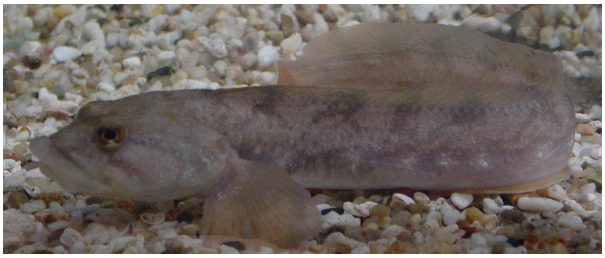
Stiftung Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven

Physiologische Grundlagen temperaturabhängiger Biogeographie bei marinen Fischen



Dipl. Biol. Gisela Lannig

Vorgelegt im Fachbereich 2 (Biologie/Chemie) der Universität Bremen
als Dissertation zur Erlangung des akademischen Grades
eines Doktors der Naturwissenschaften (Dr. rer. nat.)

Bremen 2003

Stiftung Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven

Physiologische Grundlagen temperaturabhängiger Biogeographie bei marinen Fischen

Dipl. Biol. Gisela Lannig

Vorgelegt im Fachbereich 2 (Biologie/Chemie) der Universität Bremen
als Dissertation zur Erlangung des akademischen Grades
eines Doktors der Naturwissenschaften (Dr. rer. nat.)

Bremen 2003

1. Gutachter: Prof. Dr. H.O. Pörtner
Alfred-Wegener-Institut

2. Gutachter: Prof. Dr. W.E. Arntz
Alfred-Wegener-Institut

Inhaltsverzeichnis

Abkürzungsverzeichnis

CCO	Cytochrom c-Oxidase
CLICOFI	Effects of **cli**mate induced temperature change on marine **co**astal **fi**shes (Bezeichnung des EU geförderten Projektes)
CS	Citrat-Synthase
ETS	Elektronentransportsystem
g	Erdbeschleunigung
IDH	$NADP^+$-abhängige Isocitrat-Dehydrogenase
mM	millimolar
MS222	3-Aminobenzoesäureethylester
n	Anzahl der Versuchstiere
NC	Kabeljau der Nordsee (**N**orth Sea **c**od)
NCC	Kabeljau der Norwegischen Küste (**N**orwegian **C**oastal **c**od)
NEAC	Kabeljau der Barentssee (**N**orth **E**ast **A**rctic **c**od)
NMR	Kernspinresonanz (**N**uclear **M**agnetic **R**esonance)
PO_2	Sauerstoffpartialdruck
T_c	kritische Temperatur
TCA	Trichloracetat
T_p	Pejus-Temperatur

Zusammenfassung

Im Hinblick auf globale Erwärmung wurden die physiologischen Mechanismen untersucht, die zu einer Limitierung der thermalen Toleranz und der damit verbundenen geographischen Verbreitungsgrenzen ektothermer mariner Organismen beitragen. Vergleichsstudien an marinen Fischen im latitudinalen Gradienten deuten darauf hin, dass die Limitierung der thermalen Toleranzbereiche mit einer temperaturinduzierte Anpassung der aeroben Leistungskapazität in Beziehung steht, wobei diese je nach Habitattemperatur und saisonaler Temperaturschwankung variiert.

Im ersten Teil der Arbeit wurde die Hypothese untersucht, dass sich die temperaturbedingten Anpassungsmechanismen des aeroben Stoffwechsels zum einen zwischen kaltakklimatisierten borealen und kaltadaptierten polaren Tieren und zum anderen zwischen kaltadaptierten stenothermen (Antarktis) und kaltadaptierten eurythermen (Subarktis und Arktis) Tieren unterscheiden. Hierfür wurden verschiedene Kabeljaupopulationen (*Gadus morhua*) aus der südlichen Nordsee, der Norwegischen Küste und der Barentssee sowie zwei Aalmutterarten (*Zoarces viviparus* der Nordsee und *Pachycara brachycephalum* der Antarktis) bei verschiedenen Temperaturen gehältert, und die aerobe Kapazität der Leber auf mitochondrialer und enzymatischer Ebene untersucht.

Eine Kälteakklimatisation und –adaptation führte zu einer Erhöhung der hepatischen aeroben Kapazität, aufgrund einer Größenzunahme der gesamten Leber und einer damit einhergehender Erhöhung mitochondrialen Proteingehalts. Es zeigte sich, dass mitochondriale Matrixenzyme (Citrat-Synthase und NADP+-abhängige Isocitrat-Dehydrogenase) während einer Kälteanpassung stärker reguliert werden als die Membranenzyme der Atmungskette. Diese Befunde deuten vor allem bei kaltadaptierten Fischen auf eine kälteinduzierte Verschiebung zu anabolischen Prozessen hin.

Es wurden Unterschiede in der temperaturbedingten Anpassung des hepatischen mitochondrialen Stoffwechsels zwischen kaltadaptierten antarktischen Stenothermen und kaltadaptierten Eurythermen (*G. morhua* der Barentssee) gefunden. Im Gegensatz zu dem in der Literatur beschriebenen höheren mitochondrialen Stoffwechsel von *G. morhua* der Barentssee, hatte *P. brachycephalum* bei gleicher Hälterungs- und Meßtemperatur eine geringere ATP-Synthese-Kapazität und einen vergleichbaren Sauerstoffverbrauch durch Protonen-Leckströme (*proton leakage*) wie die eurythermen Nordseetiere. Die antarktische Aalmutter zeigte im Vergleich zu kaltakklimatisierten Eurythermen nicht die für kaltstenotherme Tiere der Antarktis postulierte Reduzierung des Sauerstoffverbrauchs über Protonen-Leckströme. Diese Ergebnisse und die erfolgreiche Langzeit-Wärmeakklimatisation bei 5°C,

die zu einer verminderten aeroben Kapazität führte lassen vermuten, dass *P. brachycephalum* aufgrund ihrer benthischen und trägen Lebensweise (Hypometabolismus) einige Eigenschaften eines kaltadaptierten eurythermen Organismus beibehalten hat.

Die von Projektpartnern durchgeführten Wachstumsexperimente mit Kabeljau-populationen im latitudinalen Gradienten bei verschiedenen Temperaturen ergaben für alle Populationen ein optimales Wachstum bei c. 10°C. Zudem nahm das temperaturspezifische Wachstum von Kabeljau der Nordsee zur Barentssee ab. Nicht nur eine Kälteakklimatisation, sondern vor allem eine Kälteadaptation, scheint aufgrund einer gesteigerten aeroben Kapazität mit einem höheren Energieaufwand gekoppelt zu sein. Ebenfalls deutet der geringere Lipidgehalt in der Leber von kalt- versus warmakklimatisiertem Kabeljau der Nordsee auf höhere Stoffwechselkosten in der Kälte hin. Es wird diskutiert, dass der in der Literatur beschriebene erhöhte Standardstoffwechsel und das verminderte Wachstum eurythermer Tiere in der Kälte vorwiegend auf den mit einer Mitochondrienproliferation einhergehenden Sauerstoffverbrauch durch *proton leakage* zurückzuführen ist.

Die Funktion isolierter Mitochondrien ist bei ausreichender Sauerstoffverfügbarkeit noch jenseits der oberen kritischen Temperatur des Ganztiers gewährleistet. Die Tiere gehen jedoch oberhalb dieser Temperatur vom aeroben zum anaeroben Stoffwechsel über, und ein Überleben ist nur noch innerhalb einer begrenzten Zeit möglich. Daher ist die temperaturbedingte Limitierung des Stoffwechsels des Ganztiers vermutlich auf eine verminderte Sauerstoffversorgung der Gewebe zurückzuführen. Zur Klärung dieser Hypothese wurden Herz-Kreislauf-Parameter von *G. morhua* der Nordsee in Abhängigkeit einer progressiven Temperaturänderung gemessen. Anhand von temperaturinduzierten Änderungen in der Herzfrequenz, dem arteriellem und venösem Blutfluss und dem venösem Sauerstoffpartialdruck konnte bei Nordseekabeljau Pejus-Temperaturen bei 1,6°C und 7,3°C nachgewiesen werden, die den ökologischen Toleranzbereich mit maximaler aerober Leistungskapazität begrenzen. Ober- und unterhalb dieser Temperaturen verminderte sich die aerobe Leistungskapazität von *G. morhua* der Nordsee. Mit Erreichen der oberen kritischen Temperatur wurde eine starke Abnahme der aeroben Leistungskapazität sowie Herz-Rhythmus-Störungen beobachtet. Anhand dieser Ergebnisse wurde das in der Literatur für Crustaceen beschriebene Temperatur-Toleranzmodell der aeroben Leistungskapazität mit Optimum-, Pejus- und Pessimum-Bereichen für Fische modifiziert. Es wird diskutiert, dass die Temperaturabhängigkeit des Herz-Kreislaufsystems zu den beschriebenen saisonalen Verbreitungsgebieten von Kabeljau der Nordsee beitragen und zu einer ganzjährigen Verschiebung der Verbreitungsgrenzen aufgrund globaler Erwärmung führen kann.

Summary

The physiological mechanisms limiting thermal tolerance and therefore geographical distribution of ectothermal marine organisms were investigated in the light of global warming. Comparative studies carried out on marine ectotherms from a latitudinal cline indicate that the limits of thermal tolerance are linked with temperature induced adjustments in aerobic scope and capacity depending on latitude or seasonal temperature acclimatisation.

In the first part of this work a hypothesis is tested, that the temperature induced adjustments of aerobic scope differ between cold acclimated boreal and cold adapted polar animals as well as between cold adapted stenotherms (Antarctica) and cold adapted eurytherms (sub-Arctic and Arctic). Accordingly, aerobic mitochondrial and enzymatic capacities were investigated in liver of different cod populations (*Gadus morhua*) from the southern North Sea, the Norwegian coast and the Barents Sea and of two eelpout species (*Zoarces viviparus* from the North Sea and *Pachycara brachycephalum* from Antarctica) acclimated to different temperatures.

Cold acclimation and cold adaptation led to an increase of the hepatic aerobic capacity in cod and eelpout due to an increase in liver size accompanied by an increase in mitochondrial protein content. Furthermore, enhanced mitochondrial matrix enzymes (citrate synthase and $NADP^+$-dependent isocitrate dehydrogenase) over respiratory chain capacities were found and might support enhanced anabolic processes in cold adapted animals.

Differences in temperature induced adjustments of hepatic mitochondrial metabolism were found between cold adapted stenotherms (*P. brachycephalum*, Antarctica) and cold adapted eurytherms (*G. morhua*, Barents Sea). In contrast to higher mitochondrial respiration rates described for cold adapted eurythermal cod, *P. brachycephalum* had a lower ATP synthesis capacity and a similar oxygen consumption due to proton leakage compared to cold acclimated boreal eurytherms at same acclimation and assay temperatures. However, *P. brachycephalum* did not show the reduction in oxygen consumption due to proton leakage as postulated for cold stenotherms from Antarctica. These results and the successful long-term warm acclimation at 5°C leading to reduced aerobic capacities might indicate that *P. brachycephalum* has retained some features of cold adapted but hypometabolic eurytherms and is at an intermediate stage between full cold-stenothermy and cold-eurythermy.

The experimental growth data obtained by project partners at different temperatures for cod populations in a latitudinal cline revealed optimal growth rates between 10°C and 11°C for all populations. However, temperature-specific growth decreased in cod populations from higher latitudes. Cold acclimation but above all cold adaptation seems to cause a shift in

energy budget, unfavourable for growth and the accumulation of energy reserves. Accordingly, elevated maintenance costs could explain lower liver lipid contents in cold versus warm acclimated North Sea cod. The differences in mitochondrial respiration rates and above all in proton leakage rates between cold adapted stenotherms (Antarctica) and cold adapted eurytherms (Arctic and Sub-Arctic) when compared to cold acclimated boreal eurytherms are in line with the patterns of oxygen consumption of whole animals as described in the literature. The results indicate that elevated standard metabolism and reduced growth in the cold may be mainly attributed to elevated proton leakage correlated with increased mitochondria densities.

Studies of mitochondrial functions revealed that elevated temperatures beyond the lethal limit of the animal do not impair oxidative mitochondrial metabolism at sufficient oxygen supply. Therefore, thermal limitation of the whole animal metabolism is rather due to limited oxygen supply to tissues than to mitochondrial dysfunction. The hypothesis of an oxygen limited thermal tolerance due to limitations in cardiovascular performance was tested in Atlantic cod, *G. morhua* by recording the temperature dependency of heart rate, arterial and venous blood flow and venous oxygen tension during an acute temperature change. Pejus temperatures could be characterised that are defined to represent the ecological tolerance range by maximum scope for aerobic activity. Thermal limitation may already set in below 2°C and beyond 7°C, respectively, characterised by decreased venous P_{O2} owing to onset of a progressive mismatch between oxygen demand and circulatory performance. The model of thermal tolerance with optimum, pejus and pessimum ranges for aerobic scope defined for crustaceans was modified for fish. The temperature induced limitation in cardiovasular performance is discussed in the light of geographical distribution limits found for *G. morhua* in nature and might be the reason for the predicted northern shift in the distribution of North Sea cod with global warming.

1. Einleitung

Die geographische Verbreitung von Tieren hängt von einer Vielzahl von Faktoren ab, die bei einer Charakterisierung von Verbreitungsgrenzen berücksichtigt werden müssen. Für marine Ektotherme, deren Körpertemperatur der jeweiligen Umgebungstemperatur entspricht (mit einer Ausnahme, den Scombriodei, z.B. Thunfisch) ist neben dem Nahrungsangebot oder dem Fraßdruck maßgeblich die Temperatur für die Verbreitungsgrenzen verantwortlich. Globale Temperaturveränderungen oder extreme Wetterereignisse führten immer wieder zu einer Verschiebung der Verbreitungsgebiete mariner Organismen (Cushing 1982; Frank et al. 1990; Arntz und Tarazona 1990; Beamish 1995; Southward et al. 1995; Bakun 1996; Parsons und Lear 2001).

Strategien zur Temperaturanpassung beinhalten folglich verhaltensbedingte Mechanismen. Verhaltensanpassungen zielen in erster Linie darauf ab, extremen Temperaturen oder größeren Temperaturschwankungen auszuweichen. Pelagische Fische sind durch ihre aktive Lebensweise dazu eher in der Lage als benthische, sessile Organismen. Beispielsweise reagiert der Atlantische Kabeljau *Gadus morhua* sehr sensibel auf seine Umgebungstemperatur. Wie aus einer Arbeit von Rose (1993) hervorgeht, folgt neufundländischer Kabeljau bei seiner Wanderung im nordwestlichen Atlantik einer Wassersäule zwischen 2°C und 3°C, dem sogenannten „*migration highway*“. Dippner (1997) und O`Brien et al. (2000) berichteten von temperaturabhängigen Verbreitungsmustern von *G. morhua* in der Nordsee. Nach R. Knust (pers. Mitteilung) wird in warmen Sommern kein Kabeljau in der südlichen Nordsee gefunden. Marine Evertebraten der Gezeitenzone wandern im Winter ins Sublitoral ab (Beukema und de Vlas 1979; Günther 1992) oder ziehen sich bei extremen Temperaturen in Höhlen zurück (Eshky et al. 1996).

Die Begrenzung des Temperaturbereichs, in dem Überleben möglich ist, wird durch kritische Temperaturen (T_c) charakterisiert, ober- bzw. unterhalb derer ein aerober Stoffwechsel nicht mehr aufrechterhalten werden kann und anaerobe Endprodukte akkumulieren (siehe Übersichtsartikel Pörtner et al. 1998, 2000; Pörtner 2001). Die thermalen Toleranzbereiche mariner Ektothermer sind je nach Habitat unterschiedlich. Polare Organismen, die in Habitaten mit extrem kalten Temperaturen und geringen Temperaturschwankungen leben, gelten als kaltstenotherm und besitzen eine geringere Toleranz gegenüber hohen Temperaturen als eurytherme Organismen, in deren Habitaten die Temperatur je nach Jahreszeit variiert. So werden bei polaren im Vergleich zu borealen Tieren die kritischen Temperaturen bei niedrigeren Temperaturen erreicht (Sommer et al. 1997; van Dijk et al. 1999). Zudem ergaben sich Verschiebungen der kritischen Temperaturen

bei eurythermen Organismen zwischen Sommer- und Wintertieren (Sommer et al. 1997). Da die kritischen Temperaturen durch das Eintreten einer Anaerobiose definiert sind, müssen folglich physiologische Anpassungsmechanismen vorhanden sein, die zu einer Limitierung der thermalen Toleranz führen. Bei poikilothermen Tieren variiert die Körpertemperatur mit der Umgebungstemperatur, und da diese den Stoffwechsel beeinflusst (Q_{10}-Modell: Maß für die Beschleunigung physiologischer Prozesse bei einer Temperaturerhöhung um 10°C, Krogh 1914), erfolgt bei Kälteakklimatisation sowie –adaptation eine Temperaturkompensation über eine Erhöhung der aeroben Stoffwechselkapazität (siehe Übersichtsartikel von Prosser 1991; Guderley 1998; Pörtner et al. 1998, 2000; Pörtner 2001, 2002a,b). Unterschiedliche Mechanismen können daran beteiligt sein, wie eine Änderung der Mitochondrienstruktur (Gaebel und Roots 1989; Archer und Johnston 1991), eine Änderung der Membranzusammensetzung (Prosser 1991; Miranda und Hazel 1996) oder Aktivitätssteigerungen einzelner Enzyme (Crockett und Sidell 1990; Guderley 1990; Sokolova und Pörtner 2001; Kawall et al. 2002). Neben einer Erhöhung der Mitochondriendichte (Egginton und Sidell 1989; Londraville und Sidell 1990; Sommer und Pörtner 2002; Bouchard und Guderley 2003) wurden bei kaltakklimatisierten und kaltadaptierten Tieren vergrößerte Organe gefunden, die über den damit verbundenen, erhöhten mitochondrialen Proteingehalt zu einer Kompensation des aeroben Stoffwechsels bei niedrigen Temperaturen beitrugen (Kent et al. 1988 (Leber und Herz); Seddon und Prosser 1997 (Leber)). Diesen Mechanismen ist gemeinsam, dass durch sie zum einen die Verteilung von Sauerstoff und Metaboliten im gesamten Organismus und innerhalb von Geweben und Zellen aufrechterhalten bleibt und zum anderen die Stoffwechselaktivität erhöht wird.

Eine Erhöhung der aeroben Kapazität in der Kälte impliziert eine gleichzeitige Erhöhung des Standardstoffwechsels des Ganztiers. Nachdem Scholander et al. (1953) und Wohlschlag (1960) bei einigen polaren Fischen einen höheren Standardsauerstoffverbrauch (Ruhestoffwechsel ohne Auftreten spontaner Aktivität) fanden, als der Vergleich mit extrapolierten Daten borealer Arten mit einem Q_{10}-Faktor von 2,5 erwarten ließ, hielt der Begriff einer *metabolic cold adaptation* (metabolische Kälteadaptation, MCA) in die wissenschaftliche Literatur Einzug. Holton (1973, 1974) berichtete später von niedrigerem Standardstoffwechsel bei arktischen Fischen und führte die hohen Werte der früheren Arbeiten auf eine experimentbedingte Stresssituation zurück. Über die Hypothese der *metabolic cold adaptation* und wie ihr Grad zu quantifizieren sei, folgte eine kontroverse Diskussion (Clarke 1983, 1991; Johnston 1990; Somero et al. 1998).

Pörtner et al. (2000) unterscheiden zwischen kaltadaptierten eurythermen und kaltadaptierten stenothermen Tieren. Im Vergleich zur Antarktis ist die Arktis jüngeren Alters und nicht so stark von angrenzenden Wassermassen isoliert wie die südliche Hemisphäre, wo der Wasseraustausch durch den antarktischen Zirkumpolarstrom minimiert wird. Unter Berücksichtigung der Entstehungsgeschichte der beiden Polargebiete erscheint es daher möglich, dass sich einige arktische Arten noch im Übergang zu einer kaltadaptierten Lebensweise befinden, wohingegen Tiere aus der Antarktis die Entwicklung funktionaler Eigenschaften für ein Leben in permanenter Kälte über Millionen von Jahren optimiert haben und deshalb keine Erhöhung des Standardstoffwechsels im Sinne der *metabolic cold adaptation* zeigen (siehe Übersichtsartikel Pörtner et al. 1998; Pörtner 2002a,b). Eine solche Optimierung könnte eine Reduzierung des Sauerstoffverbrauchs über Protonen-Leckströme (passive Diffusion der Protonen über die innere Mitochondrienmembran unter Umgehung der F_0F_1-ATPase) sein (Pörtner et al. 1998; Pörtner 2000). Denn eine Erhöhung der Mitochondrienanzahl führt neben dem positiven Effekt einer gesteigerten aeroben Kapazität auch zu einem gesteigerten Sauerstoffverbrauch über Protonen-Leckströme, der zwischen 20 und 50% zum Standardstoffwechsel eines Tieres beitragen kann (Brand 1990; Brookes et al. 1998; siehe Abb. 1).

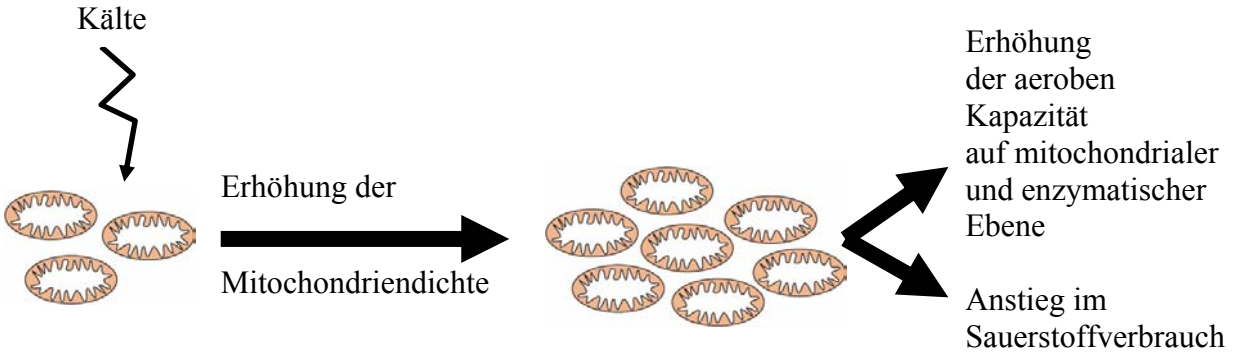


Abb. 1 Modell für eine kälteinduzierte Erhöhung der aeroben Kapazität

Die Hypothese einer *metabolic cold adaptation* wird kontrovers diskutiert, jedoch wurde deutlich, dass mit Kälteakklimatisation und -adaptation einer für den Organismus tolerierbaren Temperaturerhöhung enge Grenzen gesetzt sind (siehe Übersichtsartikel Pörtner et al. 1998, 2000). Das Eintreten von Anaerobiose bei Erreichen der kritischen Temperaturen entsteht durch ein Sauerstoffdefizit an den Mitochondrien, das auf eine unzureichende Ventilation oder Verteilung des aufgenommenen Sauerstoffs zurückzuführen ist (siehe Übersichtsartikel Pörtner 2002a). Frederich und Pörtner (2000) fanden bei der Seespinne

Maja squinado Schwellenwerte, unter- bzw. oberhalb derer der arterielle Hämolymph–PO_2 zu sinken begann noch bevor die kritischen Temperaturen (T_c) erreicht wurden. Dieser Befund deutet auf eine Limitierung der aeroben Leistung vor Erreichen der kritischen Temperatur hin. Die Abnahme im arteriellen Hämolymph-PO_2 korrelierte mit einer verminderten Ventilations- und Zirkulationsleistung der Tiere, und die Schwellenwerte wurden, in Anlehnung an Shelford´s Toleranzmodell („*Law of toleration*“, Shelford 1913, 1931) als Pejus-Temperaturen (pejus = schlechter) definiert (siehe Abb. 2). Ektotherme Organismen scheinen somit optimale Temperaturbereiche (innerhalb der Pejus-Temperaturen) zu besitzen, die vermutlich die temperaturbedingten geographischen Verbreitungsgrenzen markieren (Frederich und Pörtner 2000).

