heidelber.

I

Iedema, A. (1989) Metingen van groei, voedselopname en zuurstof consumptie van de puitaal, *Zoarces viviparus* L. Stageverslag voor studie biologie aan de Rijkssuniversiteit Groningen.

IPCC (2001) *Climate Change 2001: Synthesis Report. A Contribution of the Working Groups I, II and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.

J

Jacob, U., Mintenbeck, K., Brey, T., Knust, R. and Beyer, K. (2005) Stable isotope food web studies: a case for standardized sample treatment. Marine ecology-progress series, 287, 251-253.

Jangaard, P.M., Ackman, R.G. and Sipos, J.C. (1967) Seasonal changes in fatty acid composition of cod liver, flesh. roe, and milt lipids. Journal of Fisheries Research Board of Canada, 24, 613-627.

Jobling, M. (1994) *Fish Bioenergetics*. Chapman and Hall, New York.

Jobling, M. (1986) Gastrointestinal overload - a problem with formulated feeds? Aquaculture , 51, 257-263.

Jobling, M. (1987) Growth of Arctic charr (*Salvelinus alpinus* L.) under conidtion of constant light and temperature. Aquaculture, 60, 243-249.

Jobling , M. and Bendiksen, A.A. (2003) Dietary lipids and temperature interact to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr. Aquaculture Research, 34, 1423-1441.

Johnston, I. A. and Battram, J. (1993) Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. Marine biology. Berlin, Heidelberg, vol. 115[no. 1], pp. 7-14.

Johnston, T.P. and Bennett, A.F. (1995) The thermal acclimation of burst escape performance in fish: An intregated study of molecular and cellular physiology and organismal performance. Journal of Experimental Biology, 198, 2165-2175.

Jol, J., Kater, B. and Duin, R. (2001) *De Biologie, Ecologie en Populatiedynamica van Puitaal (Zoarces viviparus L) in de Nederlandse Kustwateren en Estuaria*. Minsterie van Verkeer en Waterstaat , Rijksinstituut voor Kust en Zee / RIKZ, Kamperland.

K

Kattner, G. and Fricke, H.S.G. (1986) Simple gas-liquid chromatographic method for simultaneous detremination of fatty acids and alcohols in wax esters of marine organisms. Journal of Chromatographie, 361, 263-268.

Kawall, H. G. and Somero, G. N. (1996) Temperature compensation of enzymatic activities in brain of antarctic fishes: Evidence for metabolic cold adaptation. Antarctic Journal of the United States [Antarct. J. U.S.] 31[2], 115-117.

Knijn, R.J., Boon, T.W., Heessen, J.L. and Hisloop, J.R.G. (1993) *Atlas of the North Sea fishes*. ICES, Cooperative research report, 194.

Kock, K.-H. and Shimadzu, Y. (1994) Trophic relationships and trends in population size and reproductive parameters in Antarctic high-level predators. *Southern Ocean Ecology: The BIOMASS Perspective* (El-Sayed, S.), pp. 287-312. Cambridge University Press, Cambridge.

Kooijman, S.A.L.M. (2000) *Dynamic Energy and Mass Budgets in Biological Systems*. University Press, Cambridge.

Korsgaard, B. (1994) Calcium metabolism in relation to ovarian functions during early and late pregnancy in the viviparous blenny Zoarces viviparus. Journal of Fish Biology, 44[4], 661-672.

Korsgaard, B. (1986) Trophic adaptations during early intraovarian development of embryos of Zoarces viviparus (L.). Journal of Experimental Marine Biology and Ecology, 98[1-2], 141-152.

Koya, Y., Ikeuchi, T., Matsubara, T., Adachi, S., and Yamauchi, K. (1994) Embryonic growth during gestation of the viviparous eelpout, Zoarces elongatus. Japanese Journal of Ichthyology, 41[3], 338-342.

Kristoffersson, R. and Oikari, A. (1975) Notes on the biology of the eel-pout, Zoarces viviparus (L.), in the brackish water of Tvaerminne, Gulf of Finland. Annales Zoologici Fennici, 12[2], 143-147.

L

La Mesa, M. and Vacchi, M. (2001) Review. Age and growth of high Antarctic notothenioid fish. Antarctic Science 13[3], 227-235.

Lambert, Y. and Dutil, J.-D. (1997) Condition and energy reserves of Atlantik cod (Gadus Morhua) during the collapse of the northern Gulf of St. Lawrence stock. Can. J. Fish. Aquat. Sci., 54, 2388-2400.

Lannig , G. , Storch, D. and Pörtner, H.O. (2005) Aerobic mitochondrial capacities in Antarctic and temperate eelpout (Zoarcidae) subjected to warm versus cold acclimation . Polar Biology, 28, 575-584.

Le Cren, E.D. (1951) The length - weight relationship and seasonal cycle in gonad weight and condition in the perch *(Perca fluviatilis)*. J. Anim. Ecol., 20, 201-219.

Lee, S., Cho, S., and Kim, D. (2000) Effects of feeding frequency and dietary energy level on growth and body composition of juvenile flounder, *Paralichthys olivaceus* (Temminck and Schlegel). Aquaculture Research. Vol. 31, no. 12, pp. 917-921.

Lipski, J. (2001) AMLR 2000/2001 Field season report - Objectives , accomplishment and tentative conclusions. 187pp. NOAA-TM-NMFS-SWFSC-314, Southwest Fisheries Science Center - Antarctic Ecosystems Research Division.

Lucassen, M., Schmidt, A., Eckerle, L.G. and Pörtner, H.-O. (2003) Mitochondrial proliferation in the permant vs. temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. Am. J. Physiol Regul Integr Comp Physiol, 285, R1410-R1420.

M

Maciejewska, K., Opalinski, K. W., and Margonski, P. (2001) Energy budget in early stages of smelt Osmerus eperlanus (L.). Bulletin of the Sea Fisheries Institute, Gdynia. Gdynia, no. 153, pp. 53-61.

Malloy, K. D. and Targett, T. E. (1991) Feeding, growth and survival of juvenile summer flounder Paralichthys dentatus : Experimental analysis of the effects of temperature und salinity. Marine ecology progress series. Oldendorf, 72[3], 213-223.

Mark, F.C., Bock, C. and Pörtner, H.O. (2002) Oxygen limited thermal tolerance in Antarctic

fish investigated by magnetic resonance imaging (MRI) and spectroscopy (^{31}P-MRS). Am. J Physiol Regul. Integr Comp Physiol, 283, R1254-R1262.

Mark, F.C., Hirse, T. and Pörtner, H.-O. (2005) Thermal sensitivity of celular energy budgets in Antarctic fish hepatocytes. Polar Biology, 28, 805-814.

Mark, F.C., Lucassen, M. and Pörtner, H.-O. (2004) Are mitochondrial uncoupling proteins involved in thermal acclimation in temperate and polar fish? Physiological Genomics, submitted.

Marshall, C.T., Yaragina, N.A., Ådlandsvik, B. and Dolgov, A.V. (2000) Reconstructing the stock–recruit relationship for Northeast Arctic cod using a bioenergetic index of reproductive potential. Can. J. Fish. Aquat. Sci., 57, 2433-2442.

Matallanas, J., Rucabado, J., Lloris, D., and Olivar, M. P. (1990) Early stages of development and reproductive biology of the South-American eelpout Austrolycus depressiceps Regan, 1913 (Teleostei: Zoarcidae). Scientia Marina (Barcelona), 54[3], 257-261.

Morescalchi, A., Morescalchi, M. A., Odierna, G., Stingo, V., and Capriglione, T. (1996) Karyotype and genome size of zoarcids and notothenioids (Teleostei, Perciformes) from the Ross Sea: Cytotaxonomic implications. Polar Biology, 16[8], 559-564.

N

Netzel, J. and Kuczynski, J. (1995) The abundances of flounder (*P. flesus* L.) and eelpout (*Z. viviparus* L.) in the Gulf of Gdansk area estimated by swept area method. ICES Council Meeting Papers. 8 pp. CM J:30.