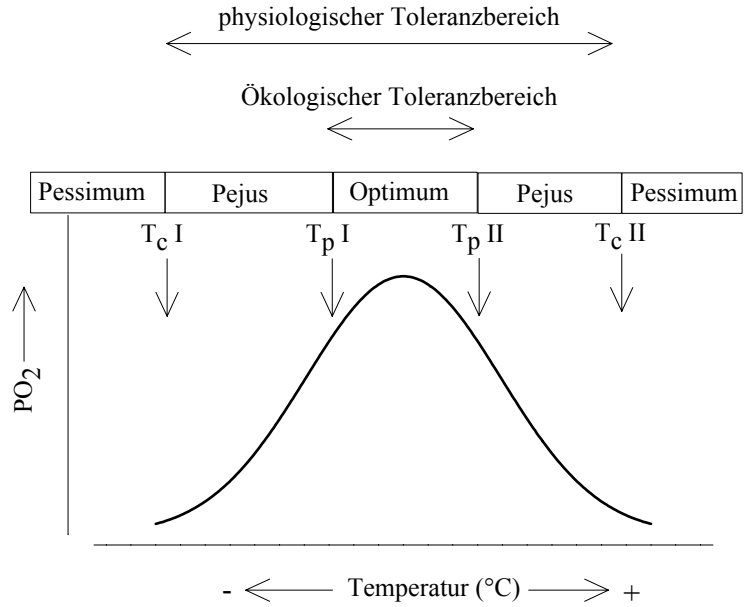


Abb. 2 Modell der Temperaturtoleranz im Hinblick auf die aerobe Kapazität (aus Frederich und Pörtner 2000).

Die oberen und unteren Pejus-Bereiche (zwischen T_p und T_c) definieren hingegen die tolerierbaren Temperaturen, bei denen Überleben gewährleistet ist, jedoch zunehmend Einschränkungen in Aktivität, Reproduktion und Wachstum auftreten. Mit Erreichen der kritischen Temperaturen ist ein Überleben nur noch innerhalb einer begrenzten Zeit möglich und führt letztendlich zum Tod der Tiere (siehe Übersichtsartikel Pörtner 2001).

Ein ähnlicher Zusammenhang zwischen Temperaturtoleranz und aerober Leistungskapazität wird bei Fischen vermutet. Bei Fischen scheint jedoch das Kreislaufsystem temperaturabhängiger als die Ventilation zu sein (van Dijk et al. 1999; Mark et al. 2002). Heath und Hughes (1973) zeigten, dass mit zunehmender Temperatur die Herzfrequenz der Regenbogenforelle *Salmo gairdneri* ein Maximum erreichte und mit

weiterer Erwärmung abnahm, wohingegen die Ventilation erhöht blieb. Die Differenz zwischen arteriellem und venösem Sauerstoffgehalt stieg an, wobei nur eine geringfügig temperaturbedingte Abnahme des arteriellen PO_2 zu erkennen war (Heath und Hughes 1973). Das zunehmend größer werdende Ungleichgewicht zwischen Sauerstoffversorgung und –verbrauch wird auf eine begrenzte Herz-Kreislauf-Kapazität zurückgeführt. Diese könnte zu einer Hypoxie im Herzen führen, da die Sauerstoffversorgung des Herzmuskels bei Fischen, die keinen Coronarkreislauf besitzen, auf venösem Blut erfolgt (Abb. 3, siehe Übersichtsartikel Farrell 1996, 2002).

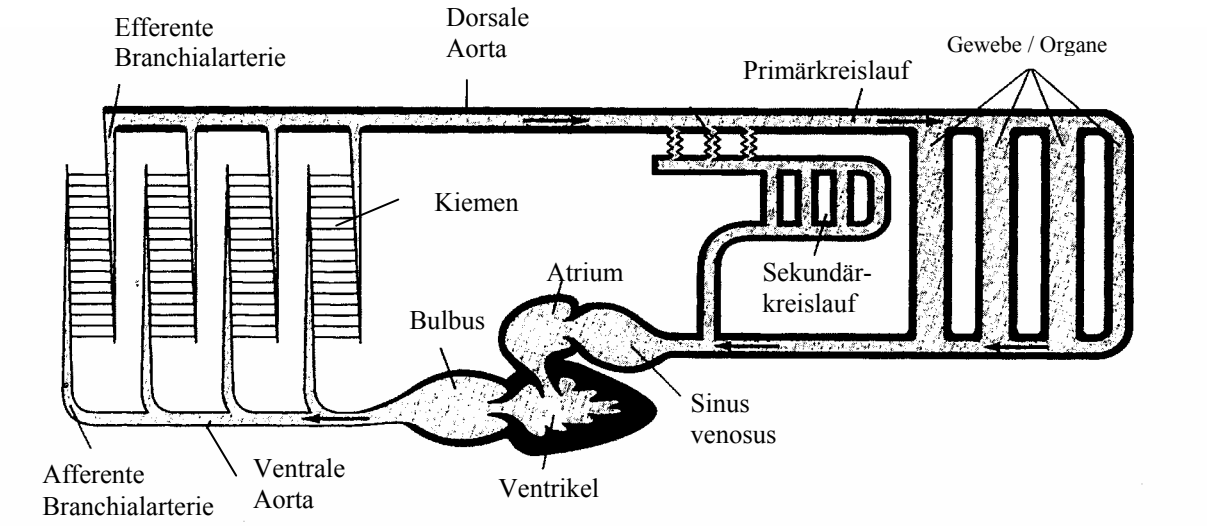


Abb. 3 Schematischer Blutkreislauf der Teleostei (aus Serendero 2000)

Eine temperaturbedingte Limitierung der Herz-Kreislauf-Kapazität wäre zum einen der Grund für das beobachtete Ungleichgewicht zwischen Sauerstoffversorgung und –verbrauch, und zum anderen wäre das Myocardium eines der ersten Organe, dessen aerobe Leistungskapazität betroffen wäre, wenn es bei extremen Temperaturen zu einer Anaerobiose sowie zu einem Zusammenbruch des Energiehaushalts kommt (siehe Übersichtsartikel Pörtner 2002a).

Eine eindeutige Beziehung zwischen Temperatur und Herz-Kreislauf-System bzw. aerober Leistungskapazität, wie von Frederich und Pörtner (2000) bei der Seespinne *M. squinado* gefunden, konnte bei Fischen noch nicht eindeutig nachgewiesen werden.

Die vorliegende Arbeit entstand im Rahmen des von EU geförderten Projekts „Effects of **c**limate induced temperature change on marine **co**astal **fi**shes“ (CLICOFI; Nr.:ENV4-CT97-0596). In diesem Projekt sollte die Wirkung von klimabedingten Temperaturänderungen auf die Verbreitung und Populationsdynamik von Fischen auf ökologischer, physiologischer und genetischer Ebene untersucht werden. Die Untersuchungen wurden an Modellorganismen zweier Familien, Zoarcidae und Gadidae, im latitudinalen Gradienten durchgeführt, um Hinweise auf temperaturbedingte adaptive Mechanismen und Toleranzgrenzen zu erhalten.

Aus der Familie der Zoarcidae wurden zwei Aalmutterarten (*Zoarces viviparus* L. aus der Nordsee und *Pachycara brachycephalum* P. aus der Antarktis) untersucht.

Z. viviparus zählt zu den Standfischen und ist in arktisch – borealen Küstengewässern von der Ostsee bis zum Weißen Meer verbreitet, wobei das Wattenmeer die südliche Verbreitungsgrenze ist (Abb. 5A; Knijn et al. 1993). Diese Art ist vivipar (Götting 1976) und lebt benthisch, in der Nordsee vorwiegend in flacheren Gebieten (Knijn et al. 1993). In der Ostsee ist sie auch in Tiefen bis zu 80 Metern zu finden (Netzel und Kuczynski 1995). Die Tiere ernähren sich von Würmern, Krebstieren und Mollusken (Knijn et al. 1993).

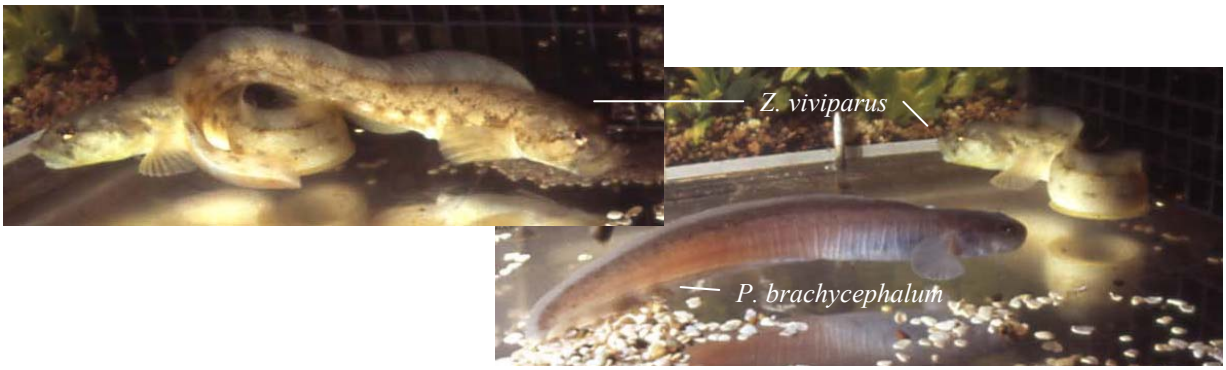


Abb. 4 Aalmutter der Nordsee (*Z. viviparus*) und der Antarktis (*P. brachycephalum*)

P. brachycephalum ist eine endemische Art und an küstennahen Hängen in einer Tiefe von 200 bis 1810 Metern zirkumantarktisch verbreitet (Abb. 5B; Anderson 1990). Über die Ökologie dieser Art ist bislang wenig bekannt, doch ähnelt ihre Lebens- und Ernährungsweise vermutlich der von *Z. viviparus*, wobei *P. brachycephalum* jedoch ovovivipar ist (Anderson 1990).

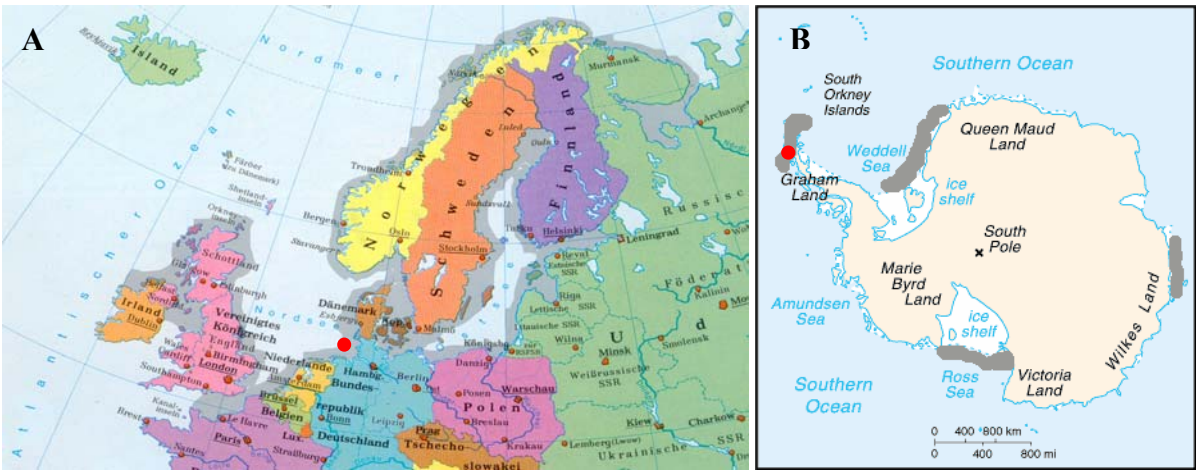


Abb. 5 Verbreitungsgebiet von *Z. viviparus* (A) und *P. brachycephalum* (B) (Untersuchungsgebiete sind rot markiert)

Aus der Familie der Gadidae wurden verschiedene Kabeljaupopulationen der südlichen Nordsee, der Norwegischen Küste und der Barentssee (Nord-Ost arktischer Kabeljau) untersucht. Atlantischer Kabeljau *Gadus morhua* L. kommt in küstennahen Schelfgebieten bis zu einer Tiefe von 600 Metern vor (Cohen et al. 1990).

Seine Verbreitungsgebiete sind die kühleren Meeresgebiete der nördlichen Biskaya, von der Nordsee über die Küste Norwegens bis in die Barentssee und über Island-Grönland bis zu den Küsten Labradors und Neufundlands (Abb. 6B; Lythgoe und Lythgoe 1974; McKeown 1984). Als kälteliebende Fische leben sie bevorzugt zwischen –0.5°C und 10°C, wobei die Temperatur jedoch mit dem Verbreitungsgebiet, der Jahreszeit und der Fischgröße variiert (Scott und Scott 1988).

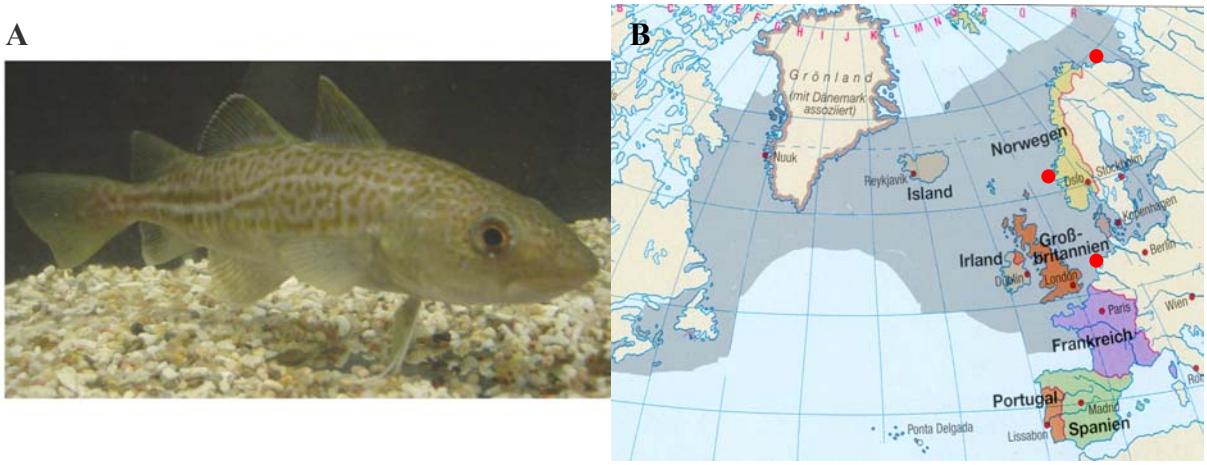


Abb. 6 Atlantischer Kabeljau, *Gadus morhua* (A) und sein Verbreitungsgebiet (B, Untersuchungsgebiete sind rot markiert)

Juveniler Kabeljau ernährt sich von Plankton und mit zunehmendem Alter von Würmern, Weichtieren und Krebsen. Die adulten Tiere fressen vorwiegend Fisch, wobei auch Kannibalismus vorkommt (Arntz 1973; Müller 1983; Scott und Scott 1988; Palsson 1994). *G. morhua* laicht vorwiegend im Frühjahr bei Wassertemperaturen von 4 bis 6°C (Müller 1983), wobei der Zeitpunkt jedoch populationsabhängig ist und eine Korrelation mit Phytoplankton-Blüten vermutet wird (Cushing 1984; Brander 1996).

Die untersuchten Kabeljaupopulationen unterscheiden sich hinsichtlich der Temperatur ihrer Verbreitungsgebiete. In der Nordsee schwankt die Oberflächentemperatur im Jahresverlauf zwischen 7 und 16°C (Laevastu 1993), und während der Sommerzeit kann sich eine Temperatursprungschicht bilden, welche zu Grundtemperaturen zwischen 6 und 8°C führt (Brander 1994; Dippner, CLICOFI-Bericht). Um Norwegen schwanken die Temperaturen zwischen 5°C und 8°C. Kabeljau der Barentssee ist bei Temperaturen von 2 bis 4°C zu finden und erfährt während seiner Laichwanderung an norwegische Küsten Temperaturen bis maximal 7°C (Brander 1994; Dippner, CLICOFI-Bericht).

Mit der vorliegenden Arbeit sollten Fragen zur Temperaturanpassung und zu temperaturbedingten Toleranzgrenzen von Fischen im latitudinalen Gradienten geklärt werden. Aufgrund der vergleichbaren Lebensweise, jedoch unterschiedlicher Habitattemperaturen zwischen den Kabeljaupopulationen und zwischen den beiden Aalmutterarten, sind diese Tiere für einen Vergleich hinsichtlich adaptiver Anpassungsmechanismen an unterschiedliche Temperaturen gut geeignet. Zudem hat der Kabeljau *G. morhua* eine große Bedeutung für die Fischerei, und möglichhe Auswirkungen einer globalen Erwärmung auf die Verbreitungsgebiete von Kabeljau würden die Fischerei beeinflussen. Nach einem Höhepunkt 1968 mit einem Gesamtfanggewicht von 3,9 Millionen Tonnen nahm die Fangquote für Kabeljau kontinuierlich ab (Brander 1996) und im letzten Jahrzehnt wurde ein Zusammenbruch mehrer Kabeljaubestände im nordwestlichen Atlantik verzeichnet (Hutchings und Myers 1994; Myers et al. 1996). Seit 1984 liegt die Kabeljaupopulation der Nordsee mit 150000 Tonnen unter dem konventionellen Minimum einer aus biologischer Sicht akzeptierbaren Quote (ICES 1991; ICES 1997), und die Fischerei für Kabeljau in der Nordsee wurde bereits stark eingeschränkt. Das beobachtete Zusammenbrechen von Kabeljaubeständen ist vermutlich hauptsächlich auf die Überfischung zurückzuführen. Ein Einfluss auf die Bestandsgröße von Kabeljau aufgrund klimatischer Veränderungen kann jedoch nicht ausgeschlossen werden, da sich die Temperatur maßgeblich auf die Fruchtbarkeit, die Rekrutierung und das Wachstum der Fische auswirkt (siehe Nakken

1994; Brander 1996; O`Brien et al. 2000). Der Einfluss klimatischer Veränderungen und der Temperatur auf die Ökologie von Kabeljau wird bereits seit Jahren untersucht, jedoch konnte noch kein mechanistisches Prinzip von Ursache und Wirkung beschrieben werden.

Die vorliegende Arbeit wird sich vor allem mit den folgenden Fragen befassen:

1. Gibt es Unterschiede in der Kälteanpassung der aeroben Kapazität zwischen kaltakklimatisierten und kaltadaptierten Tieren?
2. Unterscheidet sich der mitochondriale Stoffwechsel zwischen kaltadaptierten Eurythermen und kaltadaptierten Stenothermen?
3. Welchen Einfluss besitzt eine Kälteanpassung auf den Energiehaushalt im Hinblick auf Wachstum und Energiereserven in der Leber?
4. Ist die kaltstenotherme Aalmutter *P. brachycephalum* in der Lage, sich an höhere Temperaturen anzupassen?
5. Können thermale Toleranzgrenzen (Pejus-Temperaturen) für Fische definiert werden, und inwieweit sind diese für die Verbreitungsgrenzen von *G. morhua* in der Nordsee verantwortlich?

2. Material und Methoden

Die in dieser Arbeit verwendeten Methoden werden im folgenden Kapitel kurz dargestellt. Eine genauere Beschreibung kann den einzelnen Publikationen (Kapitel 3) entnommen werden.

2.1 Hälterung und Temperaturinkubation

Die Hälterung aller Tiere erfolgte in belüftetem natürlichem Seewasser, dessen Salinität 31 - 33‰ betrug.

Aalmutter:

Z. viviparus wurde im August 2001 und im März 2002 in der Deutschen Bucht der Nordsee gefangen und von der Biologischen Anstalt Helgoland bezogen (Abb. 5A). Exemplare von *P. brachycephalum* wurden während der Polarsternexpedition ANT XV/3 1998 nahe King George Island gefangen (Abb. 5B) und mit dem Forschungsschiff nach Bremerhaven zum Alfred-Wegener-Institut (AWI) transportiert. Beide Arten wurden bei ihren jeweiligen Umgebungstemperaturen 10°C und 0°C (Kontrolle) und für mindestens 10 Monate bei 5°C (Akklimatisation) gehältert und einmal pro Woche mit lebenden Garnelen (*Crangon crangon*) gefüttert.

Kabeljau:

Nachkommen des nordostarktischen (NEAC) und norwegischen Küstenkabeljaus (NCC) sowie Wildfänge aus der südl. Nordsee (NC, gefangen im Frühling 1999 in der Deutschen Bucht; Untersuchungsgebiete siehe Abb. 6B) wurden für ein Wachstumsexperiment im Rahmen des EU-Projekts CLICOFI (Nr.: ENV4-CT97-0596) bei den Temperaturen, 4°C (außer NEAC), 8°C, 12°C und 15°C gehältert und *ad libitum* gefüttert. Das Wachstumsexperiment mit NCC und NEAC wurde von Projektpartnern der Universität Bergen (T. Johansen und G. Naevdal) im Zeitraum vom 27. Mai 1998 bis 5. März 1999 (Experiment I) und vom 18. Januar 1999 bis 7. März 2000 (Experiment II) durchgeführt, wobei die Tiere mit kommerziellem Fischfutter (NorAqua) gefüttert wurden. Das Wachstumsexperiment mit NC wurde vom 27. April bis 10. September 1999 auf Helgoland von T. Fischer (Projektpartner am AWI) durchgeführt. Diese Wildfänge wurden mit Sprotten gefüttert, da die Tiere das kommerzielle Futter verweigert hatten (siehe Fischer 2002). Nach Beendigung der Wachstumsexperimente wurden die Tiere getötet, Leber und weißes Muskelgewebe entnommen und die Gewebe in flüssigem Stickstoff schockgefroren. Bis zur Weiterverarbeitung lagerten die Proben bei –130°C.