Nicieza, A. G., Reyes-Gavilan, F. G., and Brana, F. (1994) Differentiation in juvenile growth and bimodality patterns between northern and southern populations of Atlantic salmon (Salmo salar L.). Canadian Journal of Zoology/Revue Canadien de Zoologie, 72[9], 1603-1610.

Nienhuis, P. H., Smaal, A. C., and Knoester, M. (1994) *The Oosterschelde Estuary: An evaluation of changes at the ecosystem level induced by civil-engineering works*. Hydrobiologia.

North, A. W. (1988) Age of Antarctic fish: Validation of the timing of annuli formation in otoliths and scales. Cybium. Paris, 12[2], 107-114.

North, A.W. (1998) Growth of young fish during winter and summer at South Georgia. Polar Biology, 19, 198-205.

North, A. W., White, M. G., and Burchett, M. S. (1980) Age Determination of Antarctic Fish. CYBIUM (3E SER.) 8, 7-11.

Norton, E. C., Macfarlane, R. B., and Mohr, M. S. (2001) Lipid class dynamics during development in early life stages of shortbelly rockfish and their application to condition assessment. Journal of Fish Biology, 58[4], 1010-1024.

Nyssen, F., Brey, T., Dauby, P. and Graeve, M. (2005) Enhanced analysis of trophic position of Antarctic amphipods revealed by a 2- dimensional biomarker assay. Marine Ecology Progress, in press.

Nyssen, F., Brey, T., Lepoint, G., Bouquegneau, J.M., DeBroyer, C. and Dauby, P. (2002) A stable isotope approach to the eastern Weddell Sea trophic web: focus on benthic amphipods. Polar Biology, 25, 280-287.

O

O'Brien, C.M., Fox, C.J., Planque, B. and Casey, J. (2000) Climate variability and North Sea cod . Nature, 404, 142.

Odum, E. (1971) *Fundamentals of ecology*. Saunders, Philadelphia.

Ojaveer, H. (1997) Composition and dynamics of fish stocks in the Gulf of Riga ecosystem. Diss. Biol. Univ. Tartuensis, Tartu University Press, Tartu (Estonia) no. 31, 138 pp.

Ojaveer, H. and Lankov, A. (1997) Adaptation of eelpout, *Zoarces viviparus* (L.), to spatially changing environment on the coastal slope of the Gulf of Riga (Baltic Sea). ICES Council Meeting Papers. 12 pp. CM, EE:03.

Ojaveer, H. and Lärv, L. (2003) Eelpout, *Zoarces viviparus* L. *Fishes of Estonia* (E. Ojaveer, E. Pihu and T. Saat), pp. 316-323. Estonian Academy Publishers, Tallinn.

Ojaveer, H., Turovsky, A., and Lankov, A. (1996) Some characteristics of eelpout (Zoarces viviparus) stock in the Gulf of Riga. Proceedings of Polish-Swedish Symposium on Baltic Coastal Fisheries. Resources and Management. pp. 153-163.

Olsen, R. E. and Henderson, R. J. (1989) The rapid analysis of neutral and polar marine lipids

using double-development HPTLC and scanning densitometry. Journal of Experimental Marine Biology and Ecology, 129[2], 189-197.

Otterlei, E., Nyhammer, G., Folkvord, A., and Stefansson, S. O. (1999) Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod. Canadian Journal of Fisheries and Aquatic Sciences, 56[11], 2099-2111.

P

Pannella, G. (1980) Growth patterns in fish sagittae. *Skeletal growth of Aquatic organisms* (Rhoads, D. C. and Lutz, R. A. ed.), pp. 519-560. Pleum Press, New York.

Pannella, G. (1974) Otolith growth patterns: An aid in age determination in temperate and tropical fishes. Begenal, T. B. *The Aging of Fish*. The proceedings of an International Symposium on the Ageing of Fish, sponsored by The European Inland Fisheries Advisory Commission of FAO, The Fisheries Society of the British Isles and The Freshwater Biological Association, and held at the University of Reading, England on 19-20 July, 1973 , 28-39.

Pauly, D. (1979) Gill size and temperature as governing factors in fish growth: a generalization of von Bertalanffy's growth formula. Ber. Inst. Meereskd. Christian-Albrecht-Univ. Kiel 63, 156p.

Pauly, D. (1980) On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. J. Cons. CIEM, 39[2], 175-192.

Pearson, L.S. and Lear, W.H. (2001) Climate variability and marine ecosystemsimpacts: a North Atlantic perspective. Prog. Oceanogr. , 49, 167-188.

Peck, L.S. (2002) Ecophysiology of antarctic marine ectotherms: limits to life. Polar Biology, 25, 31-40.

Peck, L.S. (2005) Prospects for surviving climate change in Antarctic aquatic species. Frontiers in Zoology, 2.

Pekkarinen, M. (1980) Seasonal variations in lipid content and fatty acids in the liver, muscle and gonads of the eel-pout, *Zoarces viviparus* (Teleostei) in brackish water. Ann. Zool. Fenn. 17(4), 249-254.

Pepin, D. C., Orr, D. C., and Anderson, J. T. (1997) Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). SELECTED PROCEEDINGS OF THE SYMPOSIUM ON THE BIOLOGY AND ECOLOGY OF NORTHWEST ATLANTIC COD, 1997, pp. 2-10, Canadian Journal of Fisheries and Aquatic Sciences/Journal Canadien des Sciences Halieutiques et Aquatiques. Ottawa, vol. 54, no. Suppl. 1.

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

Pitcher, T.J. and Hart, P.J.B. (1982) *Fisheries ecology*. Croom Helm, London and Canberra.

Pörtner, H.O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., Lannig, G., Naevdal, G., Nedenes, A., Nyhammer, G., Sartoris, F.J., Serendero, I., Sirabella, P., Thorkildsen, S. and Zakhartsev, M. (2001) Climated induced tmperature 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*). Continental Shelf Research, 21, 1975-1997.

Purchase, C. F. and Brown, J. A. (2000) Interpopulation differences in growth rates and food conversion efficiencies of young Grand Banks and Gulf of Maine Atlantic cod (*Gadus morhua*). Canadian journal of fisheries and aquatic sciences/Journal canadien des sciences halieutiques et aquatiques. Ottawa ON, Vol. 57, no. 11, pp. 2223-2229.

Pütter, A. (1920) Studien über physiologische Ähnlichkeit VI. Wachstumsähnlichkeiten. Pflügers Arch., 180.

Pörtner, H.-O. (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften, 88, 137-146.

Pörtner, H. O. (2002a) Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. Journal of Experimental Biology, 205[15], 2217-2230.

Pörtner, H.-O. (2002b) Environmental and functional limits to muscular exercise and body size in marine invertebrate athletes. Comparative Biochemistry and Physiology Part A, 133, 303-321.

Pörtner, H.O., Lucassen, M. and Storch, D. (2005) Metabolic biochemistry, an integrative view. *The Physiology of Polar Fishes* (Steffensen, J. F. and Farrell, A. P.), series Fish Physiology .

Pörtner, H.-O., Mak, F.C. and Bock, C. (2004) Oxygen limited thermal tolerance in fish? Answered obtained by nuclear magnetic resonance techniques. Respiratory Physiology and Neurobiology , 141, 243-260.

Pörtner, H.O., Van Dijk, P.L.M., Hardewig, I. and Sommer A. (2000) Levels of Metabolic Cold Adaptation: Tradeoffs in Eurythermal and Stenothermal Ectotherms. *Antarctic Ecosystems:Models for a Wider Understanding* (Davison, W. and Williams, C. W.), pp. 109-122. Caxton Press, Christchurch, New Zealand .

Q

Quadfasel, D. (2005) The Atlantic heat conveyor slows. Nature, 438, 565-566.

Quayle, W.C., Peck, L.S., Peat, H., Ellis-Evans, J.C. and Harrigan, P.R. (2002) Extreme responses to climate change in Antarctic lakes. Science, 295, 645pp.

R

Ricker, W.E. (1975) Computations and interpretations of biological statistics of fish populations. Bulletin of the Research Board of Canada, 191, 1-382.

Ricker, W.E. (1979) Growth rates and models. Fish physiology. Bioenergetics and growth, 8.