In einem weiteren Temperaturexperiment am AWI wurde Kabeljau aus der Barentssee (NEAC) und der südlichen Nordsee (NC) für mindestens sechs Wochen bei 4°C und 12°C akklimatisiert und zweimal pro Woche mit Muschelfleisch (*Mytilus edulis*) gefüttert.

Für die *in vivo*-Versuche wurde *G. morhua* aus der südlichen Nordsee für mindestens 4 Wochen bei 10°C gehältert und zweimal pro Woche mit Muschelfleisch (*M. edulis*) gefüttert. Eine Woche vor Versuchsbeginn wurde die Fütterung eingestellt.

In der nachfolgenden Tabelle sind die jeweiligen Versuchs- und Tierdaten zusammengestellt.

Tabelle 1 Versuchsdaten (Zeitdauer, Ort, Fütterung) und Tierdaten (Tiergewicht, Lebergewicht, Konditionsfaktor (k-Faktor = Tiergewicht / Tierlänge^3*100)) der untersuchten Fischarten bzw. –Populationen bei den unterschiedlichen Hälterungstemperaturen. Daten sind als arithmetisches Mittel ± Standardabweichung dargestellt.

Aalmutter	***Z. viviparus***	***P. brachycephalum***
Beginn der Hälterung	**10°C**: August 2001 bzw. März 2002 **5°C**: Februar bzw. April 2002	**0°C**: März 1998 **5°C**: Februar 2002
Ort	AWI, Bremerhaven	
Fütterung	einmal pro Woche mit lebenden Garnelen	
Probennahme	Januar bis März 2003	
Tiergewicht (g)	**10°C**: 43 ± 22 (Kontrolle) **5°C**: 63 ± 17	**0°C**: 35 ± 12 (Kontrolle) **5°C**: 39 ± 8
Lebergewicht (g)	**10°C**: 0,85 ± 0,70 (Kontrolle) **5°C**: 2,43 ± 1,01	**0°C**: 1,16 ± 0,58 (Kontrolle) **5°C**: 1,18 ± 0,57
Konditionsfaktor	**10°C**: 0,37 ± 0,08 (Kontrolle) **5°C**: 0,41 ± 0,02	**0°C**: 0,40 ± 0,04 (Kontrolle) **5°C**: 0,36 ± 0,03

Kabeljau (*G. morhua*)	**Nordsee**	**Barentssee**
Beginn der Hälterung	**4°C**: Februar 2001 **12°C**: November 2000	**4°C**: November 2000 **12°C**: November 2000
Ort	AWI, Bremerhaven	
Fütterung	zweimal pro Woche mit Muschelfleisch	
Probennahme	Januar bis April 2001	
Tiergewicht (g)	**4°C**: 403 ± 259 **12°C**: 304 ± 93	**4°C**: 270 ± 31 **12°C**: 191 ± 52
Lebergewicht (g)	**4°C**: 10,17 ± 8,66 **12°C**: 3,02 ± 1,24	**4°C**: 3,03 ± 0,97 **12°C**: 1,41 ± 0,30
Konditionsfaktor	**4°C**: 0,73 ± 0,09 **12°C**: 0,70 ± 0,08	**4°C**: 0,47 ± 0,05 **12°C**: 0,43 ± 0,05

Kabeljau (*G. morhua*)	**Nordsee**	**Norwegische Küste**	**Barentssee**
Beginn der Hälterung (Wachstumsexperiment)	April bis September 1999	Mai 1998 bis März 1999 bzw. Januar 1999 bis März 2000	Mai 1998 bis März 1999
Ort	AWI, Helgoland	Universität Bergen, Bergen	
Fütterung	*ad libitum* mit Sprotten	*ad libitum* mit kommerziellem Fischfutter	
Probennahme	September 1999	März 1999 bzw. 2000	März 1999
Tiergewicht (g)	**4°C**: 292 ± 96 **8°C**: 371 ± 99 **12°C**: 434 ± 193 **15°C**: 342 ± 46	**4°C**: 996 ± 217 **8°C**: 1422 ± 372 **12°C**: 1441 ± 431 **15°C**: 1038 ± 191	**8°C**: 1349 ± 503 **12°C**: 1395 ± 535 **15°C**: 787 ± 256
Lebergewicht (g)	**4°C**: 25,8 ± 5,8 **8°C**: 38,9 ± 11,2 **12°C**: 47,6 ± 23,7 **15°C**: 34,5 ± 8,0	**4°C**: 85,9 ± 33,3 **8°C**: 124,7 ± 38,8 **12°C**: 105,6 ± 66,6 **15°C**: 62,5 ± 39,3	**8°C**: 85,7 ± 76,9 **12°C**: 100,1 ± 91,5 **15°C**: 35,5 ± 28,4
Konditionsfaktor	**4°C**: 1,0 ± 0,1 **8°C**: 1,0 ± 0,1 **12°C**: 1,1 ± 0,1 **15°C**: 1,0 ± 0,1	**4°C**: 1,2 ± 0,1 **8°C**: 1,4 ± 0,2 **12°C**: 1,4 ± 0,3 **15°C**: 1,4 ± 0,1	**8°C**: 1,1 ± 0,2 **12°C**: 1,2 ± 0,1 **15°C**: 1,1 ± 0,1

2.2 Respiration und Enzymaktivität isolierter Lebermitochondrien

2.2.1 Mitochondrienisolierung

Die Mitochondrienpräparation aus Lebern von *Z. viviparus*, *P. brachycephalum* und *G. morhua* (Experiment am AWI, Bremerhaven) erfolgte nach Hardewig et al. (1999b). Hierzu wurde die frisch entnommene Leber in eisgekühltem Homogenisationspuffer kleingeschnitten. Nach mehreren Homogenisations- und Zentrifugationsschritten wurden die isolierten Mitochondrien in 1 – 2 ml Assaymedium resuspendiert.

2.2.2 Mitochondriale Respiration

Der mitochondriale Sauerstoffverbrauch von *Z. viviparus* und *P. brachycephalum* wurde mit einer Sauerstoffelektrode (Clarke-Type) in temperierbaren Respirationskammern (Eschweiler, Kiel) in einem Volumen von 1 ml zwischen 5°C und 20°C gemessen. Als Substrat diente Succinat (3,3 mM). Nach Zugabe von Adenosin-5´-diphosphat (ADP; 0,3 mM) erfolgte die Aufzeichnung der oxidative Phosphorylierung (state III Respiration), die nach Umsetzung des zugegebenen ADPs in die state IV Respiration überging. Für die Ermittlung des Sauerstoffverbrauchs, der auf Protonen-Leckströme (*proton leakage*) zurückzuführen ist, wurde die mitochondriale F_0F_1-ATPase mit 2,5 µg ml^{-1} Oligomycin gehemmt. Die ermittelten Sauerstoffverbrauchswerte wurden unter Berücksichtigung der temperaturabhängigen Sauerstofflöslichkeit im Assaymedium (Johnston et al. 1994) auf nmol O min^{-1} mg^{-1} mitochondriales Protein berechnet.

2.2.3 Mitochondriale Enzymaktivität

Ein Teil der jeweiligen Mitochondriensuspension von *Z. viviparus*, *P. brachycephalum* und *G. morhua* wurde für die Bestimmung von Enzymaktivitäten der Citrat-Synthase (CS) und der $NADP^+$-abhängigen Isocitrat-Dehydrogenase (IDH) weiter verarbeitet. Für die Verifizierung der Mitochondrienausbeute aus der Leber erfolgte eine CS-Messung ebenfalls direkt in der Leber (100% CS).

Die Mitochondriensuspension wurde 2:1 mit Assaymedium (für die Bestimmung der CS-Aktivität) oder mit Stabilisationspuffer (für die Bestimmung der IDH-Aktivität) verdünnt. Für die Messung der CS-Aktivität direkt in der Leber wurden 0,02 g Leber zu 1 ml Assaymedium gegeben. Nach Ultraschallbehandlung und anschließender Zentrifugation wurde in den jeweiligen Überständen die Enzymaktivitäten (CS nach Sidell et al. 1987, und

IDH nach Alp et al. 1976) zwischen 5°C und 20°C photometrisch gemessen. Die ermittelten Enzymaktivitäten wurden auf mg mitochondriales Protein bzw. auf g Leber bezogen.

2.3 Enzymaktivitäten gefrorener Muskel- und Leberproben

In den Gewebeproben der drei Kabeljaupopulationen aus dem Wachstumsexperiment wurden die Enzymaktivitäten von Citrat-Synthase (CS), Cytochrom c-Oxidase (CCO) und des Elektronentransportsystems (ETS, zwei Enzyme der Atmungskette, NADH-Q-Reduktase und Cytochrom b-Reduktase) bestimmt. Die gefrorenen Gewebeproben wurden unter flüssigem Stickstoff mit einem Mörser aufgeschlossen. Nach Überführung in die jeweiligen Homogenisationspuffer folgte eine enzymspezifische Zentrifugation bei 0°C (CS: 9500g, 10min; CCO: 1000g, 10min; ETS: 300g, 10min), wobei für CS eine Ultraschallbehandlung voranging. In den Überständen wurde anschließend die Aktivität von CS (nach Sidell et al. 1987), CCO (nach Moyes et al. 1997) und ETS (nach Madon et al. 1998) zwischen 2°C und 18°C photometrisch bestimmt und auf g Gewebe kalkuliert.

2.4 Mitochondrialer Proteingehalt

Die mitochondriale Proteinkonzentration der Leber wurde anhand der Biuret Methode nach Gornall et al. (1949) ermittelt. Da der hohe Fettanteil der Leber die Messung beeinflusste, erfolgte eine Proteinfällung mittels 3 M TCA. Die Proben wurden anschließend mit Diethyläther und Ethanol (4:1) gewaschen, zentrifugiert und die Proteinkonzentration photometrisch bestimmt. Die ermittelten Proteinwerte (mg pro g Leber) wurden anschließend über die Citrat-Synthase-Messungen in der Mitochondriensuspension und direkt in der Leber korrigiert, da die Ausbeute an Mitochondrien aus der Leber über die Mitochondrienisolierung nicht vollständig ist.

2.5 Lipid-, Wasser- und Proteingehalt der Leber

Der Lipid- und Wassergehalt der Leber wurde für die drei Kabeljaupopulationen aus den Wachstumsexperimenten für die Akklimatisationstemperaturen 4°C und 12°C bestimmt. Die Ermittlung des Gesamtlipids erfolgte nach der Chloroform-Methanol-Wasser Methode von Bligh und Dyer (1959). Für die Berechnung des hepatischen Wasseranteils wurden Leberstücke bei 60°C zu konstantem Gewicht getrocknet. Die ermittelten Datensätze sind als prozentualer Anteil zum Frischgewicht der Leber dargestellt. Eine Abschätzung des Gesamtproteingehalts erfolgte über die Subtraktion des prozentualen Lipid- und Wasseranteils und wurde auf g Gesamtleberprotein kalkuliert.

2.6 Allometrische Skalierung

Das Tiergewicht beeinflusst sowohl die spezifischen Enzymaktivitäten im Muskel als auch die Lebergröße, weshalb eine Normalisierung der Datensätze auf ein einheitliches Tiergewicht erfolgte. Zudem wurden die Enzymaktivitäten und die Proteingehalte der Leber für die gesamte Leber, normalisiert auf ein Tiergewicht, kalkuliert, da die spezifischen Datensätze durch den Lipidgehalt beeinflusst werden. Die angewandten Normalisierungsverfahren sind ausführlich in den Publikationen I und III beschrieben.

2.7 *In vivo*-Versuche an *G. morhua*

Die *in vivo*-Versuche an *G. morhua* aus der Nordsee wurden in zwei Versuchsserien durchgeführt. In der ersten Serie wurde der Sauerstoffpartialdruck im Blut von Kiemenarterien unter Anwendung von faseroptischen Sauerstoffoptoden (Microx I, Pre Sens GmbH) während einer akuten Temperaturerhöhung von 1°C h^{-1} gemessen. Die Eichung der Optoden erfolgte jeweils bei der Kontrolltemperatur von 10°C. Hierzu wurden die 0% und 100% Werte mittels einer gesättigten Natriumsulfit-Lösung bzw. O_2-gesättigtem Seewasser erstellt. Zur Temperaturkorrektur der gemessenen Werte (in % Luftsättigung) dienten erstellte Eichgeraden zwischen 0°C und 20°C bei verschiedenen Sauerstoffkonzentrationen, die über ein Gemisch aus Luft und Stickstoff eingestellt wurden.

Die zweite Versuchsserie diente der Messung von Herz-Kreislauf-Parametern in Abhängigkeit von der Temperatur. Hierbei erfolgte ebenfalls eine Temperaturänderung von 1°C h^{-1}, wobei jedoch ausgehend von der Akklimatisationstemperatur (10°C) neben einer Temperaturerhöhung auch eine Temperaturerniedrigung vorgenommen wurde. Relative Änderungen von arteriellem und venösem Blutfluss wurden mit Hilfe von flussgewichteter Bildgebung in einem Kernspin-Tomographen (NMR) bestimmt, wobei zeitgleich die Aufzeichnung der Herzfrequenz erfolgte. In anschließenden Versuchen wurde unter denselben Bedingungen der venöse Sauerstoffpartialdruck im *ductus Cuvier* bestimmt, wobei jedoch die Temperaturerniedrigung und –erhöhung an verschiedenen Tieren durchgeführt wurde (siehe dazu Publikation IV). Eine Temperaturkorrektur der gemessenen Sauerstoffpartialwerte wie in der ersten Serie war diesmal nicht nötig, da weiterentwickelte Optoden vom Tx-Typ mit integrierter Temperaturkompensation verwendet wurden.

Für die jeweiligen operativen Eingriffe (siehe folgende Abschnitte) wurden die Tiere mit 0,08 g l^{-1} 3-Aminobenzoesäureethylester (MS222) anästhetisiert. Die Anästhesie wurde während der Operation aufrechterhalten, indem die Kiemen mit belüftetem Seewasser mit einer Konzentration von 0,04 g l^{-1} MS222 umspült wurden. Anschließend erfolgte die

Überführung der Tiere in die jeweilige Messkammer, welche an einen kontinuierlichen belüfteten Seewasserkreislauf angeschlossen war. Die Temperatur wurde frühestens nach 24stündiger Erholungszeit erhöht bzw. erniedrigt.

2.7.1 Herzfrequenz

Die Katheterisierung der afferenten Branchialarterie des letzten Kiemenbogens ermöglichte eine kontinuierliche Aufzeichnung der Herzrate. Hierzu wurde ein mit Heparin (50 IU ml^{-1}) und 0,9% Natrium-Chlorid-Lösung gefüllter PE50-Schlauch ca. 0,5 cm in die Arterie eingeführt und fixiert. Über einen verbundenen Druckabnehmer (UFI, Motro, Kanada), der an einen Brückenverstärker Mac Lab System (AD Instruments, Australien) angeschlossen war, konnte die Herzfrequenz über den pulsatilen Blutdruck ermittelt werden.

2.7.2 Blutfluss

Die Bestimmung des Blutflusses bei *G. morhua* erfolgte mit einer flussgewichteten, bildgebenden NMR-Methode. Dafür wurde eine spezielle Kammer aus Plexiglas (Länge: 63 cm; Durchmesser: 12 cm) mit Fixierungsmöglichkeiten für das Tier entwickelt. In Abbildung 7 ist zu sehen, dass mittels beidseitiger Klammern, die an einer Führung variabel zu befestigen waren, die Beweglichkeit des Kabeljaus eingeschränkt werden konnte.

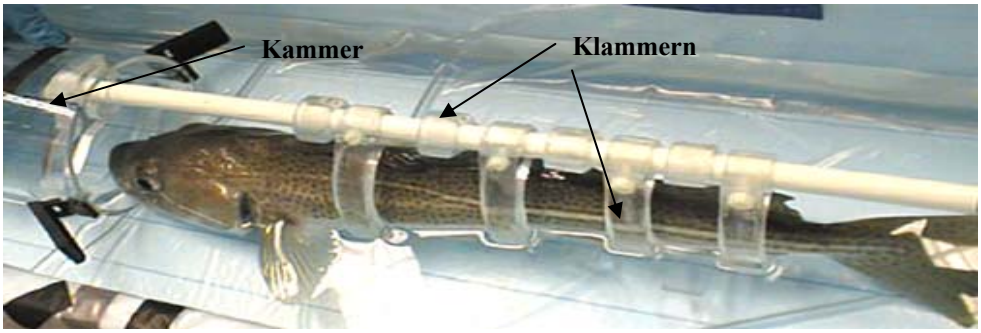


Abb. 7 Kammer Set-up für die zweite Versuchsreihe der *in vivo*-Versuche an *G. morhua*. Erklärung siehe Text.

Der Fisch konnte somit seine horizontale Position innerhalb der Kammer nicht verändern, jedoch Schwanz und Brustflossen bewegen. Mit dieser Kammer war eine definierte Positionierung gewährleistet und Bewegungsartefakte in den MR-Bildern minimiert. Das Kammer-Design, mit variabler Befestigungsmöglichkeit, wurde für die Messungen des venösen PO_2 im *ductus Cuvier* beibehalten.

Die Position des Tieres im Magneten (Bruker Biospec 47/40, Bruker, Ettlingen) wurde mit Hilfe einer Gradienten-Echo-Sequenz in alle drei Raumrichtungen (Tri-Pilot) kontrolliert.

Die Schnittebene für die flussgewichteten Bilder wurde so gewählt, dass sie durch den Rumpf des Fisches am Ende der Schwimmblase verlief (siehe Abb. 8). Die Signalaufnahme erfolgte über einen 1H-Zylinder-Resonator (20 cm Durchmesser), der auf hohe Leitfähigkeiten, wie von Meerwasser, optimiert ist. Relative Änderungen des Blutflusses in der *Aorta dorsalis* und *Vena caudalis* wurden mit der Snapshot FLASH Methode (Haase 1990) aufgenommen (Parameter: Bildmatrix 128 x 128, Bildgröße 6 x 6 cm, Schichtdicke 5 mm, Repetitionszeit 9 ms, Echozeit 3,1 ms, Pulslänge 2000 µs (hermite), Pulswinkel 45° - 60°, Zeit für eine aus 32 Bildern gemittelten Aufnahme 32 s).

Abbildung 8 zeigt eine Schichtaufnahme von *G. morhua* unter Kontrollbedingungen bei 10°C. Die Gefäße, *A. dorsalis* und *V. caudalis*, sind gut als helle Punkte unterhalb der Wirbelsäule zu erkennen. Der dunkle Bereich unter den Gefäßen beruht auf dem Kontrast von Luft aus der Schwimmblase. Die Kombination aus Kammerdesign (eine Klammer ist links im Bild zu erkennen) und einer schnelleren Bildgebungsmethode ermöglichte erstmals flussgewichtete Aufnahmen und damit eine Bestimmung des Blutflusses an pelagischen Fischen.

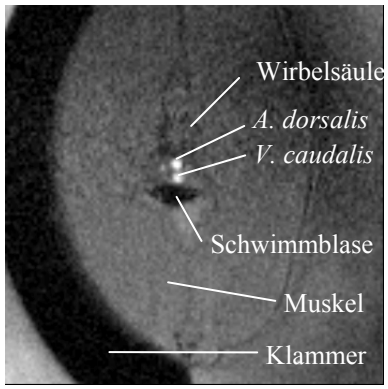


Abb. 8 Flussgewichtete MR-Schichtaufnahme von *G. morhua* unter Kontrollbedingung. Erklärungen siehe Text

2.7.3 Sauerstoffpartialdruck

Die Implantation faseroptischer Sauerstoffsensoren in Blutgefäße von *G. morhua* ermöglichte die on-line Messung des Sauerstoffpartialdrucks in Abhängigkeit von akuten Temperaturänderungen. Die Spitzen der Optoden waren mit einem Teflonderivat ummantelt, in das 1 µm große Graphitpartikel eingebunden waren. In Vorversuchen hatte sich gezeigt, dass Abbauprodukte des Hämoglobins die Messung über die Zeit beeinflussten, da sich die Farbstoffe des Blutes auf der Sensorspitze anlagerten.

In die mittels einer Einmalkanüle (0,6 mm x 30 mm) penetrierte afferente bzw. efferente Kiemenarterie des betäubten Kabeljaus wurde eine Optode (Microx I Typ) ca. 0,5 cm in die Arterie eingeführt und am Kiemenbogen und der Rückenflosse fixiert.

Vor der Implantation in den *ductus Cuvier* wurde die Optodenspitze in 100 IU ml^{-1} Heparinlösung getaucht, um eine Blutgerinnung zu vermindern. Entlang des über einen dorso-ventralen Schnitt im Cleithrum freigelegten *ductus Cuvier* wurde ein ca. 2 cm langer PE50-Schlauch fixiert. Nachdem die Vene mittels einer Einmalkanüle penetriert worden war, wurde

die Optode durch den fixierten PE50-Schlauch etwa 1 cm retrograd in den *ductus Cuvier* geführt und am PE-Schlauch sowie am Fisch fixiert.

Die Messwerte wurden einmal pro Minute über einen angeschlossenen Computer aufgezeichnet und anschließend in Sauerstoffpartialdruck (torr) umgerechnet.

2.8 Statistik

Die Daten sind als arithmetische Mittel ± Standardabweichung angegeben. Unterschiede wurden mit der Varianzanalyse und anschließendem Tukey-Test (ANOVA, Sigma Stat 2.0, SPSS; USA) auf Signifikanz getestet (Signifikanzniveau 95%). Diskontinuitäten im Verlauf der Daten wurden nach Yeager and Ultsch (1989) ermittelt und die sich daraus ergebenden Regressionen wurden mit dem F-Test auf signifikante Unterschiede getestet. Regressionen wurden mit Sigma Plot 8.0 (SPSS, USA) kalkuliert.

3. Publikationen

Liste der veröffentlichten und beabsichtigten Publikationen mit Erklärung über den von mir geleisteten Anteil.

Publikation I

Lannig G, Eckerle LG, Serendero I, Sartoris FJ, Fischer T, Knust R, Johansen T, Pörtner HO (2003) Temperature adaptation in eurythermal cod (*Gadus morhua*): a comparison of mitochondrial enzyme capacities in boreal and Arctic populations.

Marine Biology 142: 589-599

Diese Arbeit entstand im Rahmen des EU geförderten Projekts „Effects of **cli**mate induced temperature change on marine **co**astal **fi**shes“ (CLICOFI; Nr.: ENV4-CT97-0596). Ich habe einen Teil der Experimente durchgeführt und die vom Zweit- und Drittautor ermittelten Daten überarbeitet. Das Manuskript wurde von mir geschrieben, dessen Überarbeitung in Kooperation mit dem siebten und achten Autor erfolgte.

Publikation II

Sartoris FJ, Bock C, Serendero I, Lannig G, Pörtner HO (2003) Temperature dependent changes in energy metabolism, intracellular pH and blood oxygen tension in the Atlantic cod, *Gadus morhua*.

Journal of Fish Biology 62: 1-15

Diese Arbeit entstand ebenfalls im Rahmen des EU geförderten Projekts CLICOFI (Nr.: ENV4-CT97-0596). Ein Teil der Experimente (Sauerstoffpartialdruck im Blut) wurde von mir durchgeführt, und ich habe die vom Drittautor ermittelten Daten überarbeitet.

Publikation III

Lannig G und Pörtner HO Aerobic mitochondrial capacities in Antarctic and temperate eelpout (Zoarcidae) subjected to warm versus cold acclimation.

Die Idee der Versuche entstand in Zusammenarbeit mit dem Koautor. Die Experimente wurden von mir geplant und durchgeführt. Das Manuskript habe ich geschrieben und mit dem Koautor überarbeitet.

Publikation IV

Lannig G, Bock C, Sartoris FJ, Pörtner HO Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L. studied by magnetic resonance imaging (MRI) and on-line venous oxygen monitoring.

Diese Arbeit entstand ebenfalls im Rahmen des EU geförderten Projekts CLICOFI (Nr.: ENV4-CT97-0596). Der diesen Experimenten zugrunde liegende Ansatz entstand in Zusammenarbeit mit dem dritten und vierten Autor. Die Experimente wurden von mir geplant und in Zusammenarbeit mit dem Zweitautor durchgeführt. Das Manuskript habe ich geschrieben und mit den Koautoren überarbeitet.

Weitere Publikationen:

Pörtner HO, 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 FJ, 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

Diese Arbeit ist eine Publikation im Rahmen des EU geförderten Projekts „Effects of **cli**mate induced temperature change on marine **co**astal **fi**shes“ (CLICOFI; Nr.: ENV4-CT97-0596) und entstand in Zusammenarbeit aller Autoren.

Marine Biology (2003) 142: 589–599
DOI 10.1007/s00227-002-0967-6

G. Lannig · L.G. Eckerle · I. Serendero
F.-J. Sartoris · T. Fischer · R. Knust · T. Johansen
H.-O. Pörtner

Temperature adaptation in eurythermal cod (*Gadus morhua*): a comparison of mitochondrial enzyme capacities in boreal and Arctic populations

Received: 8 April 2002 / Accepted: 19 September 2002 / Published online: 30 November 2002

Abstract Activities of citrate synthase (CS), cytochrome *c* oxidase (CCO) and the electron transport system (ETS) were investigated in white muscle and liver of laboratory-maintained cod, *Gadus morhua*, from the North Sea, Norwegian coast and Barents Sea for an analysis of temperature acclimation and adaptation in aerobic metabolism. Cold acclimation within each population led to elevated activities of CS, CCO and ETS in white muscle. In liver, however, only North Sea cod showed cold-compensated CS activities, with CCO and ETS unchanged. In contrast, cold-acclimated Norwegian cod displayed unchanged enzyme activities, and Arctic cod showed elevated activities for CS, but decreased activities for CCO and no change in ETS. Between-population comparisons revealed clear evidence for permanent cold adaptation in white muscle of northern (Norwegian coast and Barents Sea) compared to boreal (North Sea) cod populations, reflected by higher activities of CS, CCO and ETS at the same acclimation temperature. Cold-compensated, mass-specific enzyme activities in liver were found in northern compared to boreal cod for CS and CCO, however, for ETS, after warm acclimation only. When evaluated as capacity in total liver, such activities were only found in northern populations in the case of CS at all temperatures, and for CCO and ETS at 15°C only. Hepatosomatic index (I_H) and liver lipid contents were highest in North Sea cod, with similar I_H but lower lipid contents in cold- versus warm-acclimated animals. An acclimation effect on I_H was found in Norwegian cod only, with higher I_H but unchanged lipid contents in the cold. In conclusion, permanent cold adaptation of muscle aerobic metabolism prevails in cod populations at higher latitudes, which is in line with permanently elevated rates of oxygen consumption observed in a parallel study. These differences reflect higher maintenance costs in cold-adapted versus cold-acclimated cod.

Communicated by O. Kinne, Oldendorf/Luhe

G. Lannig · L.G. Eckerle · I. Serendero · F.-J. Sartoris
T. Fischer · R. Knust · H.-O. Pörtner (✉)
Alfred-Wegener-Institute for Marine and Polar Research,
Columbusstrasse, 27568 Bremerhaven, Germany

E-mail: hpoertner@awi-bremerhaven.de
Tel.: +49-471-48311307
Fax: +49-471-48311149

T. Johansen
Fisheries and Marine Biology, University of Bergen, Norway

Introduction

Temperature is a crucial environmental factor setting the limits for life and influencing the activity of organisms including the rates of growth and reproduction. Fish live in environments with temperatures ranging from around 1.9°C in polar waters to about 34°C in the tropics. Living in polar oceans goes hand in hand with small annual temperature variations. Since low temperatures depress metabolic rates and performance levels, compensatory physiological adjustments may occur. Many poikilotherms have the ability to adjust the rates of biological function by using homeokinetic mechanisms. Enhanced enzyme activities are found in cold-acclimated as well as in cold-adapted animals (Torres and Somero 1988; Crockett and Sidell 1990; Sokolova and Pörtner 2001; Kawall et al. 2002). Alterations in intracellular ionic and membrane phospholipid compositions, quantitative alterations of functional protein levels, functional differences between isozymes (Hochachka and Somero 1984), an increase in mitochondrial densities (Egginton and Sidell 1989; Johnston et al. 1998) or cristae densities (St-Pierre et al. 1998) and/or in organ size [Kent et al. 1988 (liver and heart); Seddon and Prosser 1997 (liver)] have been reported to compensate for the effects of low temperatures.

The adjustment of aerobic scope, reflected by mitochondrial densities and capacities, is identified as a crucial step in thermal adaptation, and mitochondrial enzyme levels are an important determinant of aerobic capacity for ATP production. These adjustments are

suggested to differ between cold-acclimated and cold-adapted as well as between cold-eurythermal and cold-stenothermal animals (Pörtner et al. 2000; Pörtner 2002a). Cold-eurythermal species are predominantly found in the Northern Hemisphere, as the fauna in Arctic oceans has a much younger evolutionary history with respect to cold adaptation than that in Antarctic seas and is less isolated from adjacent seas. Accordingly, some species in sub-Arctic and Arctic waters may still be in transition to life in the permanent cold, while those in the Antarctic have developed features of permanent cold adaptation over millions of years (Pörtner 2002a).

For a comparison of the effects of cold acclimation versus cold adaptation in eurythermal fish the present study was designed to investigate the degree of cold compensation of aerobic enzyme activities in white muscle and liver tissue of three cod populations (*Gadus morhua*) in a latitudinal cline between the southern North Sea (NC), the Norwegian coast (NCC) and the north-eastern Arctic or Barents Sea (NEAC). According to recent evidence these populations are genetically distinct (Nielsen et al. 2001), a finding likely related to their different spawning sites, but possibly, also to life in different climates. The cod population from the North Sea experiences large seasonal temperature fluctuations between 6°C and 14°C, whereas cold-adapted cod from the Norwegian coast and Arctic live in more stable, colder habitats, at temperatures between 5°C and 8°C for NCC and 2°C and 4°C for NEAC (J.W. Dippner, personal communication). The comparison of sub-populations of the same species in a latitudinal cline should avoid pitfalls associated with comparisons of aerobic capacities between different, distantly related fish species. To estimate metabolic activity, enzyme capacities of aerobic metabolism were analyzed: citrate synthase (CS), an indicator for citric acid cycle activity; the so-called ETS (electron transport system), which comprises the activity of two enzymes of the respiratory chain, NADH oxidase and cytochrome *b* reductase (complex I and III); as well as cytochrome *c* oxidase (CCO), which represents complex IV.

Materials and methods

Animals and experimental procedure

Gadus morhua of three populations, southern North Sea cod (NC), Norwegian coastal cod (NCC) and northeastern Arctic cod (NEAC) were acclimated to different temperatures (4, 8, 12 and 15°C, except for NEAC which was only acclimated to 8, 12 and 15°C). Fishes were tagged individually and fed ad libitum for growth rate analysis (NCC and NEAC with commercial food pellets (NorAqua) and NC with sprats, as these cod refused to feed on the food pellets. Experiments with offspring from broodstocks of NCC and NEAC were carried out at the University of Bergen between 18 January 1999 and 7 March 2000 at 4°C and 12°C and between 27 May 1998 and 5 March 1999 at 8°C, 12°C and 15°C. A growth experiment with NC was carried out on the island of Helgoland (Alfred-Wegener-Institute) between 8 July and 9 September, 1999, with cod caught in the German Bight in spring 1999. At the end of the growth experiments, the fish were killed at an age between 1 and 2 years and samples of white axial muscle and liver were frozen in liquid nitrogen. Final body and liver mass, condition and gonadosomatic indices of the experimental fish are summarized in Table 1.

Analyses

Samples of white axial muscle and liver, of female cods only, were prepared for homogenization by grinding in a mortar under liquid nitrogen. CS activity was measured using a modification of the

Table 1 *Gadus morhua*. Final body and liver mass, condition indices (*k*-factor), hepatosomatic indices (I_H) and gonadosomatic indices (I_G) of cod from different acclimation groups and populations. Data are means ± SD; liver masses and I_H are only given for fishes used for the determination of liver composition and enzyme activities

	North Sea cod	Norwegian coastal cod	Northeastern Arctic cod
(*n*)	(7–9)	(6–19)	(5–10)
Body mass (g)			
4°C	292 ± 96	996 ± 217	
8°C	371 ± 99	1422 ± 372	1349 ± 503
12°C	434 ± 193	1441 ± 431	1395 ± 535
15°C	342 ± 46	1038 ± 191	787 ± 256
Liver mass (g)			
4°C	25.8 ± 5.8	85.9 ± 33.3	
8°C	38.9 ± 11.2	124.7 ± 38.8	85.7 ± 76.9
12°C	47.6 ± 23.7	105.6 ± 66.6	100.1 ± 91.5
15°C	34.5 ± 8.0	62.5 ± 39.3	35.5 ± 28.4
k-factor			
4°C	1.0 ± 0.1	1.2 ± 0.1	
8°C	1.0 ± 0.1	1.4 ± 0.2	1.1 ± 0.2
12°C	1.1 ± 0.1	1.4 ± 0.3	1.2 ± 0.1
15°C	1.0 ± 0.1	1.4 ± 0.1	1.1 ± 0.1
Hepatosomatic index (I_H)			
4°C	10.0 ± 1.3	8.2 ± 1.8	
8°C	10.5 ± 1.4	8.5 ± 1.2	5.9 ± 3.2
12°C	10.8 ± 0.9	6.7 ± 2.3	5.2 ± 2.4
15°C	9.9 ± 1.2	5.4 ± 2.6	4.3 ± 1.9
Gonadosomatic index (I_G)			
4°C	0.3 ± 0.1	2.2 ± 0.9	
8°C	0.4 ± 0.1	11.6 ± 4.4	3.7 ± 1.9
12°C	0.4 ± 0.1	11.5 ± 7.3	4.3 ± 2.2
15°C	0.4 ± 0.1	11.5 ± 3.0	2.5 ± 1.1

procedure from Sidell et al. (1987): tissue powder (approximately 125 mg) was placed in 9 vol of buffer (75 mM Tris-HCl, pH 7.6, 1 mM EDTA) and mixed briefly using a vortex. After 5 min of ultrasonic treatment at 0°C (output control 8, duty cycle 50%, Branson Sonifier 450), the samples were centrifuged at 9500 *g* for 10 min at 0°C and the extract was withdrawn from below the fatty layer. The effect of this procedure on dilution factors was taken into account for all enzymes (see below). CS activity was determined in 100 mM Tris-HCl buffer (pH 8), containing 5 mM DTNB, 20 mM acetyl-CoA and 20 mM oxaloacetate. Absorbance at $\lambda = 412$ nm was monitored in a thermostatted spectrophotometer (Beckman DU 7400) at 5, 8, 12, 15 and 18°C. For tissues from NC and for all liver samples, measurements were performed at 5, 12 and 18°C only. CS activities, expressed in units per gram tissue wet mass were calculated using an extinction coefficient (ϵ_{412}) for CS of 13.61 l mmol^{-1} cm^{-1}.

For CCO determinations, powdered tissue (approximately 125 mg) was briefly homogenized with an Ultra Turrax in 9 vol of buffer (20 mM Tris-HCl, pH 7.4, 1 mM ETDA, 0.1% Tween 20) according to Hardewig et al. (1999). In contrast to muscle samples, liver samples were centrifuged for 10 min at 1000 *g* and 0°C and the extract was withdrawn from below the fatty layer by use of a syringe. CCO activity was measured according to Moyes et al. (1997) in a buffer containing 20 mM Tris-HCl at pH 8, 0.5% Tween 20 and 50 µM reduced cytochrome *c*. Cytochrome *c* was dissolved in 20 mM Tris-HCl at pH 8 and reduced by the addition of sodium dithionite, which was removed by gel filtration in a Sephadex G-25 column (Hardewig et al. 1999). CCO activity was measured by monitoring the decrease in extinction at 550 nm. Activity, expressed in units per gram wet mass, was calculated using an extinction coefficient (ϵ_{550}) of 19.1 l mmol^{-1} cm^{-1}.

The method for measuring ETS activity was modified from Madon et al. (1998). Powdered tissue (approximately 30 mg) was homogenized with an Ultra Turrax in 200 vol of 0.1 M phosphate buffer at pH 8.5, 0.75 mM $MgSO_4$ and 0.2% Triton X-100 and centrifuged for 10 min at 300 *g* and 0°C, and the extract was withdrawn from below the fatty layer. ETS activity was determined in 0.1 M phosphate buffer at pH 8.5, 0.85 mM NADH, 2 mM INT [2-(p-jodophenyl)-3-(p-notrophenyl)-5-phenyl-tetrazoliumchlorid] and 0.03% Triton X-100 by following the reduction of INT, which yields an increase in absorbance at 490 nm. ETS activity, in units per gram tissue wet mass, was calculated using an extinction coefficient (ϵ_{490}) of 15.9 l mmol^{-1} cm^{-1}.

CCO and ETS activities were determined in a thermostatted spectrophotometer (Beckman DU 7400) at 2, 5, 8, 12, 15 and 18°C for white muscle and at 12°C for liver. For ETS measurements, a significant decrease in enzyme activity occurred over time; therefore, a fresh extract was prepared for analysis at each temperature.

Total lipid, water and protein content of liver were determined for all populations at 4°C and 12°C acclimation temperatures. Total lipid content of liver was measured by the chloroform–methanol–water method of Bligh and Dyer (1959). To obtain hepatic water content, pieces of liver were oven dried at 60°C to constant mass. The results of total lipid and water content of liver are expressed as a percentage of liver wet mass. Total protein content of liver was estimated after subtraction of lipid and water contents from 100% and was also expressed as a percentage of liver wet mass.

Allometric scaling

Body and liver mass varied strongly between individual fishes and populations (see Table 1 for original data) and, accordingly, allometric relationships may have influenced the levels and comparisons of enzyme activities. To eliminate the effect of body mass on muscle-specific enzyme activities, values measured for white muscle were converted to enzyme activities expected for a fish weighing 1.2 kg, using a formula modified after Pelletier et al. (1993):

- normalized activity = measured activity × (1.2/body mass)^m,

where *m* is the slope of the linear regression in a double logarithmic plot of enzyme activity per gram tissue against body mass in kilograms. Since experimental body-mass ranges were too small to detect significant relationships, values of *m* were adopted from Pelletier et al. (1993), who determined –0.22 for CS and –0.31 for CCO in white muscle of Atlantic cod (*G. morhua*) captured in the St. Lawrence estuary, near Matane (Quebec). The same factor as for CCO was used to estimate the normalized activity of ETS. In our own experiment similar, but non-significant slopes, were found for NCC (largest dataset) of –0.23 for CS with $P = 0.3286$, –0.23 for CCO with $P = 0.1249$ and –0.15 for ETS with $P = 0.4815$.

A significant negative relationship was found between enzyme activities per gram liver and total liver wet mass. Accordingly, liver-mass-specific enzyme activities were converted to specific enzyme activities expected for a liver weighing 40 g by use of the following formula:

- normalized activity = measured specific activity × (40/liver mass)^m,

where the liver mass exponent *m* was determined from the slope of the linear regression of measured enzyme activity per gram liver wet mass versus liver mass in grams in a double logarithmic plot (Fig. 1). Furthermore, since specific enzyme activities are

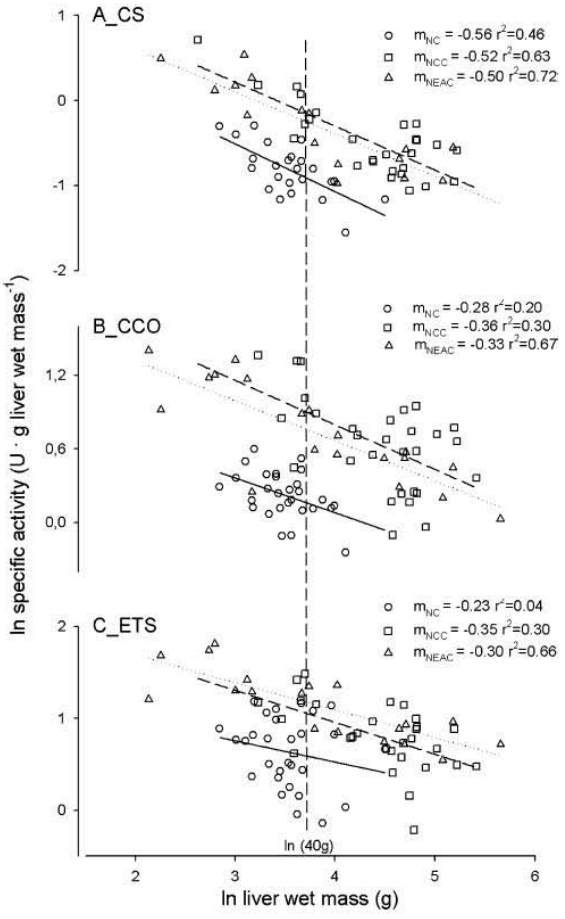


Fig. 1A–C *Gadus morhua*. Double logarithmic plots of measured specific enzyme activities in liver [citrate synthase (*CS*, **A**), cytochrome *c* oxidase (*CCO*, **B**) and electron transport system (*ETS*, **C**)] depending on liver mass in grams of different cod populations (*circle*, *NC* southern North Sea cod, $n = 24$–29; *square*, *NCC* Norwegian coastal cod, $n = 27$–29; *triangle*, *NEAC* north-eastern Arctic cod, $n = 16$–19; *m* slopes of the regressions)

influenced by the lipid contents of the liver we calculated total enzyme activities in whole liver normalized to a 1.2 kg cod with the following formula:

- normalized activity – total activity in liver × (1.2/body mass)^m,

where the body mass exponent m was determined from the slope of the linear regression in a double logarithmic plot of activity in whole liver versus body mass in kilograms (Fig. 2B–D).

Calculation of hepatosomatic index with:

- I_H – liver mass(g)/body mass(g)×100

was done with normalized liver mass for a 1.2 kg cod by the following formula:

- normalized liver mass – exact liver mass × (1.2/body mass)^m,

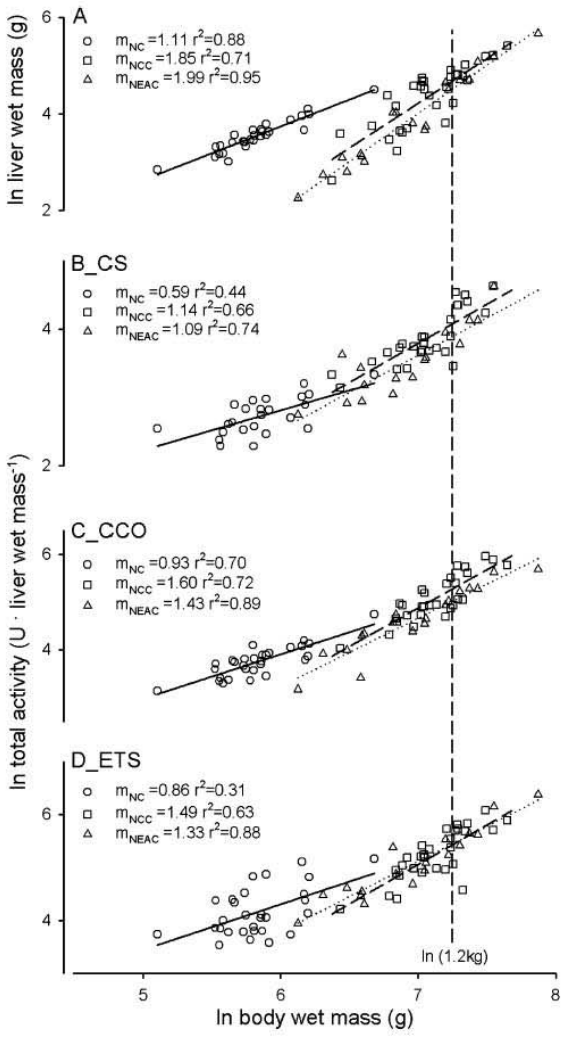


Fig. 2A–D *Gadus morhua*. Double logarithmic plots of liver mass (**A**) and enzyme activities in whole liver [citrate synthase (*CS*, **B**), cytochrome *c* oxidase (*CCO*, **C**) and electron transport system (*ETS*, **D**)] depending on body mass in grams of specimens from different cod populations (*circle*, *NC* southern North Sea cod, n – 23–29; *square*, *NCC* Norwegian coastal cod, n – 27–32; *triangle*, *NEAC* northeastern Arctic cod, n – 16–19; m slopes of the regressions)

where the body mass exponent m was determined from the slope of the linear regression in a double logarithmic plot of liver mass versus body mass in kilograms (Fig. 2A).

Since linear regressions were not significantly different between acclimation groups, mass exponents were calculated from regressions for all acclimation groups within one population.

Statistical analyses

All values are given as means (±standard deviation). Statistical significance was tested at the P – 0.05 level using analysis of variance (ANOVA, SigmaStat) and post hoc procedures (Tukey test). Before statistical testing, log transformations were used, whenever necessary to meet statistical assumptions of the homogeneity of variances. However, non-transformed data are shown in text and figures. The f-test was used for regression comparisons.

Results

White muscle

Activities of CS, CCO and ETS per gram white muscle (normalized to a 1.2 kg cod, for non-transformed data see Table 2) with respect to acclimation temperature and population are shown in Fig. 3. Cold acclimation (4°C and 8°C) led to higher CS activities than those at 15°C in white muscle of all three populations (Fig. 3A). A reduction of environmental temperature from 15°C to 4°C (NC and NCC) or 8°C (NEAC) caused enzyme activities to rise by a factor of 2.27 for CS, 1.72 for CCO and 1.65 for ETS in North Sea cod; by a factor of 2.15 for CS, 1.18 for CCO and 1.32 for ETS in Norwegian coastal cod (CCO and ETS not significant); and by a factor of 1.90 for CS, 1.46 for CCO and 1.62 for ETS in northeastern Arctic cod. An acclimation effect was also found for Arrhenius activation energies (E_a) of CS, with data shown in Table 3. E_a in NC and NEAC increased at cold temperatures, whereas E_a in NCC displayed a non-significant trend to decrease with falling acclimation temperature. The highest E_a levels in the cold (8°C) were found in NEAC; however, NC displayed higher levels of E_a than NCC at 4°C.