Rocha, L.A., Robertson, D.R., Rocha, C.R., Van Tassell, J.L., Craig, M.T. and Bowen, B.W. (2005) Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. Molecular Ecology, 14, 3921.

Roff, D. A. (1982) Reproductive strategies in flatfish: A first synthesis. Canadian Journal of Fisheries and Aquatic Sciences, 39[12], 1686-1698.

Rosas, C., Martinez, E., Gaxiola, G., Brito, R., Sanchez, A., and Soto, L. A. (1999) The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (Linnaeus) juveniles. Journal of Experimental Marine Biology and Ecology, 234[1], 41-57.

S

Sampaio, L. A. and Bianchini, A. (2002) Salinity effects on osmoregulation and growth of the euryhaline flounder Paralichthys orbignyanus. Journal of Experimental Marine Biology and Ecology, 269[2], 187-196.

Schmidt, J. (1916) *Zoarces viviparus* L. and local races of the same. Comptes - Rendus des Travaux du laboratoire de Carlsberg, 13.

Scofiani, N.M. and Hawkins, A.D. (1985) Field studies of energy budgets. Fish Energetics: New Perspectives, 283-307.

Scott, F.J., Davidson, A.T. and Marchant, H.J. (2000) Seasonal variation in plankton, submicrometre particles and size-fractionated dissolved organic carbon in Antarctic coastal waters. Polar Biology, 23, 635-643.

Shekk, P. B., Kulikova, N. I., and Rudenko, V. I. (1990) Age-related changes in reaction of the Black Sea golden gray mullet, Liza aurata , to low temperature. Journal of Ichthyology, 30[1], 132-147.

Shelford, V.E. (1931) Some concepts of bioecology . Ecology, 12, 455-467.

Sidell, B.D. (1991) Physiological roles of high lipid content in tissue of Antarctic fish species. *Biology of Antarctic Fish* (Di Prisco, G., Maresca, B., and Tota, B.), pp. 220-231. Springer- Verlag, Berlin, Heidelberg, New York.

Sidell, B.D. and Hazel, J.R. (2002) Triacylglycerol lipase activities in tissue of Antarctic fishes. Polar Biology, 25, 517-522.

Southward, A.J., Hawking, S.J. and Burrows, M.T. (1995) Seventy years' observation of changes in distribution and abundance of zooplankton and intertidal organisms in the western english channel in relation to rising sea temperature. Journal of Thermal Biology, 20, 127-155.

Steffensen, J.F. (2002) Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artefact? Artefact! Comparative Biochemistry and Physiology, Part *A*, 132, 789-795.

Storch, D., Lannig, G. and Pörtner, H.-O. (2005) Temperature dependent protein synthesis

capacities in stenothermal (Antarctic) and eurythermal (North Sea) fish (Zoarcidae). Journal of Experimental Biology, 208, 2409-2420.

Stryer, L. (1994) *Biochemie*. Spektrum Adademischer Verlag, Heidelberg, Berlin, Oxford.

T

Thomas, R. E., Gharrett, J. A., Carls, M. G., Rice, S. D., Moles, A., and Korn, S. (1986) Effects of fluctuating temperature of mortality, stress, and energy reserves of juvenile coho salmon. Transactions of the American Fisheries Society, 115[1], 52-59.

U

Urich, K. (1990) *Vergleichende Biochemie der Tiere*. Gustav Fischer Verlag, Stuttgart, New York.

V

Van der Veer, H., Kooijman SALM, and Van der Meer, J. (2003) Body size scaling relationships in flatfish as predicted by Dynamic Energy Budgets (DEB theory): implications for recruitment. Journal of Sea Research. 50[2-3], 255-270.

Van Dijk, P. L. M., Tesch, C., Hardewig, I., and Poertner, H. O. (1999) Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). Journal of Experimental Biology 202[24], 3611-3621.

Vetemaa, M. (1998) Life of the viviparous blenny. Eesti loodus/Estonian nature. Tallinn, no. 3, pp. 132-133.

W

Walther, G.-H., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological response to recent climate change. Nature, 416, 389-395.