An acclimation effect on CCO activities in white muscle (Fig. 3B) was found for NC and NEAC only, with significantly higher activities at 4°C and 8°C (NC) and at 8°C and 12°C (NEAC) compared to 15°C. CCO activities of Norwegian cod remained unchanged over the range of investigated acclimation temperatures. No significant differences were found between E_a values at various acclimation temperatures within populations or between populations of the same acclimation level. Values varied around 55.92±3.50 kJ mol^{-1} (measured between 2°C and 18°C, data not shown).

ETS rates in white muscle of all three populations (Fig. 3C) were significantly higher in the cold, with higher activities at 4°C and 8°C (NC), at 8°C (NCC) and at 8°C and 12°C (NEAC) compared to acclimation at 15°C.

In a between-population comparison, CS activities tended to be higher in NEAC than in NC and NCC at all

Table 2 *Gadus morhua.* Non-transformed enzyme activities in white muscle and liver of cod from different acclimation groups and populations. Data are means ± SD (*CS* citrate synthase; *CCO* cytochrome *c* oxidase; *ETS* electron transport system;)

	North Sea cod	Norwegian coastal cod	Northeastern Arctic cod
White muscle			
(*n*)	(5–8)	(6–14)	(3–8)
CS (U g $^{-1}$ wet mass)			
4°C	2.5 ± 0.8	2.4 ± 0.9	
8°C	2.4 ± 0.8	2.0 ± 0.5	2.6 ± 1.2
12°C	1.6 ± 0.4	1.2 ± 0.3	1.9 ± 0.7
15°C	1.1 ± 0.3	1.1 ± 0.2	1.5 ± 0.2
CCO (U g $^{-1}$ wet mass)			
4°C	1.9 ± 0.4	1.9 ± 0.2	
8°C	1.8 ± 0.4	1.4 ± 0.4	1.9 ± 0.5
12°C	1.4 ± 0.3	1.6 ± 0.2	1.8 ± 0.2
15°C	1.0 ± 0.3	1.6 ± 0.3	1.5 ± 0.2
ETS (U g $^{-1}$ wet mass)			
4°C	1.9 ± 0.3	1.7 ± 0.5	
8°C	1.8 ± 0.8	1.9 ± 0.4	1.5 ± 0.5
12°C	1.3 ± 0.3	1.3 ± 0.3	1.8 ± 0.4
15°C	1.1 ± 0.3	0.3 ± 1.3	1.1 ± 0.3
Liver			
(*n*)	(5–8)	(6–8)	(4–8)
CS (U g $^{-1}$ wet mass)			
4°C	0.6 ± 0.1	0.5 ± 0.2	
8°C	0.5 ± 0.1	0.6 ± 0.1	0.9 ± 0.6
12°C	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
15°C	0.4 ± 0.1	0.9 ± 0.3	0.7 ± 0.4
CCO (U g $^{-1}$ wet mass)			
4°C	1.4 ± 0.2	1.2 ± 0.3	
8°C	1.3 ± 0.2	1.8 ± 0.3	1.5 ± 0.3
12°C	1.2 ± 0.1	1.9 ± 0.4	1.9 ± 0.6
15°C	1.2 ± 0.2	2.9 ± 0.8	2.9 ± 0.9
ETS (U g $^{-1}$ wet mass)			
4°C	2.3 ± 0.6	1.7 ± 0.3	
8°C	2.8 ± 0.4	2.1 ± 0.4	2.6 ± 0.8
12°C	1.4 ± 0.4	2.3 ± 0.8	2.7 ± 0.8
15°C	1.4 ± 0.4	3.2 ± 0.8	3.9 ± 1.4
Lipid-free liver			
(*n*)	(4–6)	(6)	(6)
CS (U g $^{-1}$ lipid-free mass)			
4°C	1.8 ± 0.3	1.0 ± 0.2	
12°C	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.5
CCO (U g $^{-1}$ lipid-free mass)			
4°C	4.1 ± 0.4	2.6 ± 0.4	
12°C	4.7 ± 0.8	4.6 ± 0.8	3.9 ± 0.9
ETS (U g $^{-1}$ lipid-free mass)			
4°C	7.5 ± 2.1	4.2 ± 1.0	
12°C	7.0 ± 3.1	6.7 ± 1.2	7.2 ± 1.2

acclimation temperatures, however, differences remained insignificant. At all acclimation temperatures, the population comparisons showed significantly lower CCO activities in white muscle of North Sea cod compared to Norwegian and Arctic cod. Lower ETS rates were found in white muscle of NC compared to NCC at 8, 12 and 15°C acclimation and compared to NEAC at 12°C acclimation only.

Overall, the data suggest enhanced oxidative capacity in white muscle due to permanent cold adaptation in Norwegian coastal and northeastern Arctic cod compared to North Sea cod with mean factors of 1.10 (CS), 1.25 (CCO) and 1.64 (ETS) between NC and NCC and of 1.37 (CS), 1.59 (CCO) and 1.19 (ETS) between NC and NEAC (calculated for an acclimation temperature of 8°C). For CS and CCO activities, a continuum in the level of cold adaptation was apparent that followed the latitudinal cline between NC, NCC and NEAC.

Liver

Weight-specific CS, CCO and ETS activities normalized to 40 g liver wet weight depending on acclimation temperature and population are shown in Fig. 4. Figure 5 displays total activity in liver normalized to a 1.2 kg cod. Acclimation effects on CS activity in liver were different between populations. CS of NC showed similar specific activities between acclimation groups (Fig. 4A), but a significant increase with decreasing acclimation temperature became apparent for CS in total liver (Fig. 5A). Norwegian cod acclimated to 4°C displayed significantly lower levels of specific CS activity compared to 8, 12 and

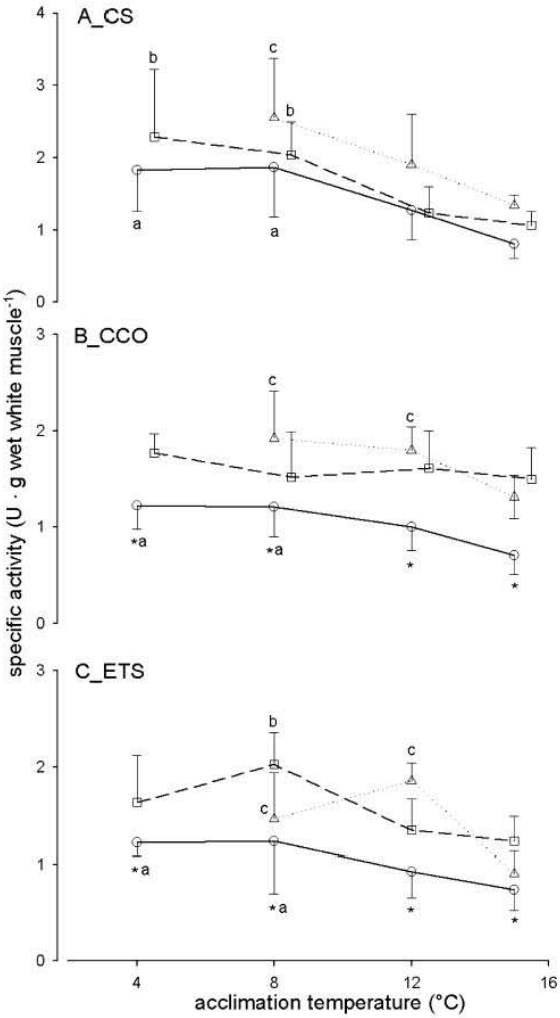


Fig. 3A–C *Gadus morhua.* Activities of citrate synthase (*CS*, **A**), cytochrome *c* oxidase (*CCO*, **B**) and electron transport system (*ETS*, **C**) in white muscle (measured at 12°C and normalized to 1.2 kg cod) of different cod populations acclimated to 4, 8, 12 and 15°C. For better viewing squares were shifted to the right in A and B (*circle* southern North Sea cod, $n = 5$–8; *square* Norwegian coastal cod, $n = 6$–14; *triangle* northeastern Arctic cod, $n = 3$–8; *a*, *b* and *c* indicate data significantly different from values at 15°C for each population; *asterisk* indicates data of NC significantly different from NCC and NEAC, for ETS the difference to NEAC is significant at 12°C only)

15°C (Fig. 4A), but, due to a rise in liver size, similar values in total liver at all acclimation temperatures (Fig. 5A). Both, specific and total CS activities in NEAC liver rose in the cold and were significantly higher at 8°C than at 15°C.

No differences in E_a values were found between acclimation temperatures of the same population and between populations at the same acclimation temperature, with

Table 3 *Gadus morhua.* Arrhenius activation energies E_a (kJ mol^{-1}) of citrate synthase activity in white muscle of different acclimation groups and populations. Data are means ± SD; E_a values measured between 5°C and 18°C. Means with *different superscripts* denote significant acclimation effects within one population; means with *asterisks* differ significantly between populations at the same acclimation temperature

	North Sea cod	Norwegian coastal cod	Northeastern Arctic cod
(*n*)	(6)	(6–14)	(3–4)
4°C	$45.3 \pm 6.7^{a*}$	$27.3 \pm 8.7^{*}$	
8°C	$31.7 \pm 4.1^{b*}$	32.6 ± 17.5	$54.6 \pm 4.1^{a*}$
12°C	33.0 ± 1.7^{b}	38.3 ± 15.1	33.3 ± 9.9^{b}
15°C	31.9 ± 1.6^{b}	43.7 ± 23.3	35.1 ± 3.2^{b}

average values of about 32.1 ± 2.3 kJ mol^{-1} for all specimens (measured between 5°C and 18°C, data not shown).

Specific activities of CCO in liver were similar at the different acclimation temperatures in NC; however, total liver activities tended to rise between 15°C and 8°C. In contrast, CCO in NEAC was reduced in the cold (Fig. 4B and Fig. 5B, specific and total, respectively). In NCC, specific CCO was reduced in cold versus warmer acclimation temperatures (Fig. 4B) but remained similar with acclimation in total liver (Fig. 5B).

Specific ETS activities in liver of NC were higher at 4°C and 8°C compared to 15°C. Total ETS activities were higher at 8°C only compared to 15°C and fell again at 4°C, resulting in no difference to 15°C. (Fig. 4C and Fig. 5C, specific and total, respectively). In NCC, specific ETS was significantly lower in cold- versus warm-acclimated fish (Fig. 4C). Total ETS in NCC and specific as well as total ETS in NEAC showed unchanged values between acclimation temperatures.

Differences in CS activities between populations were found at all acclimation temperatures. Specific and total CS activities in liver of NC were significantly lower than those of NCC and NEAC. At 8, 12 and 15°C specific CCO and at 12°C and 15°C specific ETS activities were significantly lower in NC than in NCC and NEAC (Fig. 4B, C); however, this pattern was only apparent at 15°C for total liver CCO and ETS (Fig. 5B, C).

The normalized hepatosomatic index (I_H; Fig. 6) showed no significant acclimation effect in NC and NEAC (for the latter investigated between 12°C and 8°C only), but I_H rose in NCC during cold acclimation (Fig. 6). Overall, significantly higher I_H values were found in NC compared to NCC and NEAC.

Lipid and water contents and estimated protein contents of liver for acclimation temperatures of 4°C and 12°C are shown in Fig. 7 (except those for NEAC at 4°C). NC livers had significantly more lipid at 12°C than at 4°C, whereas lipid contents of NCC livers remained unchanged at the different temperatures. Population comparisons showed higher liver lipid contents for NC compared to NCC and NEAC (Fig. 7A), an observation possibly not related to temperature but to the much higher lipid contents in sprats (around 70%) than in the commercial pellets (around 12%). The water contents of

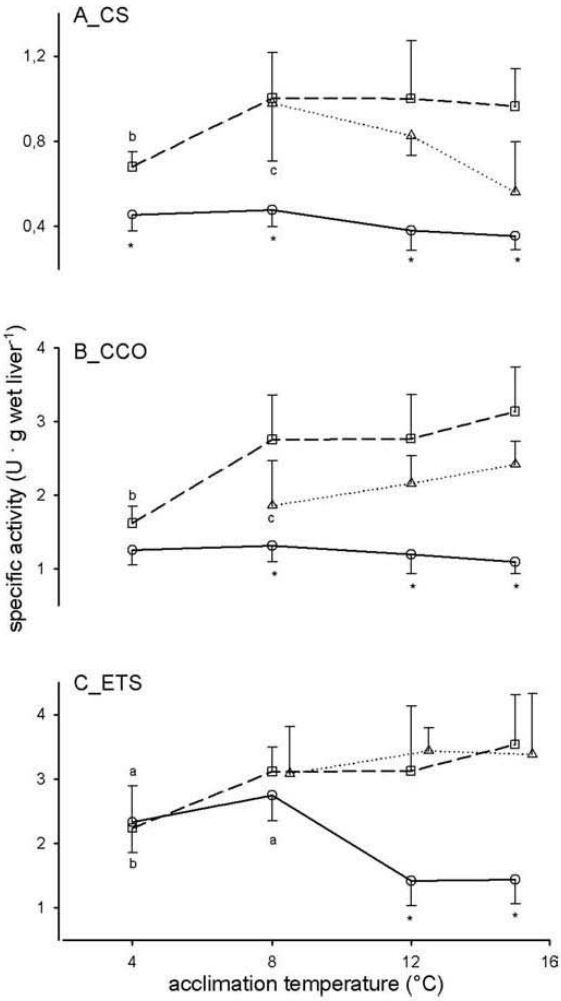


Fig. 4A–C *Gadus morhua.* Specific activities of citrate synthase (*CS*, **A**), cytochrome *c* oxidase (*CCO*, **B**) and electron transport system (*ETS*, **C**) in liver (measured at 12°C and normalized to 40 g liver; ETS values of NC were not normalized, since the regression in Fig. 1C remained non-significant) of different cod populations acclimated to 4, 8, 12 and 15°C (*circle* southern North Sea cod, $n = 5–8$; *square* Norwegian coastal cod, $n = 6–14$; *triangle* northeastern Arctic cod, $n = 3–8$; *a* and *c* indicate data significantly different from values at 15°C for NC and NEAC; *b* indicates data significantly different from those at 8, 12 and 15°C for NCC; *asterisk* indicates data of NC significantly different from those of NCC and NEAC at the same acclimation temperature)

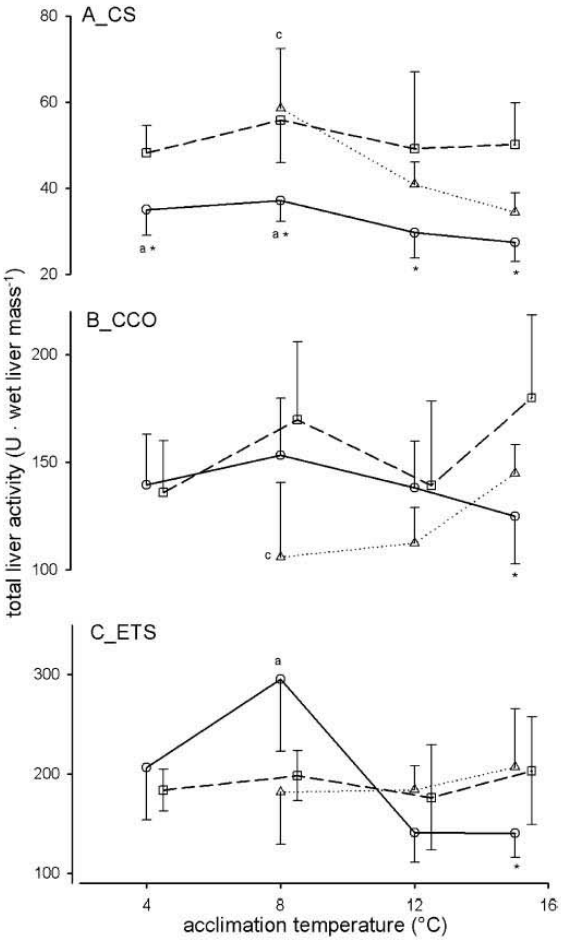


Fig. 5A–C *Gadus morhua.* Total activities of citrate synthase (*CS*, **A**), cytochrome *c* oxidase (*CCO*, **B**) and electron transport system (*ETS*, **C**) in liver (measured at 12°C and normalized to a 1.2 kg cod) of different cod populations acclimated to 4, 8, 12 and 15°C. For better viewing squares were shifted to the right in B and C (*circle* southern North Sea cod, $n = 5–8$; *square* Norwegian coastal cod, $n = 6–14$; *triangle* northeastern Arctic cod, $n = 3–8$; *a* and *c* indicate data significantly different from values at 15°C for NC and NEAC; *asterisk* indicates data significantly different from those of other populations at the same acclimation temperature)

liver mirrored the patterns of lipid contents (Fig. 7B), with lower contents in NC livers at 12°C compared to 4°C, and unchanged values regardless of acclimation temperature in NCC livers. Population comparisons revealed significantly lower liver water contents in NC compared to NCC and NEAC. The estimated total protein contents of liver showed neither a significant temperature effect nor significant differences between populations (Fig. 7C), although due to elevated lipid levels, specific protein contents of NC livers at 12°C tended to be lower than at 4°C and also below the total liver protein contents of NCC and NEAC at 12°C.

Discussion

Cold acclimation

Elevated activities of the investigated aerobic enzymes were found after cold acclimation in white muscle of all

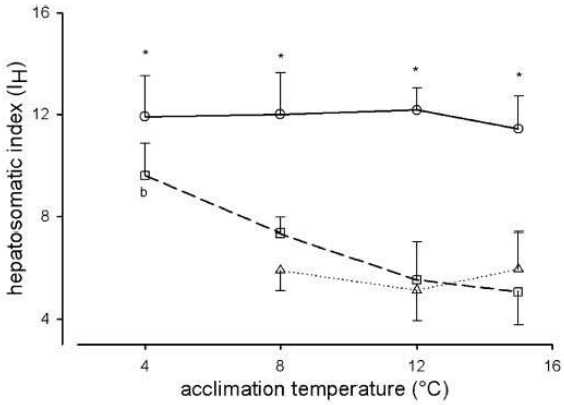


Fig. 6 *Gadus morhua.* Hepatosomatic index (I_H, normalized to a 1.2 kg cod) of different cod populations acclimated to 4, 8, 12 and 15°C (*circle* southern North Sea cod, $n = 4–8$; *square* Norwegian coastal cod, $n = 6–9$; *triangle* northeastern Arctic cod, $n = 5–7$; *b* indicates data significantly different from those at 12°C and 15°C for NCC; *asterisk* indicates data of NC significantly different from those of NCC and NEAC at the same acclimation temperature)

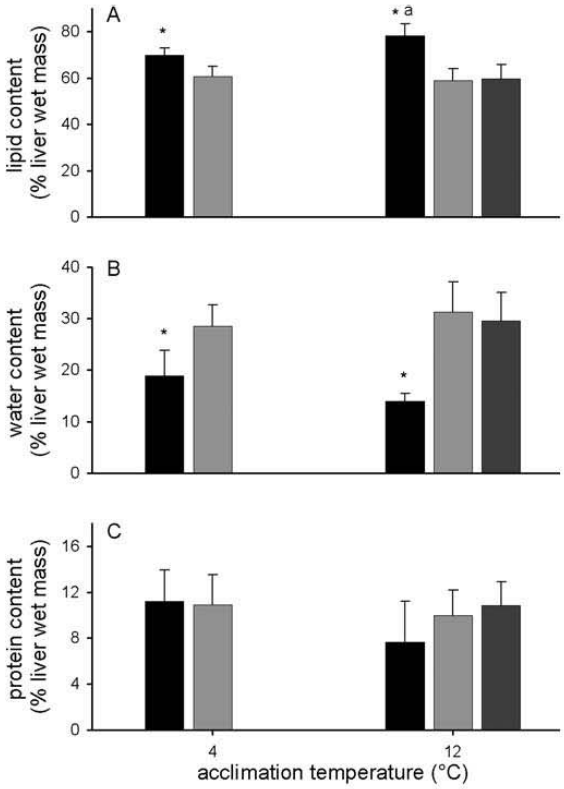


Fig. 7A–C *Gadus morhua.* Total lipid (**A**), water (**B**) and protein contents (**C**) of liver of different cod populations acclimated to 4°C and 12°C (*black* southern North Sea cod, $n = 4–8$; *light gray* Norwegian coastal cod, $n = 4–9$; *dark gray* northeastern Arctic cod, $n = 5–7$; *a* indicates data significantly different from those at 4°C for NC; *asterisk* indicates data of NC significantly different from those of NCC and NEAC at the same acclimation temperature)

cod populations (Fig. 3). Factorial increments were in the medium range compared to those described for other fish species. Usually, factorial changes were lower for CCO and ETS than for CS activities. An excess capacity of citrate synthase in the cold might provide excess citrate for enhanced lipid biosynthesis (for review cf. Pörtner 2002a,b). For comparison, cold acclimation also led to higher CS activities in axial and pectoral muscle of threespine stickleback, *Gasterosteus aculeatus* (Guderley and Leroy 2001), by a factor of approximately 2 (axial) and 1.5 (pectoral) and in muscle of rainbow trout, *Oncorhynchus mykiss*, by a factor of 2.82 (white muscle) and 3.98 (red muscle) (Cordiner and Eggington 1997). These changes are reversed during heat exposure as in white sucker, *Castotomus commersoni*, where CS activity in white muscle fell by around 30% during the first day and remained constant thereafter (Hardewig et al. 2000). Elevated CCO activities in white muscle after cold acclimation were found in North Sea eelpout, *Zoarces viviparus* (Hardewig et al. 1999), and in juvenile cod, *Gadus morhua* (Foster et al. 1993), as well as in red muscle of cold-acclimated carp, *Cyprinus carpio* L. (Wodtke 1981). Van den Thillart and Modderkolk (1978) found a significant increase in CCO activities, which was accompanied by a parallel increase of mitochondrial respiration rates (state III, ADP-activated) per gram red and white muscle of goldfish, *Carassius auratus auratus*, with decreasing acclimation temperature.

CCO and ETS activities (the latter measured as the combined activities of complex I and III) as measures of oxidative capacity have been investigated in many tissues and whole organisms from plankton to mammals (Jansky 1961; Simon and Robin 1971; Ikeda 1989; Elderkin et al. 1998); however, very little information is available for the response of ETS activities to temperature in animals. Tully et al. (2000) estimated metabolic rate responses to changes in temperature by measuring ETS activity (combined complex I, II and III) in abdominal muscle of lobster, *Homarus gammarus*. They found similar ETS activities in muscle of cold- (8°C) and warm-acclimated (18°C) lobster when assayed at 13°C, indicating no compensation for changes in environmental temperature. These findings are in contrast to our demonstration of cold-compensated ETS activities in cod white muscle (Fig. 3C). The poor compensation ability of lobsters is consistent with the well-described seasonality in their activity, growth and depth distribution (Tully et al. 2000). In our study, CS, CCO and ETS activities in white muscle changed in parallel. This indicates that aerobic capacities of white muscle are enhanced in cold-acclimated cod.

In liver, enzyme activities failed to show such a clear pattern of cold acclimation. Specific CS and CCO activities in liver of NC remained unchanged at the different acclimation temperatures, and only specific ETS

was enhanced in the cold. However, total liver CS of NC rose with cold acclimation, whereas total CCO and likely ETS remained unchanged over the investigated acclimation range. In NCC, all enzymes even showed lower specific activities at 4°C compared to 8, 12 and 15°C, whereas total enzyme activities remained similar during acclimation, due to the rise in liver mass. In NEAC, specific and total CS activities were enhanced after cold acclimation, whereas CCO and ETS activities decreased with decreasing acclimation temperature (not significant for ETS). A lack of change or even a loss of specific CCO activities (U g^{-1} liver) had also been shown in liver of cold-acclimated eelpout, *Z. viviparus* (Hardewig et al. 1999), goldfish, *C. auratus* (van den Thillart and Modderkolk 1978), and ide, *Leuciscus idud melanotus* (Rafael and Braunbeck 1988). In cold-acclimated channel catfish, *Ictalurus punctatus*, Kent et al. (1988) found a positive compensation of total liver CS activities, whereas total CCO remained similar between fish acclimated to 25°C and 15°C. Comparisons of specific activities and DNA content based on liver wet mass showed no compensation of CS activity, whereas CCO activity and DNA content were reduced in the cold. Furthermore, the authors found increased CS in the cold but decreased CCO when based on milligrams liver protein and increased CS but similar CCO activities when based on milligrams liver DNA. Overall, their findings were related to a twofold increase in liver mass in the cold accompanied by an increase of total protein leaving protein concentrations per gram tissue unchanged.

These observations in liver are principally in line with the trends in cod white muscle for CS on the one hand and for CCO and ETS on the other. CS showed stronger cold compensation than the membrane-bound systems. Hepatic-specific activities of the latter even appear to fall during cold acclimation in the Northern cod populations, as observed in cold-acclimated catfish. This trend was linked to increasing liver size (at unchanged lipid levels) in NCC, but not (between 15°C and 8°C) in NEAC. Since no data are available for NEAC acclimated to 4°C, comparison with this population is outside its normal temperature range. A rise in I_H similar to that observed in NCC can therefore not be excluded during extreme cold acclimation of NEAC (Fig. 6). In NC an increase in functional liver size in the cold appears likely, but was hidden by the concomitant drop in lipid levels. Here, total CS activity (Fig. 5A) was enhanced in the cold, with a concomitant increase in liver protein. Similar to the catfish, no acclimation effects were found on total CCO (Fig. 5B) despite enhanced liver protein content (Fig. 7C). A rise in total ETS was only observed at 8°C. This result likely reflects a decrease of specific enzyme activities per milligram tissue protein as described by Kent et al. (1988) for catfish (see above).

In NEAC, total CS activities were more enhanced in the cold than in NC (Fig. 5A). Since liver-mass-specific CS activities (Fig. 4A) were also higher in the cold, a rise in enzyme number and/or enzyme turnover number may have occurred during cold acclimation of NEAC. The concomitant drop in CCO activity again suggests a metabolic reorganization of cold-acclimated mitochondria, emphasizing the general trend discussed above. Aerobic capacity (state III respiration) of mitochondria reflects the integration of the isolated systems into the larger unit. In fact, the degree of cold compensation of state III respiration of isolated liver mitochondria after acclimation to 4°C was small in both NC and NEAC (T. Fischer, G. Lannig, R. Knust, H.O. Pörtner, unpublished data), a finding in line with the picture drawn here. Capacity increments at the mitochondrial level are most strongly suggested by the rise in CS activities in both NC and NEAC in the cold, but the functional impact of this rise may be compromised to some extent by falling CCO and ETS levels. CS, CCO and ETS activities in NCC indicate unchanged or even falling aerobic capacities during cold acclimation, where a drop in liver-mass-specific rates is compensated for by a rise in liver size.

Cold adaptation

The findings of permanent cold compensation in NCC and NEAC white muscle are consistent with inter-specific comparisons by Crockett and Sidell (1990), who found 1.5- to 5-fold higher CS and CCO activities in muscles from sluggish, bottom-dwelling as well as from more active, pelagic Antarctic species (*Notothenia gibberifrons*, *Trematomus newnesi*) compared to their temperate counterparts (*Myoxocephalus octodecimspinosus*, *Tautoga onitis*). Cold compensation linked to enhanced CS activities in white muscle of Antarctic compared to California mesopelagic fishes was also observed by Torres and Somero (1988). In contrast, comparisons of enzyme activities in Antarctic and tropical fish by Kawall et al. (2002) failed to show cold compensation in white muscle, likely due to different lifestyles (lower activity rates in Antarctic fish), but clear evidence for cold compensation in the polar species was visible when brain CS activities were compared.

In liver, once again, the picture is less clear, owing to parallel changes in organ size, composition and enzyme capacities. Liver-mass-specific levels of all enzymes increase between NC, NEAC and then NCC (Fig. 4) for ETS at 12°C and 15°C acclimation only. Some of this is due to different lipid levels, which were high especially in NC (Fig. 7A). Accordingly, total liver capacities are no longer as clearly different, especially for CCO and ETS. Ratios of total enzyme activities between populations after 8°C and 15°C acclimation are shown in Table 4 and provide clear evidence for positive cold compensation in total CS activities due to permanent cold adaptation independent of acclimation temperature. However, no significant differences between total CCO and ETS activities of populations were seen at 8°C, whereas at 15°C total levels were higher in NEAC and

Table 4 *Gadus morhua*. Ratios of total enzyme activities in liver of different cod populations acclimated to 8°C and 15°C (*CS* citrate synthase; *CCO* cytochrome *c* oxidase; *ETS* electron transport system; *asterisk* indicates significantly enhanced ratios)

	Norwegian: North Sea		Arctic: North Sea	
	8°C	15°C	8°C	15°C
CS	1.5*	1.8*	1.6*	1.3*
CCO	1.1	1.4*	0.7	1.2*
ETS	0.7	1.5*	0.6	1.5*

NCC than in NC. These findings again suggest distinct changes of CS on the one hand and of respiratory chain components on the other, as discussed in the context of cold acclimation (see previous subsection). Data on isolated mitochondria in NC and NEAC (T. Fischer, G. Lannig, R. Knust, H.O. Pörtner, unpublished data) emphasize that elevated liver-mass-specific activities, especially of CS, but also of CCO and ETS, are in line with highly cold-compensated mitochondrial capacities in the northern cod populations. Total hepatic capacities of CCO and ETS were similar or even lower (in the case of CCO in NEAC) in cold- compared to warm-adapted cod, counteracting the effect of enhanced CS levels. Nonetheless, they may still allow for some rise in liver total aerobic capacity and may be further influenced by population-specific changes in membrane properties.

Metabolic control in the cold

Enhanced enzyme capacities in white muscle in the cold, as found in cold-acclimated as well as in cold-adapted cod, indicate a rise in aerobic capacity due to either mitochondrial proliferation or enhanced capacities of individual mitochondria; both mechanisms are likely involved. Mitochondrial proliferation has been found in cold-acclimated eurythermal (Campbell and Davies 1978; Egginton and Sidell 1989) as well as in cold-adapted, Southern and Northern Hemisphere species (Londraville and Sidell 1990; Johnston et al. 1998; Sommer and Pörtner 2002).

A mechanism involved in capacity changes of individual enzymes is the minimization or maximization of Arrhenius activation energy (E_a) (Hochachka and Somero 1984; Pörtner et al. 2000; Pörtner 2002a). The traditional understanding is that E_a falls in the cold, indicated by a drop in E_a values of a wide range of enzymes (Hazel and Prosser 1974). This picture has recently been questioned, and it has been argued that a rise in E_a of non-equilibrium enzymes like isocitrate dehydrogenase, in mitochondrial proton leakage and, in consequence, in mitochondrial and whole-animal oxygen demand contributes to low energy turnover in polar, especially Antarctic, species and explains the level of extreme stenothermy (Pörtner et al. 2000; Pörtner 2002a). The higher E_a of CS (Table 3) in cold- versus warm-acclimated white muscle of NC and NEAC, being also higher in cold-adapted NEAC compared to cold-acclimated NC, may serve to limit some of the enhanced metabolic flux caused by mitochondrial proliferation.

Cost of cold compensation

In cod, liver is the primary site of lipid storage (Love 1970; Lambert and Dutil 1997). As food composition differed between NC on the one hand and NEAC and NCC on the other hand, temperature-induced modifications of lipid content can reliably be analyzed only within populations. In accordance with the much higher lipid contents of sprats, comparison between populations revealed significantly lower liver lipid contents in cold-adapted (NCC and NEAC) versus boreal cod (NC) regardless of acclimation temperature. Different gonadosomatic indices (I_G; Table 1) may also explain some of the differences in hepatic lipid content between populations.

However, significantly lower hepatic lipid contents were also found in cold-compared to warm-acclimated NC, reflecting lower energy reserves in the cold despite unlimited food availability (Fig. 7A). This effect was not found in NCC, which showed similar lipid levels at 4°C and 12°C acclimation. Considering its enhanced I_H at low acclimation temperatures, NCC were able to accumulate more lipid at 4°C than at 12°C. A cold-induced shift to lipid anabolism and turnover appears typical for cold-adapted species (for review cf. Pörtner 2002b).

Overall, our findings of elevated aerobic capacities in the cold are in line with the hypothesis that life in the cold is more costly at high northern latitudes due to the eurythermy of many Arctic and sub-Arctic species, in contrast to the largely stenothermal Antarctic fauna (Pörtner et al. 2000, 2001; Pörtner 2002a,b). Mitochondrial proliferation and the cost of maintenance of a higher number of mitochondria [e.g. maintenance of ion (proton) gradients] and aerobic enzyme capacities would contribute to a rise (a higher "idling") of basal metabolism (for review see Pörtner et al. 2000; Pörtner 2002a,b). This increase would cause a shift in energy budget, unfavorable to the accumulation of energy reserves, for growth and for reproduction. Accordingly, elevated maintenance costs could explain lower liver lipid contents in cold-acclimated NC (Fig. 7A). Moreover, Pörtner et al. (2001) found that growth performance in NC, NCC and NEAC, acclimated to 4, 8, 12 and 15°C, was optimal close to 10°C for all populations. However, temperature-specific growth decreased at higher latitudes. Certainly, mitochondrial changes are only part of the overall picture, but it is crucial to understand adjustments of aerobic capacities in cold-acclimated and cold-adapted animals. Further effort is required to investigate the mechanistic links between enzyme capacities and whole-animal oxygen demand in more detail and to come to a more complete understanding of the mechanisms of thermal adaptation,

their constraints and limitations as well as their energetic consequences.

Acknowledgements A contribution to the ELOISE project: "Effects of climate induced temperature change on marine coastal fishes (CLICOFI)", funded by the European Union program "Climate and environment", contract no. ENV4-CT97-0596. The authors wish to thank W. Wosniok (Institute for Statistics, University Bremen) for helping in the statistical and allometric series. The authors hereby declare that experiments performed during this study comply with current legislation.

References

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Campbell C, Davies P (1978) Temperature acclimation in the teleost, *Blennius pholis*: changes in enzyme activity and cell structure. Comp Biochem Physiol B 61:165–167

Cordiner S, Eggington S (1997) Effect of seasonal temperature acclimatization on muscle metabolism in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol Biochem 16:333–343

Crockett EL, Sidell BD (1990) Some pathways of energy metabolism are cold adapted in antarctic fishes. Physiol Zool 63:472–488

Egginton S, Sidell B (1989) Thermal acclimation induces adaptive changes in subcellulas structure of fish skeletal muscle. Am J Physiol 256:R1–R10

Elderkin C, Schneider D, Stoeckel J, Padilla D (1998) A method for measuring in situ oxygen consumption rates of freshwater gastropods. J North Am Benthol Soc 17:338–347

Foster AR, Hall SJ, Houlihan DF (1993) The effect of temperature acclimation on organ/tissue mass and cytochrome *c* oxidase activity in juvenile cod (*Gadus morhua*). J Fish Biol 42:947–957

Guderley H, Leroy P (2001) Family origin and the response of threespine stickleback, *Gasterosteus aculeatus*, to thermal acclimation. J Comp Physiol B 171:91–101

Hardewig I, van Dijk PLM, Moyes CD, Pörtner HO (1999) Temperature-dependent expression of cytochrome-*c* oxidase in Antarctic and temperate fish. Am J Physiol 277:R508–R516

Hardewig I, van Dijk PLM, Leary SC, Moyes CD (2000) Temporal changes in enzyme activity and mRNA levels during thermal challenge in white sucker. J Fish Biol 56:196–207

Hazel JR, Prosser CL (1974) Molecular mechanisms of temperature compensation in poikilotherms. Physiol Rev 54:620–676

Hochachka PW, Somero GN (1984) Biochemical adaptation. Princeton University Press, Princeton, N.J.

Ikeda T (1989) Estimated respiration rate of myctophid fish from the enzyme activity of the electron-transport-system. J Oceanogr Soc Jpn 45:167–173

Jansky L (1961) Total cytochrome oxidase activity and its relation to basal and maximal metabolism. Nature 189:921–922

Johnston IA, Calvo J, Guderley H, Fernandez D, Palmer L (1998) Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. J Exp Biol 201:1–12

Kawall HG, Torres JJ, Sidell BD, Somero GN (2002) Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. Mar Biol 140:279–286

Kent J, Koban M, Prosser C (1988) Cold-acclimation-induced protein hypertrophy in channel catfish and green sunfish. J Comp Physiol B 158:185–198

Lambert Y, Dutil J (1997) Can simple indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? Can J Fish Aquat Sci 54:104–112

Londraville R, Sidell B (1990) Ultrastructure of aerobic muscle in Antarctic fishes may contribute to maintenance of diffusion fluxes. J Exp Biol 150:205–220

Love R (1970) The chemical biology of fishes. Academic, New York

Madon SP, Schneider DW, Stoeckel JA (1998) In situ estimation of zebra mussel metabolic rates using the electron transport system (ETS) assay. J Shellfish Res 17:195–203

Moyes CD, Mathieu-Costello OA, Tsuchiya N, Filburn C, Hansford RG (1997) Mitochondrial biogenesis during cellular differentiation. Am J Physiol 272:1345–1354

Nielsen EE, Hansen MM, Schmidt C, Meldrup D, Gronkjaer P (2001) Population of origin of Atlantic cod. Nature 413:272

Pelletier D, Guderley H, Dutil JD (1993) Does the aerobic capacity of fish muscle change with growth rates? Fish Physiol Biochem 12:83–93

Pörtner HO (2002a) Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol A 132:739–761

Pörtner H (2002b) Physiological basis of temperature dependent biogeography: tradeoffs in muscle design and performance in polar ectotherms. J Exp Biol 205:2517–2530

Pörtner HO, van Dijk PLM, Hardewig I, Sommer A (2000) Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: Davison W, Williams CW (eds) Antarctic ecosystems: models for a wider understanding. Caxton, Christchurch, New Zealand, pp 109–122

Pörtner HO, 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 FJ, 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*). Cont Shelf Res 21:1975–1997

Rafael J, Braunbeck T (1988) Interacting effects of diet and environmental temperature on biochemical parameters in the liver of *Leuciscus idud melanotus* (Cyprinidae: Teleostei). Fish Physiol Biochem 5:9–19

Seddon W, Prosser C (1997) Seasonal variations in the temperature acclimation response of the channel catfish, *Ictalurus punctatus*. Physiol Zool 70:33–44

Sidell BD, Driedzic WR, Stowe DB, Johnston IA (1987) Biochemical correlations of power development and metabolic fuel preferenda in fish hearts. Physiol Zool 60:221–232

Simon L, Robin E (1971) Relationship of cytochrome oxidase activity to vertebrate total and organ oxygen consumption. Int J Biochem 2:569–573

Sokolova IM, Pörtner HO (2001) Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. obtusata* from different latitudes and shore levels. Mar Biol 139:113–126

Sommer AM, Pörtner H-O (2002) Metabolic cold adaptation in the lugworm *Arenicola marina* (L.): comparison of a White Sea and a North Sea population. Mar Ecol Prog Ser 240:171–182

St-Pierre J, Charest P-M, Guderley H (1998) Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimation of rainbow trout *Oncorhynchus mykiss*. J Exp Biol 201:2961–2970

Torres J, Somero G (1988) Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. Mar Biol 98:169–180

Tully O, O'Donovan V, Fletcher D (2000) Metabolic rate and lipofuscin accumulation in juvenile European lobster (*Homarus gammarus*) in relation to simulated seasonal changes in temperature. Mar Biol 137:1031–1040

van den Thillart G, Modderkolk J (1978) The effect of acclimation temperature on the activation energies of state III respiration and on the unsaturation of membrane lipids of goldfish mitochondria. Biochim Biophys Acta 510:38–51

Wodtke E (1981) Temperature adaptation of biological membranes. Compensation of the molar activity of cytochrome *c* oxidase in the mitochondrial energy-transducing membrane during thermal acclimation of the carp (*Cyprinus carpio* L.). Biochim Biophys Acta 640:710–720

Journal of Fish Biology (2003) **62**, 1239–1253
doi:10.1046/j.1095-8649.2003.00099.x, available online at http://www.blackwell-synergy.com

Temperature-dependent changes in energy metabolism, intracellular pH and blood oxygen tension in the Atlantic cod

F. J. SARTORIS*, C. BOCK, I. SERENDERO, G. LANNIG AND H. O. PÖRTNER

Alfred-Wegener-Institut für Polar- und Meeresforschung, Postfach 120161, Columbusstraße, 27568 Bremerhaven, Germany

(Received 10 April 2002, Accepted 18 March 2003)

The effect of acute increase in temperature on oxygen partial pressure (P_{O_2}) was measured in the gill arches of Atlantic cod *Gadus morhua* between 10 and 19° C by use of oxygen micro-optodes. Oxygen saturation of the gill blood under control conditions varied between 90 and 15% reflecting a variable percentage of arterial or venous blood in accordance with the position of each optode in the gill arch. The data obtained suggested that arterial P_{O_2} remained more or less constant and arterial oxygen uptake did not become limiting during warming. A progressive drop in venous P_{O_2}, however, was observed at >10° C indicating that excessive oxygen uptake from the blood is not fully compensated for by circulatory performance, until finally, P_{O_2} levels fully collapse. In a second set of experiments energy and acid–base status of white muscle of Atlantic cod *in vivo* was measured by magnetic resonance (^{31}P-NMR) spectroscopy in unanaesthetized and unimmobilized fish in the temperature range between 13 and 21° C. A decrease in white muscle intracellular pH (pH_i) with temperature occurred between 10 and 16° C (ΔpH per ° C = −0·025 per ° C). In white muscle temperature changes had no influence on high-energy phosphates such as phosphocreatine (PCr) or ATP except during exposure to high critical temperatures (>16° C), indicating that white muscle energy status appears to be relatively insensitive to thermal stress if compared to the thermal sensitivity of the whole animal. The data were consistent with the hypothesis of an oxygen limitation of thermal tolerance in animals, which is set by limited capacity of oxygen supply mechanisms. In the case of Atlantic cod circulatory rather than ventilatory performance may be the first process to cause oxygen deficiency during heat stress.

Key words: blood oxygen level; energy metabolism; *Gadus morhua*; microoptodes; NMR; temperature.

INTRODUCTION

A marine species, which will probably be affected by climate change is the Atlantic cod *Gadus morhua* L., an inhabitant of the continental shelf from the shoreline to 600 m depth or more. Its distribution covers both sides of the North Atlantic and adjacent seas, from the Bay of Biscay to Novaya Zemlya,

*Author to whom correspondence should be addressed. Tel.: +49 471 4831 1312; fax: +49 471 4831 1149; email: fsartoris@awi-bremerhaven.de

as well as the Baltic and White Seas. Although most North Atlantic cod stocks are currently considered to be overfished, temperature change may have contributed to the decrease in abundance (Brander, 1996). A decrease in Atlantic cod recruitment in the North Sea has been associated with higher-than-average temperatures during the last decade (O'Brian *et al.*, 2000). The effect of temperature change, however, is not necessarily the same in all areas of distribution (Daan, 1974; Boutilier, 1998). Since ambient temperature is the most important factor determining Atlantic cod migration and distribution in the Atlantic, global warming might have opposite effects on Atlantic cod populations on the northern and southern edges of their distribution (Pörtner *et al.*, 2001; Sirabella *et al.*, 2001).

As water temperature rises towards the upper tolerance limits, the animals are faced with a decrease in dissolved oxygen and, at the same time, with an increased oxygen demand due to elevated maintenance costs. Therefore, the balance between energy consumption and oxygen-dependent energy production may become disturbed at extreme temperatures. Nonetheless, regulated physiological parameters such as metabolic rate, blood and tissue oxygenation, acid–base status and cellular energy levels may display substantial changes before harmful effects occur (Pörtner, 1993; Pörtner & Grieshaber, 1993).

Recently, whole animal aerobic scope has been addressed as the first process to become limiting as temperature reaches low or high extremes, reflecting limited capacity of respiratory systems to ensure sufficient oxygen supply to tissues at 'pejus' temperatures (T_p, 'getting worse') (Frederich & Pörtner, 2000; Pörtner, 2001). Loss in aerobic scope at T_p is followed by transition to anaerobic metabolism at critical temperature limits (T_c, onset of anaerobic metabolism) (Zielinski & Pörtner, 1996; Sommer *et al.*, 1997; Frederich & Pörtner, 2000; Pörtner, 2001). In the case of the crustacean *Maja squinado* the envelope characterized by T_p agreed well with the ambient temperature range of this species and therefore, is interpreted to indicate the limits of long-term survival in the natural environment and to be ecologically relevant. Oxygen limitation at extreme temperatures appears as the unifying principle determining temperature-dependent limits of geographical distribution of marine ectotherms (Pörtner *et al.*, 1998, 2000, 2001). Moreover, the physiological and biochemical processes of temperature adaptation would explain trade-offs within energy budgets with the respective consequences for temperature-dependent changes in growth and reproduction (Pörtner *et al.*, 2001).

In the present study 'online' analyses of blood oxygen tensions in gill vessels of Atlantic cod were carried out using implanted optical oxygen sensors. The aim was to determine the temperature dependence of blood oxygen transport. These measurements occurred without fixation or anaesthetization of the fish and handling stress could be reduced to a minimum. In parallel experiments, the technique of *in vivo* magnetic resonance (^{31}P-NMR) spectroscopy was used to investigate the energetic consequences of thermal stress by measuring changes in the levels of intracellular pH and high-energy phosphates in white muscle of Atlantic cod. Previously, *in vivo* ^{31}P-NMR in fishes, particularly freshwater species, has been applied to determine changes in energy metabolism and intracellular pH (pH_i, van den Thillart *et al.*, 1989*a*, *b*; van Ginneken *et al.*, 1995, 1996; Borger *et al.*, 1998). Marine fishes such as *Harpagifer antarcticus*

Nybelin have also been investigated (Moerland & Egginton, 1998). By use of a horizontal magnet such measurements became possible in unrestrained and non-anaesthetized marine fishes during long-term experiments (>1 week) under controlled and well-defined physiological conditions (Bock *et al.*, 2001, 2002).