Weatherley, A.H. and Gill, H.S. (1987) *The Biology of Fish Growth*. Academic Press, London.

Wiecaszek, B. (1998) On the morphometry and growth rate in the viviparous eel-pout, *Zoarces viviparus* (Zoarcidae) from waters of different salinity. Italian Journal of Zoology, vol. 65, pp. 211-214.

Wieser, W. (1986) *Bioenergetik: Energietransformationen bei Organismen*. Georg Thieme Verlag, Stuttgart, New York.

Winberg, G. G. (1970) Energy flow in aquatic ecological system. Pol. Arch. Hydrobiol. Vol. 17(30), no. 1-2, pp. 11-19. 1970.

Winberg, G.G. (1956) Rate of metabolism and food requirements of fishes. Fish. Res. Brd. Canada Transl. Ser., 194, 253pp.

Wood, C.M. and McDonald , D.G. (1997) *Global warming: implications for freshwater and marine fish*. Cambridge University Press, Cambridge .

Wourms, J.P. (1991) Reproduction and development of Sebastes in the context of the evolution of piscine viviparity. Environmental biology of fishes, 30, 111-126.

Z

Zakhartsev, M.V., De Wachter, B., F. J. Sartoris, F.J., Pörtner, H.-O. and Blust, R. (2003) Thermal physiology of the common eelpout (*Zoarces viviparus*). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 173, 365-378.

Zhu Xiaoming, Xie Shouqi, and Cui Yibo. (2000) Effect of ration level on growth and energy budget of the gibel carp, *Carassius auratus gibelio*. Oceanologia et limnologia sinica/Haiyang Yu Huzhao. Qingdao, 31[5], 471-479.

Acknowledgements

Ich freue mich, dass mich so viele Menschen auf ihre Weise bei dieser Arbeit unterstützt haben und ich hier DANKE sagen kann, wie ich es vielleicht vorher zu oft versäumt habe. In der namentlichen Aufzählung hoffe ich, dass ich niemanden vergesse und beginne:

Ich danke **Prof. Wolf E. Arntz** für die Betreuung und Begutachtung dieser Arbeit, für seine hilfreichen Kommentare und klugen Anmerkungen, für sein „Adlerauge", dem nichts (keine überflüssige Leerstelle, kein vertippter Eigenname) entgeht, und den ökologischen Hintergrund. Ich möchte mich für das Verständnis für alle möglichen Kurzfristigkeiten bedanken, besonders kurz vor Abgabe der Arbeit.

Prof. Hans- O. Pörtner für die Betreuung und Begutachtung dieser Arbeit für die hilfreichen Kommentare und klugen Anmerkungen, den physiologischen Hintergrund und seine lösungsorientierte Art.

Dr. Rainer Knust für die direkte Betreuung und Anleitung der Arbeit.

Dr. Martin Graeve für alle Geheimnisse der Lipidanalyse und deren Auswertung, in die er mich eingewiesen hat und für seine Geduld für meine unbedarften Fragen. Den ganzen Rufschaden, den die Chemiker während meines Grundstudiums in Düsseldorf verbrochen haben, hast Du nicht nur wiedergutgemacht, sondern hast mein Bild von Chemikern deutlich ins Positive zurückverschoben.

Dr. M. E. Anderson for sharing the same interests in eelpouts and letting me participate to his great knowledge of this funny fishes, for conforming species determinations and for his hospitality in South Africa.

Prof. Dr. Gunter O. Kirst für seine spontane Bereitschaft, als Prüfer einzuspringen und an meinem Dissertationskolloquium teilzunehmen.

Dr. Barbara Niehoff und **Kai Wätjen** dafür, dass ihr Beisitzer in meinem Dissertationskolloquium seid und ihr mich auch sonst immer gut unterstützt habt.

Einen besonderen Dank möchte ich an das inoffizielle „Eva-Promotions-Motivationsteam“ aussprechen, an:

Dr. Franz Sartoris (Chef) und den Punk-**Pantopoden**, dafür daß „Alles gut wird“ und **Dr. Christian Bock**, der durch seine bloße Anwesenheit Ärger relativiert hat (das muß das „Physikalische“ an Dir sein) und **Dr. Gisela Lannig** für das beste Lächeln der Welt.