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Atlantic cod were caught by bottom trawling at 20–40 m depth in the German Bight near Helgoland (54°09′ N; 7°53′ E) during the summer of 1998 (fish used in the NMR studies) and in February and May of 1999 (fish used for the oxygen tension measurements). In the laboratory the fish were kept for several weeks in large aquaria (1 m^3) in natural sea water (salinity = 32) at a temperature of 10–12° C and a 12L : 12D photoperiod. The animals used in the *in vivo* NMR studies had a mean ± S.D. total length (L_T) of 40 ± 5 cm while the L_T lengths of the fish used for the oxygen tension measurements was 37 ± 7 cm. The fish were fed twice a week with frozen cockles *Cerastoderma edule*. Feeding was terminated 3 days prior to the start of experimentation.

GILL BLOOD OXYGEN PARTIAL PRESSURE (P_{O_2})

Analyses were carried out following the principles of cannulation outlined by Larsen *et al.* (1997). Initially, the fish were anaesthetized with MS222 (0·08 g l^{-1}) and during preparation, the gills were perfused with aerated sea water containing 0·05 g l^{-1} MS222. Subsequently, the fish were placed in the experimental chamber and exposed to a constant flow of aerated sea water at 5 l min^{-1}. Water temperature and salinity were controlled and recorded online throughout the whole experimental period. The oxygen tension in the gill arches was measured using oxygen microoptodes (Pre Sens, Neuburg a. d. Donau, Germany). The fibre tip of the optode was coated with an oxygen-sensitive layer containing a luminescent oxygen indicator. In addition, the tip was coated with Teflon to avoid clotting and oxygen-independent fluorescent signals caused by the accumulated material. Prior to insertion the optode was calibrated and drift and temperature dependence were recorded. The optode was inserted *c.* 0·5 cm into the efferent branchial blood vessel and fixed with a drop of cyanoacrylate glue. Due to the small diameter of the optode tip (<100 μm) it was safe to assume that blood flow was not hampered by the implanted sensor. Oxygen levels in the blood under control conditions varied between 90 and 15% air saturation indicating variable contributions of venous blood to P_{O_2} readings depending on the position of the optode in the respective gill arch. The good long-term stability (linear drift <0·44% h^{-1}) of the optodes enabled online monitoring of P_{O_2} for >1 week with a measurement frequency of once per minute.

After a recovery period of 24 h water temperature was changed at 1° C h^{-1}. In preliminary experiments temperature was increased until a 'critical temperature' was reached, defined as the temperature when a drastic decrease in blood oxygen tension could be observed. At this temperature limit (19–22° C) recovery of the fish was often not possible even if the fish was immediately transferred to the control temperature. Since one of the intentions of this study was to define a physiological threshold temperature with ecological relevance such as the T_p (Frederich & Pörtner, 2000) incubation was always terminated at 19° C. At the end of the experiments optodes were removed and recalibrated. The signal was corrected for the temperature-dependent drift of the optode redetermined in aerated sea water.

IN VIVO ^{31}P-NMR STUDIES

All experiments were carried out using a 4·7 T magnet with a horizontal, 40 cm diameter bore with actively shielded gradient coils (Bruker Biospec 47/40 DBX System).

A 5 cm surface coil was placed on top of the chamber and positioned directly above the tail of the animal for ^{31}P-NMR spectroscopy of muscle tissue. The position of the phosphocreatine signal was calibrated to 0 ppm relative to an external methylenediphosphate (MDP) standard. *In vivo* ^{31}P-NMR spectra were acquired continuously over 1200 scans using 100 s bp pulse (60°), repetition time of 0·6 s, size = 8K, sweep width 4096 Hz resulting in a measurement time of 10 min. Spectra were processed using an automatic fit routine as described in Bock *et al.* (2001).

The fish were anaesthetized in aerated sea water containing MS222 (0·08 g l^{-1}) and placed in a Perspex flow-through chamber (Bock *et al.*, 2002). The fish were orientated towards the incoming water. Although the space available inside the chamber was minimized the fish could swim slowly when facing the incoming water. After full recovery from anaesthesia the chamber was closed. A constant flow of sea water of 2·5 l min^{-1} was maintained, which was high enough to ensure sufficient oxygen supply at each temperature but low enough to limit swimming speed to a minimum level. The animals remained unrestrained and were free to swim slowly inside the chamber. The fish were allowed to recover for at least 24 h under control conditions before the start of the experimental protocol. Steady state conditions, as defined by permanently low inorganic phosphate (Pi) signals and constant phosphocreatine : inorganic phosphate (PCr : Pi) ratios, were confirmed by repeated recordings of *in vivo* ^{31}P-NMR spectra. Temperature control (stability ± 0·5° C in the range between 10 and 19° C) was achieved as described by Bock *et al.* (2002). In accordance with preliminary trials and depending on the response of the animals, temperature was increased in two or three steps of 1° C over 3 h, from 10 to 13° C, then from 13 to 16° C and then from 16 to 19° C. When a new steady state was reached (13, 16 and 19° C) the temperature was kept constant for at least 12 h. Some (three out of nine animals) did not survive temperatures >16° C. For the remainder, the temperature was brought back to 10° C at the end of the experiment and the animals were returned to the aquarium.

For pH_i analyses, subsequent spectra were added until the Pi signal could be clearly distinguished from noise and the position of the signal was determined relative to PCr. pH values were calculated and corrected for temperature from chemical shift data as described by Bock *et al.* (2001). The Gibbs' free energy change of ATP hydrolysis ($dG/d\zeta$) was calculated according to the methodology outlined by Pörtner *et al.* (1996). Free inorganic phosphate was assumed to be 1 mmol l^{-1} in resting animals (van Dijk *et al.*, 1999). The quantification of the concentrations of total Pi, creatine (Cr) and ATP was complicated by movement artefacts, since the sensitive volume perceived by the surface coil depends on the spatial orientation of the fish. To avoid problems arising from fish movements the following methodology was developed. In an initial experiment the movement of an Atlantic cod in the experimental chamber under control conditions had been restrained so that the fish stopped swimming, which allowed NMR spectra to be obtained without changes in spatial orientation. From this spectra the α-, β- and γ-ATP signals were quantified using the MDP concentration. The MDP standard was calibrated against the signal obtained from a 15 mmol l^{-1} PCr standard solution. The α- and γ-ATP signals include α- and β-ADP signals; therefore changes in these integrals will be solely dependent on the movement of the fish under the experimental conditions applied. Changes in the β-ATP signal (or PCr signal) without concomitant changes in the α- and γ-ATP signals, therefore excludes movement artefacts and allows for the quantification of ATP degradation due to metabolic processes. In turn, changes in α- and γ-ATP signals relative to MDP can be used to correct for the influence of movements by the fish.

STATISTICAL ANALYSIS

Data were checked for outliers beyond the 95% CL using Nalimov's test (Noack, 1980). Statistical significance was tested at the $P \leq 0{\cdot}05$ level using ANOVA and the *post-hoc* Student–Newman–Keuls test for independent samples. Regression coefficients were calculated using Sigma Plot 2000 (SPSS). All means are given ±S.D.

RESULTS

GILL OXYGEN TENSION

All fish survived the experiments when temperatures remained below thermal tolerance limits. In a preliminary experiment the stability of the microoptodes was tested for >1 week demonstrating the suitability of this technique for the online measurement of Po_2 in blood vessels of fishes. After removing the optode from the gill vessel it was possible to recalibrate and use it again. As the optode preparation reads mixed arterial and venous blood, true venous Po_2 readings are at the low end of the range. Based on this qualitative assumption and with regard to the different temperature influences on blood with high or low oxygen contents, the different saturation levels were interpreted as variable contributions of venous blood. The temperature dependence of high, *i.e.* arterial, Po_2 in gill blood of Atlantic cod under control conditions at 10° C is shown in Fig. 1(b). Periods with relatively constant Po_2 values were interspersed with periods characterized by large fluctuations. With rising temperature only a slight decrease occurred until Po_2 fell abruptly at ≥23° C [Fig. 1(a)]. Along with the sudden

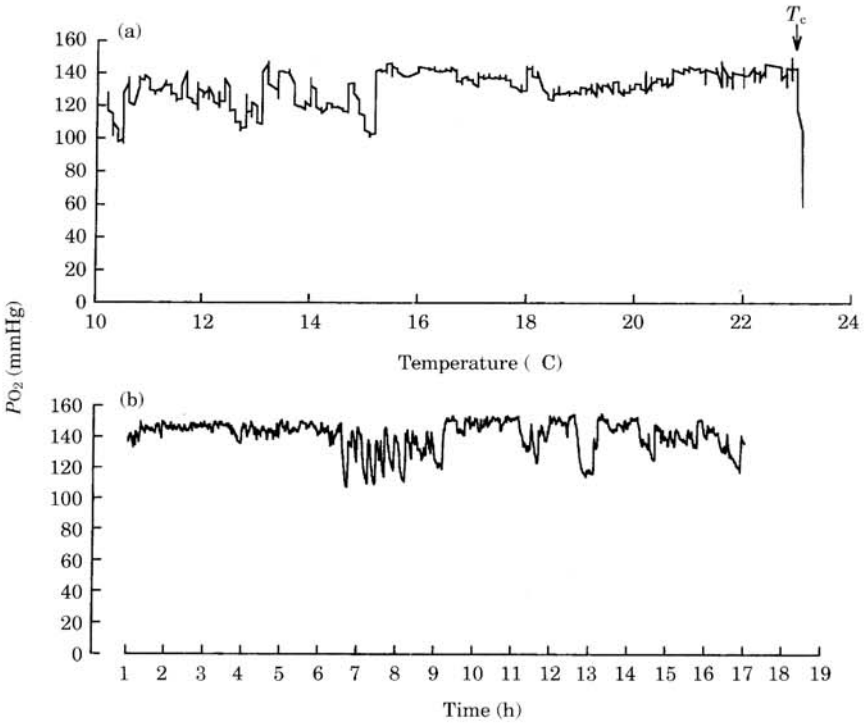


FIG. 1. (a) Changes in oxygen partial pressure (Po_2) over time in Atlantic cod gill blood during warming by 1° C h^{-1}. The decrease in Po_2 at 23° C was as abrupt as was the final decrease in intracellular pH (pH_i) observed in the nuclear magnetic resonance (NMR) studies. The decrease was not predictable from the moderate change in blood Po_2 observed during progressive warming. (b) Typical time course of changes in Po_2 over time in gill blood of an individual Atlantic cod under control conditions at 10° C.

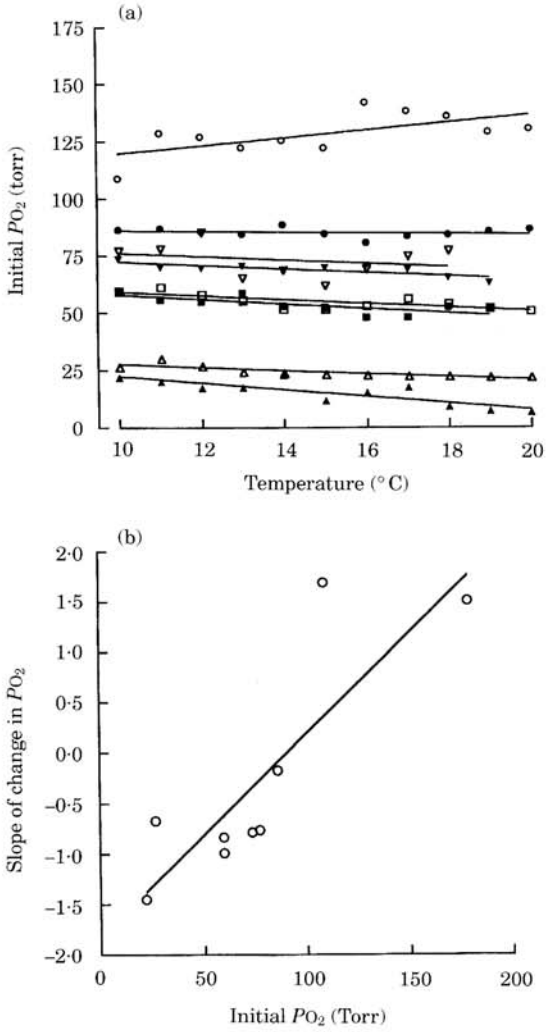


FIG. 2. Oxygen partial pressure decrease in Atlantic cod gill blood during warming by 1° C h^{-1}. (a) The lower the initial P_{O_2}, the larger the temperature-dependent decrease during warming for each individual fish. Each of the lines refer to an individual Atlantic cod with a different initial P_{O_2}. (b) The relationship between the initial P_{O_2} value in the gill and the slope of temperature-dependent decrease in blood P_{O_2} ($y = 0{\cdot}02x - 1{\cdot}81$, $r^2 = 0{\cdot}85$, $P < 0{\cdot}01$).

drops in PCr, ATP and pH_i levels at high thermal limits, as observed in the ^{31}P-NMR studies, this incident was sudden and not predictable from any progressive changes in oxygen tension below the heat tolerance limit. This was not only the case when arterial blood was recorded but was also seen in venous blood (unpubl. data). The data reveal that the temperature influence on arterial P_{O_2} is much less than on venous P_{O_2} which displayed a significant linear decrease during progressive warming (Figs 2 and 3). For each individual fish it appears that the

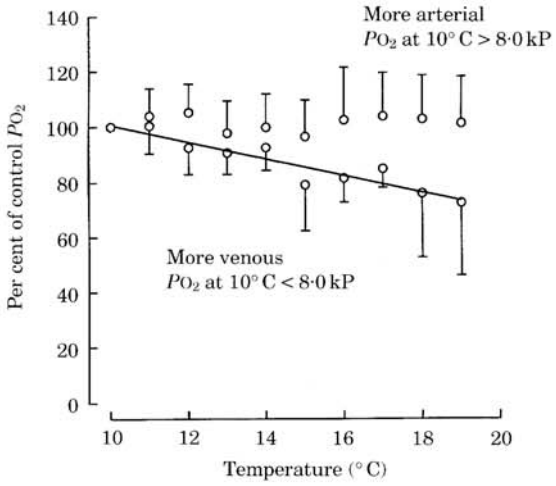


FIG. 3. Mean ± S.D. oxygen partial pressure decrease in Atlantic cod gill blood during warming by 1° C h^{-1}. With a large initial fraction of venous blood contributing to P_{O_2} readings, stronger temperature dependence became visible ($y = -3{\cdot}02x + 130{\cdot}78$, $r^2 = 0{\cdot}94$, $P < 0{\cdot}001$).

lower the initial P_{O_2}, the larger is the temperature-dependent decrease during warming. Overall, a larger contribution of venous blood [at P_{O_2} values <8·0 kPa (60 torr)] showed an increasing P_{O_2} decrement during warming, while with a larger influence of arterial blood [P_{O_2} values >8·0 kPa (60 torr)] temperature and blood oxygen tension largely remained unrelated (Figs 2 and 3).

TISSUE ENERGETICS AND ACID–BASE STATUS

The experimental set up used in ^{31}P-NMR experiments allowed for online recording of pH_i and levels of ATP and PCr in white muscle of unrestricted and non-anaesthetized Atlantic cod for >1 week. Recovery from slight anaesthetization and handling stress occurred within 2 h. *In vivo* ^{31}P-NMR spectra of white muscle of Atlantic cod immediately after placing the fish in the flow-through chamber are shown in Fig. 4(a) and after resting conditions were reached at 10° C in Fig. 4(b). Stress-free conditions are indicated by a high PCr : Pi ratio. The PCr : Pi ratio of free-swimming Atlantic cod spectra was extremely high (40–50) and, in consequence, determination of control pH_i was hampered by the low Pi signal. Integration of the three ATP signals yielded similar values. Since the α- and γ-ATP signals comprise α- and β-ADP, a high β-ATP signal indicates high ATP and low free ADP values. Both factors attest to excellent and stable physiological status under control conditions.

Reliable determinations of white muscle pH_i from chemical shifts values were only possible after averaging numerous spectra. A typical time course of the change in intracellular pH with increasing temperature is presented in Fig. 5. A mean pH_i of *c.* 7·45 was measured at 10° C. The mean values of intracellular pH of Atlantic cod at 10, 13, 16 and 19° C are shown in Fig. 6(a). The decrease in

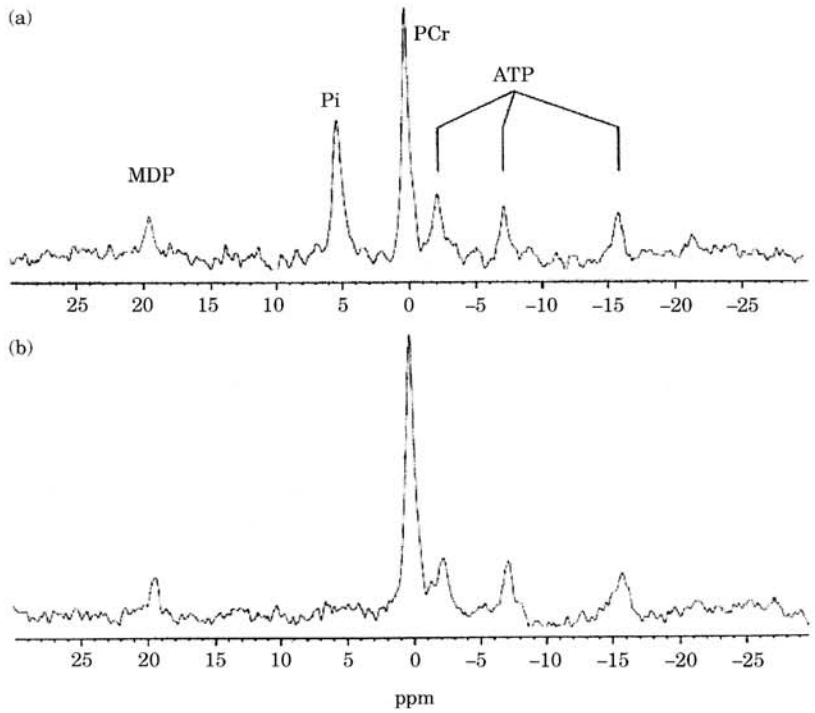


FIG. 4. *In vivo* ^{31}P-NMR spectra of white muscle of Atlantic cod: (a) obtained immediately after placing the fish in the flow-through chamber and (b) resting condition of the unrestrained Atlantic cod. Note the high phosphocreatine (PCr) level compared to the inorganic (Pi) level, which indicate low muscle tonus and low stress conditions.

pH with temperature calculated from these data was *c.* −0·025 ΔpH per °C between 10 and 16° C. The mean change in intracellular pH for each temperature step decreased from −0·029 per °C (10–13° C) to −0·022 per °C (13 and 16° C) and tended to level off between 16 and 19° C. Temperature changes had no influence on PCr or ATP levels (mean ± S.D. control values were 27·3 ± 4·2 μmol g^{-1} for PCr and 4·8 ± 0·8 μmol g^{-1} for ATP) except beyond a high tolerance threshold reached at ≥16° C which was variable between individual fish. The PCr and ATP levels dropped drastically, in similar ways to those observed for P_{O_2} levels. There was a significant trend for the levels of free ADP to increase with rising temperature. The dynamics of the Gibbs' free energy change of ATP hydrolysis followed the same pattern as PCr and ATP levels [Fig. 6(b)], with a severe drop (from 62 to 56 kJ mol^{-1}) that became visible once the upper tolerance limit was surpassed. Within 1 h the fish lost balance and died. Concomitant with this disturbance of energy balance a decrease in muscle pH_i could be observed as shown in Fig. 5 for an individual Atlantic cod reaching a tolerance limit at 16° C. Direct cooling (within 15 min) could not reverse this process, indicating an irreversible heat damage.

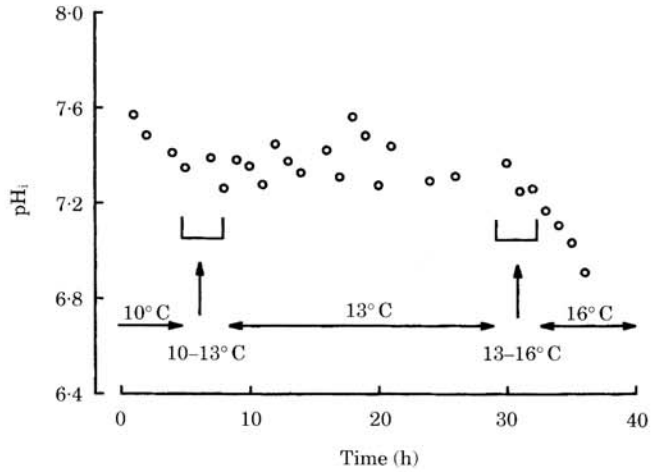


FIG. 5. Typical time course of changes in intracellular pH (pH_i) in the white muscle of an individual Atlantic cod (each symbol is the mean value of five spectra). The abrupt decrease in pH_i seen after a few hours of incubation at 16° C was not preceded by changes in the energy status of the muscle. At variable temperatures this pattern was typical for all individuals but indicated large differences between critical thermal limits of individual fish (see Fig. 2).

DISCUSSION

METHODOLOGY

For a comparison of NMR data and the level of blood oxygenation, the oxygen tensions in gill blood of Atlantic cod were measured with microoptodes. In preliminary experiments it was found that the method was suitable for prolonged measurements. The patterns observed close to lethal limits matched those observed in the NMR at similar temperatures. This indicated that the range of tolerance is not largely influenced by the implantation of the optode. The optode preparation probably reads mixed arterial and venous blood with true venous Po_2 readings at the low end of the depicted range (Fig. 3).