Ich möchte mich bei **Ute Jacob (Teamleiterin)** für jedes einzelne „out take“ im Zug und woanders, die Farbe ROSA, den Rock’n’Roll in der Wissenschaft, hübsche Zwischenblätter,einen Schlafsack, tolles Umschlagdruck- Coaching und eine gute Zeit zu Wasser & zu Lande bedanken. (.... und das „Making of a Doktorarbeit“ wird noch ganz bestimmt gedreht werden und bestimmte Ratgeber werden auch noch geschrieben werden).

Danke dir, **Tanja Joschko** für Bett und Schlüssel und gute Laune.

Ilka Hoppe, du bist im Prinzip mitverantwortlich, dass ich jetzt diese Arbeit schreibe. Danke! Ich bin froh, dass es dich gibt.

Kai Wätjen und **Fiete (Wolf) Isbert** danke ich für unseren langen gemeinsamen Weg.

Olaf Heilmayer: Vielen Dank für die Hilfestellung bei meinen (☺) ersten Respirationsversuchen, stets schnelle nette Hilfe und Schokolade und Dein nettes Wesen, gute Tips, abendliches Catering und freundliche Worte.

Randy Glasbergen, der unter www.glasbergen.com es oft geschafft hat, mich zum Schmunzeln zu bringen

Dr. Felix Mark Danke für gute Diskussionen und Gespräche und die zelluläre Seite der Aalmutter.

Danke, **Dr. Frank Melzner**, du kommst gleich unten noch mal.

Dank Dir, **Katja Mintenbeck**, für nette Stunden im Fischlabor, fürs Fische füttern und Blumen gießen.

Marco Böer: Danke für Eichgraden und dafür, dass du mir im Lipidlabor gezeigt hast, wie alles läuft und was ich tun muss.

Many grateful THANKS to my english teaching, never verzweifelig team:

Glenn Lurman, **Magdalena Gutowska** and **Dr. Frank Melzner**, thank you for doing a great job in reviewing my poor english and never giving up to cheer me up, sowie **Ortrud Gernand** für ihre Arbeit meine Arbeit „flüssiger” und runder zu machen

Vielen Dank an die unentbehrliche “Drum herum” Logistik:

Uwe Abken, **Martin** und **Klaus** für die Polaris und die Kutterfahrten.

Crew der Polarstern und der **Heincke** für ihre Hilfe auf den Fahrten.

Danke an die im Hintergrund wirkenden Kräfte im wissenschaftlichen Betrieb:

Timo Hirse für alles! .. und dafür dass du so bist, wie ich Dich mag und eine tolle Polarsternfahrtgemeinschaft.

Super-Nils Koschnik für eine tolle Polarsternfahrt und super nettes und super lustiges gemeinsames Arbeiten.

Steffi Bröhl für Heincke- und Kutterfahrtgesellschaft.

Boris Klein für die Kerkerbastelgruppe, 180 am Bauch massierten Aalmuttern und Pumpen und Toffiffee.

Kerstin Beyer für spontane Hilfe, für Kekse und Schokolade und für nette Gespräche auf der Bendex Fahrt.

Wiebke Buck und **Anja Pappert** für eure Hilfe bei einigen CHN Durchläufen, bzw. „Reaktorwechseln“.

Danke an die Unwahrscheinlichkeiten im Leben und Danke an:

Dr. Marc Lavaleije für eine email – Adresse und

Dr. Marc Fonds für tolle Tipps zu meinem Aalmutterwachstumsversuch und den ausführlichen Auskünften trotz verstrichener „neolithischer“ Zeiträume.

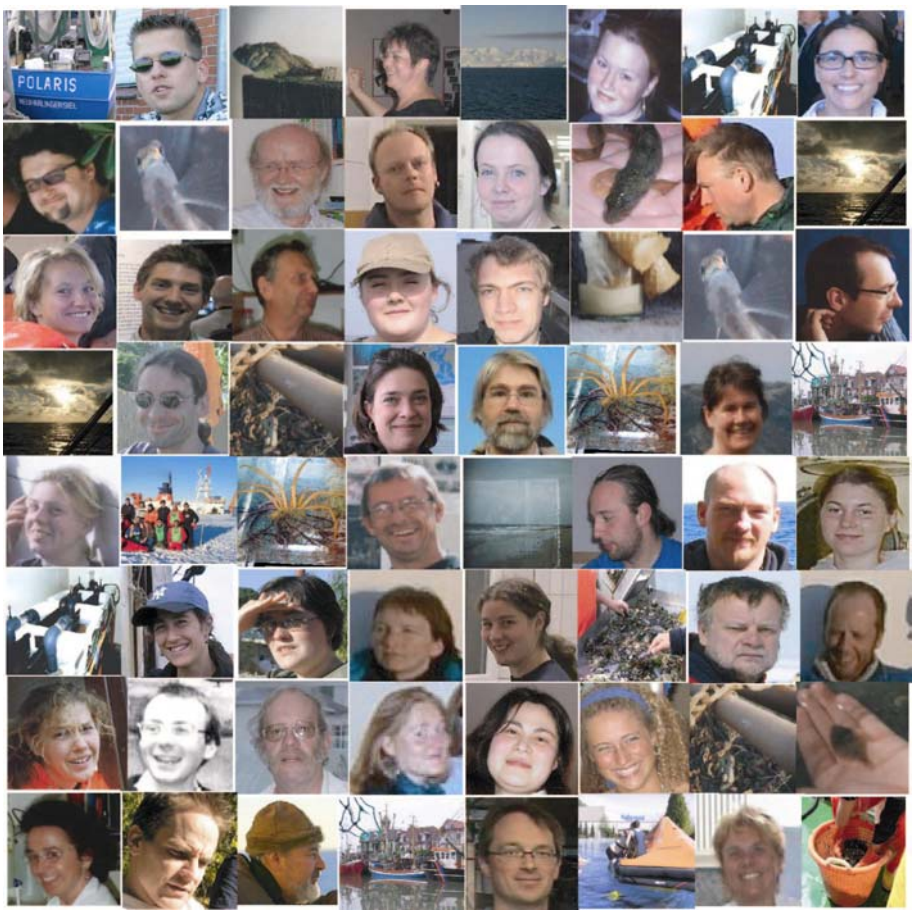
Nicht-namentlich, möchte ich mich bei „meinen“ beiden Arbeitsgruppen bedanken, ich habe mich gefreut, bei Euch eine gute Stimmung und nette Atmosphäre zu finden.

Dann möchte ich mich bei den vielen helfenden Händen der FÖJlerinnen und des FÖJlers, sowie der zahlreichen Praktikantinnen und studentischen Hilfskräften bedanken – es wird euch nicht oft gesagt, aber ihr seid und wart eine große Hilfe und Unterstützung. Danke an:

Sylke Wohlrab, **Yvonne Cherubini**, **Frauke Seemann**, **Eike Adolf**, **Annika Wulff**, **Maria Klein**, **Hulda Kristin Magnusdottir**, **Ina Wosnitza** und **Rebekka Metzger**

ACKNOWLEDGEMENTS

Am meisten möchte ich mich bei **Matthias** bedanken. Wir haben die Zeit dieser Arbeit gemeinsam durchlebt und ich weiß, dass es nicht immer einfach war. Du hast mich immer unterstützt und hast gute Gedanken zu dieser Arbeit beigetragen. Danke für Deinen Entwurf des Umschlags. Da wir den Rest unseres Lebens miteinander verbringen, werde ich viel Zeit haben, Dir oft zu danken. Du und ich, wir beide. LIEBE!



Eva Brodte
Reuterstrasse 12
28217 Bremen

Bremen, den 02. Januar 2006

Erklärung gem. § 5 (1) Nr. 3 PromO

Ich erkläre hiermit,
1. dass ich mich vor dem jetzigen Promotionsverfahren keinem anderen Promotionsverfahren unterzogen habe

und

2. dass ich außer dem jetzt laufenden Promotionsverfahren auch kein anderes beantragt habe.

Eva Brodte