The use of a horizontal magnet made it possible to measure intracellular pH and energy metabolites in non-anaesthetized and unrestrained slowly swimming Atlantic cod with a mean L_T of *c.* 40 cm, at the expense of some decrease in signal to noise ratio over time owing to spontaneous activity. Thereby, any potential influence of long-term anaesthesia on blood, tissue and acid–base parameters as reported for rainbow trout *Oncorhynchus mykiss* (Walbaum) (Iwama *et al.*, 1989) and the Antarctic fish *Pagothenia borchgrevinki* (Boulenger) (Ryan, 1992) could be avoided. The spectra obtained after 10 min showed a satisfactory signal to noise ratio. The levels of PCr ($27{\cdot}3 \pm 4{\cdot}2\,\mu mol\,g^{-1}$), ATP ($4{\cdot}8 \pm 0{\cdot}8\,\mu mol\,g^{-1}$) and the Gibbs' free energy of ATP hydrolysis ($61{\cdot}7 \pm 0{\cdot}44\,kJ\,mol^{-1}$) matched the values found by van Dijk *et al.* (1999) in eelpouts (Zoarcidae) ($PCr \cong 25\,\mu mol\,g^{-1}$; $ATP \cong 3{\cdot}7\,\mu mol\,g^{-1}$; $\Delta G/\Delta\xi \cong 61{\cdot}0\,kJ\,mol^{-1}$). In Atlantic cod, however, the PCr : Pi ratio was higher (40–50) (Fig. 4) than reported by Borger *et al.* (1998) for common carp *Cyprinus*

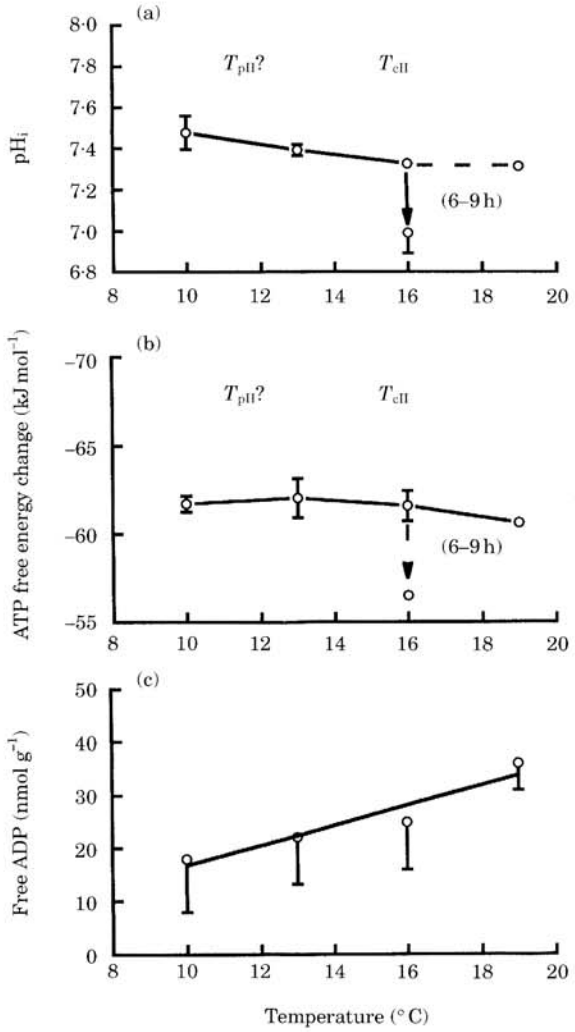


FIG. 6. Relationship between intracellular pH (pH_i) (a), Gibbs' free energy change (b) and the levels of free ADP ($y = -2{\cdot}15 + 1{\cdot}89x$, $r^2 = 0{\cdot}95$, $P \leq 0{\cdot}05$) (c) during warming in white muscle of Atlantic cod ($n = 4$ at 10, 13 and 19° C and $n = 9$ at 16° C). Temperature was increased in two or three steps of 1° C over 3 h, from 10 to 13° C, then from 13 to 16° C and then from 16 to 19° C. When a new steady state was reached (13, 16 and 19° C) the temperature was kept constant for at least 12 h. pH regulation followed the alphastat pattern consistently between 10 and 16° C in all investigated fish but not beyond 16° C. Some (three out of nine fish) did not survive temperatures >16° C which is reflected by a sudden drop in pH_i and Gibbs' free energy change (after 6–9 h at 16° C) indicating the upper critical temperature (T_{cH}) to be reached at 16° C (see Fig. 5).

carpio L. (PCr : Pi ≅ 30) and by Bock *et al.* (2001) for eelpouts (PCr : Pi ≅ 15). The high PCr : Pi ratio in Atlantic cod results as a consequence of extremely low Pi values. In consequence, pH_i determination was hampered by the low Pi signal and required extensive averaging of numerous spectra. This reflects

a thoroughly resting musculature, but was somewhat unexpected since very high ratios of PCr : Pi (Chiba *et al.*, 1988, 1990*a*, *b*) are usually found under anaesthesia due to the relaxation effect on muscle tonus. In Atlantic cod high PCr : Pi values and physiological pH_i values were maintained for >1 week. These emphasizes the good physiological condition of the animals during long-term experimentation.

BLOOD OXYGEN STATUS

During warming arterial Po_2 remained largely constant and thus arterial oxygen uptake (*i.e.* ventilation) did not become limiting even during severe heat stress [Fig. 1(a)]. This contrasts with the situation in crustaceans where a breakpoint in ventilation rate and corresponding changes in arterial haemolymph Po_2 and heart rate could be observed at T_p (Frederich & Pörtner, 2000). In Atlantic cod, the increased oxygen demand accompanying a temperature increment results in a depletion of the venous oxygen reserve as evidenced by a progressive drop in assumed venous Po_2. This finding strongly indicates that excessive oxygen uptake from the blood is not fully compensated for by circulatory performance (Figs 2 and 3). With a progressive decrease in venous oxygen reserves with increasing temperature, a clear T_p cannot be identified. Similar to the observation of a drastic change in pH and energy status in the NMR spectra, final thermal limitation is indicated by a sudden drop in oxygen tension. This is probably a consequence of circulatory collapse at the T_c resulting from a progressive insufficient oxygen supply not being able to meet the rising oxygen demand anymore. The Po_2 pressure head driving diffusion into the cell should rise to cover this demand; however, the progressive fall in venous Po_2 indicates an increasingly inadequate pressure head for maintenance of full mitochondrial aerobic scope.

It can be concluded that circulatory rather than ventilatory performance sets the limit of thermal tolerance in Atlantic cod. The heart of Atlantic cod relies on oxygen delivered by the venous blood. Since the oxygen tension in the venous blood decreases with increasing temperature this might have influenced the cardiac performance of the heart. In the rainbow trout the venous Po_2 threshold to support cardiac performance was between 7·8 and 9·9 torr (1·0 and 1·32 kPa) (Steffensen & Farrell, 1998). As the optode preparation reads mixed arterial and venous blood true venous Po_2 readings are most likely at the low end of the range shown in Fig. 3, close to a low initial Po_2 value of 25 torr (3·3 kPa). Considering the sudden fall in blood Po_2 at the T_c, a venous Po_2 of *c.* 8 torr (1·1 kPa) should be close to the limiting value just prior to the T_c in Atlantic cod, a similar estimate to that reported by Steffensen & Farrell (1998). The development of low venous Po_2 values probably relates to the observation that heart rate in fishes increases with temperature (Farrell & Jones, 1992) but does not exceed a maximum value (120 bpm in salmonids) even at high temperatures (Farrell, 1991). A limiting role of the circulatory system is also suggested by a capacity-limited increase in blood flow during warming in the Antarctic eelpout *Pachycara brachycephalum* (Pappenheim) (Mark *et al.*, 2002). The temperature-dependent capacity limit of blood circulation in Atlantic cod remains to be investigated and compared with the patterns of venous Po_2 for

a quantification of the T_p. The limiting role of circulatory performance with a non-limiting capacity for gill oxygen uptake in Atlantic cod also emphasizes the temperature-dependent use of different haemoglobin isoforms as a means to optimize oxygen transport in Atlantic cod (O. Brix, pers. comm.).

ENERGY AND ACID–BASE STATUS

Not only the limited capacity of the circulatory system but also temperature-dependent changes in intracellular pH may be an indicator of T_p (as indicated by deviation from alphastat pH regulation, Mark *et al.*, 2002) as well as T_c (indicated by a sudden drop in pH_i, PCr and ATP and in Gibbs' free energy of ATP hydrolysis) thresholds in Atlantic cod. Even if the literature is not uniform concerning the validity of the alphastat hypothesis, the work recently of Ultsch & Jackson (1996) and Pörtner *et al.* (1998) demonstrated that at least intracellular pH is generally regulated according to alphastat especially in the normal temperature range of the species and in between the T_c range (Sommer *et al.*, 1997; Bock *et al.*, 2001; Mark *et al.*, 2002). The magnitude of temperature-induced pH changes indicates an alphastat pattern of pH_i regulation in Atlantic cod, which is visible up to 16° C with a trend towards lower slopes at higher temperatures (Fig. 6). A slight deviation from alphastat pH regulation may already set in, but not very clearly at >13° C. Adopting the principles elaborated by Mark *et al.* (2002) in a parallel study on *P. brachycephalum*, the T_p in Atlantic cod would be found close to 16° C, just below the T_c. These relationships remain to be investigated. In general, changes in temperature-dependent pH regulation might be a suitable early physiological indicator of thermal limitation as also shown in lugworms *Arenicola marina* (Sommer *et al.*, 1997). Mark *et al.* (2002) showed that the deviation from alphastat pH regulation occurred at the upper T_p in *P. brachycephalum*. The width of the window between T_p (deviation from alphastat pH regulation) and T_c (drastic decrease of pH owing to anaerobic metabolism) might thus be reflected in patterns of changes in intracellular pH. The mechanism behind the shift in temperature-dependent pH regulation at T_p but below the T_c is not yet understood, but probably involves a change in membrane properties.

The importance of alphastat pH regulation for the maintenance of energy homeostasis has been outlined by Zielinski & Pörtner (1996) and by Pörtner *et al.* (1998). With alphastat control of intracellular pH, the levels of ATP free energy should be maintained during cooling. The temperature-dependent changes in intracellular pH and the maintenance of Gibbs' free energy change in the white muscle of Atlantic cod during warming within the thermal tolerance window supports this conclusion.

At T_c, which was found at a temperature variable between individuals but >16° C, the onset of anaerobic metabolism and a sudden drop of pH_i and Gibbs' free energy change suggest complete loss of aerobic scope and insufficient oxygen supply to even cover standard metabolic rate. In accordance with earlier studies (van Dijk *et al.*, 1999) no progressive changes in the levels of white muscle high-energy phosphates such as PCr and ATP during temperature perturbations were observed except during exposure to temperature extremes beyond the T_c when after variable periods of incubation the fish lost balance

and PCr and ATP started to decrease. At this 'point of no return' even immediate cooling could not reverse this process and the fish died within 1 h. Recordings of Po_2 in the blood indicated complete failure of the circulatory system at this point. The acidosis and energetic collapse observed, match the definition of the T_c, which were defined based on work on invertebrates and fishes (Pörtner *et al.*, 1998; Pörtner, 2001). As found earlier in marine invertebrates, the T_c indicates transition to anaerobic energy production and thus to a time-limited situation. It is equivalent to the long-term lethal temperature. Since the period of hypoxia tolerance is limited, and will be even more so at high temperatures, the period of survival beyond the T_c appears very short in Atlantic cod once venous oxygen stores are depleted.

In an earlier study of eelpouts (van Dijk *et al.*, 1999), oxygen limitation became visible first in aerobic organs such as the liver before effects could be seen in white muscle. Only when circulation and ventilation collapse and the animal is close to death, do drastic changes occur in the white muscle. This is probably due to the lower metabolic rate and thus O_2 demand in white muscle. In conclusion, white muscle in resting fishes appears to be less sensitive to thermal stress than more aerobic organs such as the liver or brain. Future studies of thermal tolerance in fishes should therefore focus on the role of aerobic organs in setting the thermal limits of ectotherms. As a precondition, methodological problems associated with non-invasive NMR studies in smaller organs *in vivo* need to be solved.

By applying new methods for the long-term study of physiological parameters in non-anaesthetized Atlantic cod from the North Sea some evidence for an oxygen limitation of thermal tolerance in this species could be provided. In general, the temperature limits of physiological performance should correspond to the geographical distribution limits according to environmental temperature. The correlation between temperature and the hierarchy and time limits of thermal limitation (T_p and T_c) as outlined by Pörtner (2001) indicates that from an ecological point of view the T_p probably sets the distribution limits of a species. Although, not as clearly defined as in a crustacean (Frederich & Pörtner, 2000) or in Antarctic eelpout (Mark *et al.*, 2002), the present data indicate that T_p in Atlantic cod may be reached between 13 and 16° C, before a capacity limitation of circulation causes a fatal drop in venous oxygen levels and, finally, collapse and death at the T_c. This finding is in line with the conclusion that animals refused food above 16° C, possibly as a consequence of lost aerobic scope.

We like to thank R.-M. Wittig for excellent technical assistance. Support by a BMBF grant (project 03 PL02A, NMR laboratory) is gratefully acknowledged. A contribution to the ELOISE project: Effects of climate induced temperature change on marine coastal fishes (CLICOFI), funded by the European Union program 'Climate and Environment', contract No. ENV4-CT97-0596. ELOISE publication No.

References

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

Bock, C., Sartoris, F. J. & Pörtner, H. O. (2002). In vivo MR spectroscopy and MR imaging on non-anaesthetized marine fish: techniques and first results. *Magnetic Resonance Imaging* **20,** 165–172.

Borger, R., de Boeck, G., van Auderke, J., Dommisse, R., Blust, R. & van den Linden, A. (1998). Recovery of the energy metabolism after a hypoxic challenge at different temperature conditions: a ^{31}P-nuclear magnetic resonance spectroscopy study with common carp. *Comparative Biochemistry and Physiology A* **120,** 143–150.

Boutilier, R. G. (1998). Physiological ecology in cold ocean fisheries: a case study in Atlantic cod. In *Cold Ocean Physiology* (Pörtner, H. O. & Playle, R., eds), pp. 463–489. Cambridge: Cambridge University Press.

Brander, K. (1996). Effects of climate change on cod (*Gadus morhua*) stocks. In *Global Warming: Implications for Freshwater and Marine Fish* (Wood, C. M. & McDonald, D. G., eds), pp. 255–278. Cambridge: Cambridge University Press.

Chiba, A., Hamaguchi, M., Kosaka, M., Asai, T., Tokuno, T. & Chichibu, S. (1988). Anesthetic effects of MS 222 on the loach measured with ^{31}P-NMR. *Medical Biology* **117,** 445–448.

Chiba, A., Hamaguchi, M., Kosaka, M., Asai, T., Tokuno, T. & Chichibu, S. (1990*a*). In vivo ^{31}P-NMR analysis of the electric anesthetized loach, *Cobitis biwae*. *Comparative Biochemistry and Physiology A* **97,** 385–389.

Chiba, A., Hamaguchi, M., Kosaka, M., Tokuno, T., Asai, T. & Chichibu, S. (1990*b*). Energy metabolism in unrestrained fish with in vivo ^{31}P-NMR. *Comparative Biochemistry and Physiology A* **96,** 253–255.

Daan, N. (1974). Growth of North Sea Cod, *Gadus morhua*. *Netherlands Journal of Sea Research* **8,** 27–48.

van Dijk, P. L. M., Hardewig, I., Tesch, C. & 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,** 3611–3362.

Farrell, A. P. (1991). From hagfish to tuna – a perspective on cardiac function. *Physiological Zoology* **64,** 1137–1164.

Farrell, A. P. & Jones, D. R. (1992). The heart. In *Fish Physiology*, Vol. XII (Hoar, W. S., Randall, D. J. & Farrell, A. P., eds), pp. 1–88. New York: Academic Press, Inc.

Frederich, M. & Pörtner, H. O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in the spider crab, *Maja squinado* (Decapoda). *American Journal of Physiology* **279,** R1531–R1538.

van Ginneken, V., van den Thillart, G., Addink, A. & Erkelens, C. (1995). Fish muscle energy metabolism measured during hypoxia and recovery: an in vivo ^{31}P-NMR study. *American Journal of Physiology* **268,** R1178–R1187.

van Ginneken, V., van den Thillart, G., Addink, A. & Erkelens, C. (1996). Synergistic effect of acidification and hypoxia: in vivo ^{31}P-NMR and respirometric study in fishes. *American Journal of Physiology* **271,** R1746–R1752.

Iwama, G. K., McGeer, J. C. & Pawluk, M. P. (1989). The effects of five fish anaesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenalin in rainbow trout. *Canadian Journal of Zoology* **67,** 2065–2073.

Larsen, B. K., Pörtner, H. O. & Jensen, F. B. (1997). Extra- and intracellular acid-base balance and ionic regulation in cod (*Gadus morhua*) during combined and isolated exposures to hypercapnia and copper. *Marine Biology* **128,** 337–346.

Mark, F., Bock, C. & 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* **283,** R1254–R1262.

Moerland, T. & Egginton, S. (1998). Intracellular pH of muscle and temperature: insight from *in vivo* ^{31}P NMR measurements in a stenothermal Antarctic teleost (*Harpagifer antarcticus*). *Journal of Thermal Biology* **23,** 275–282.

Noack, S. (1980). *Statistische Auswertung Von Meß- und Versuchsdaten mit Taschenrechner und Tischcomputer*. Berlin: Walter de Gruyter.

O'Brian, C. M., Fox, C. J., Planque, B. & Casey, J. (2000). Climate variability and North Sea cod. *Nature* **404,** 142.

Pörtner, H. O. (1993). Multicompartmental analyses of acid-base and metabolic homeostasis during anaerobiosis: invertebrate and lower vertebrate examples. In *Surviving Hypoxia: Mechanisms of Control and Adaptation* (Hochachka, P. W., Lutz, P. L., Sick, T., Rosenthal, M. & van den Thillart, G., eds), pp. 139–156. Boca Raton, FL: CRC Press Inc.

Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88,** 137–146.

Pörtner, H. O. & Grieshaber (1993). Characteristics of the critical Po_2 (s): gas exchange, metabolic rate and the mode of energy production. In *The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life* (Bicudo, J. E. P. W., ed.), pp. 330–357. Boca Raton, FL: CRC Press Inc.

Pörtner, H. O., Finke, E. & Lee, P. G. (1996). Metabolic and energy correlates of intracellular pH in progressive fatigue of squid (*L. brevis*) mantle muscle. *American Journal of Physiology* **271,** R1404–R1414.

Pörtner, H. O., Hardewig, I., Sartoris, F. J. & van Dijk, P. (1998). Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? *Cold Ocean Physiology* (Pörtner, H. O. & Playle, R., eds), pp. 88–120. Cambridge: Cambridge University Press.

Pörtner, H. O., van Dijk, P. L. M., Hardewig, I. & Sommer, A. (2000). Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In *Antarctic Ecosystems: Models for Wider Ecological Understanding* (Davison, W. & Williams, C. H., eds), pp. 109–122. Christchurch: Caxton Press.

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 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.

Ryan, S. N. (1992). Susceptibility of the Antarctic fish *Pagothenia borchgrevinki* to MS-222 anaesthesia. *Polar Biology* **11,** 583–589.

Sirabella, P., Giuliani, A., Colosimo, A. & Dippner, J. (2001). Breaking down the climate effects on cod recruitment. *Marine Ecology Progress Series* **216,** 213–222.

Sommer, A., Klein, B. & Pörtner, H. O. (1997). Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina*. *Journal of Comparative Physiology B* **167,** 25–35.

Steffensen, J. F. & Farrell, A. P. (1998). Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to hypoxia. *Comparative Biochemistry and Physiology A* **119,** 585–592.

van den Thillart, G., van Waarde, A., Muller, H. J., Erkelens, C., Addink, A. & Lugtenburg, J. (1989*a*). Fish muscle energy metabolism measured by in vivo ^{31}P-NMR during anoxia and recovery. *American Journal of Physiology* **256,** R922–R929.

van den Thillart, G., Körner, F., van Waarde, A., Erkelens, C. & Lutgenburg, J. (1989*b*). A flow through probe for *in vivo* ^{31}P NMR spectroscopy of unanesthetized aquatic vertebrates at 9.4 Tesla. *Journal of Magnetic Resonance* **84,** 573–579.

Ultsch, G. R. & Jackson, D. C. (1996). pH and temperature in ectothermic vertebrates. *Bulletin of the Alabama Museum of Natural History* **18,** 1–41.

Zielinski, S. & Pörtner, H. O. (1996). Energy metabolism of the intertidal worm *Sipunculus nudus* below a critical temperature. *Journal of Comparative Physiology B* **166,** 492–500.

Aerobic mitochondrial capacities in Antarctic and temperate eelpout (Zoarcidae) subjected to warm versus cold acclimation

Gisela Lannig and Hans-O. Pörtner*

Alfred Wegener Institute for Marine and Polar Research, Columbusstrasse,
27568 Bremerhaven, Germany

*Author to whom correspondence should be addressed: Tel.: +49-471-4831-1307; fax: +49-471-4831-1149; email: **hpoertner@awi-bremerhaven.de**

Summary

The mitochondrial patterns reflecting tissue aerobic capacity during seasonal acclimatization and latitudinal adaptation to cold have been well investigated in tissues of marine ectotherms. In contrast, little is known about changes in aerobic capacity of polar fish during warm acclimation. We therefore investigated temperature dependent mitochondrial respiration and activities of citrate synthase (CS) and $NADP^+$ -dependent isocitrate dehydrogenase (IDH) in liver of Antarctic eelpout, *Pachycara brachycephalum* acclimated to 5°C and 0°C (controls) for at least 10 months, compared to those in boreal eelpout, *Zoarces viviparus* (North Sea) acclimated to 5°C and to 10°C (controls).

In North Sea eelpout cold acclimation to 5°C had no effect on specific mitochondrial state III respiration and IDH activity, but caused a decrease in specific CS activity (expressed per mg mitochondrial protein). In Antarctic eelpout no differences were found between acclimation groups, neither in specific mitochondrial respiration nor in specific enzyme activities. Antarctic eelpout maintained at 5°C showed lower specific oxidative phosphorylation rates (state III) but higher specific enzyme activities than North Sea eelpout at 5°C.

Due to increased liver sizes in both species in the cold with a concomitant rise in liver mitochondrial protein content, elevated state III rates were found in total liver of both cold- versus warm-exposed *P. brachycephalum* and *Z. viviparus*, with the highest rates in boreal eelpout acclimated to 5°C. CS and IDH activities in total liver were similar in *Z. viviparus* acclimated to 5°C and 10°C, but decreased in warm acclimated versus control *P. brachycephalum*. Enzyme capacities in total liver were again higher in eelpout from the Antarctic than from the North Sea.

In conclusion, cold compensation of aerobic capacities in the liver seems to be linked to an increase in organ size with unchanged specific mitochondrial protein content. Despite its life in the permanent cold, *P. brachycephalum* was able to reduce liver aerobic capacities in the warm and, thus, displayed a capacity for temperature acclimation.

Introduction

The geographical distribution of ectothermic species is related to the ambient temperature regime, and tolerance to fluctuations of habitat temperature exists only within certain limits (for review see Pörtner, 2001; Pörtner, 2002a). Living in the extreme Antarctic environment appears to be associated with reduced tolerance to higher temperatures. Low upper lethal temperatures have been observed in the Antarctic brachiopod, *Liothyrella uva* between 3°C and 4.5°C (Peck, 1989). Pörtner et al. (1999a) found a short-term upper lethal temperature of 4°C and a long-term upper limit of around 2°C in the bivalve *Limopsis marionensis*. An upper-lethal temperature of 6°C was found in three species of the Antarctic fish *Trematomus* by Somero and de Vries (1967). Antarctic eelpout, *P. brachycephalum* and the Notothenioid, *Lepidonotothen nudifrons* survived temperatures up to 9-10°C (van Djik et al., 1999; Hardewig et al., 1999) and Mark et al. (2002) even reported a lethal temperature of *P. brachycephalum* at around 13°C. Reduced heat tolerance in cold-stenothermal ectotherms is likely linked to cold induced functional adaptations, which compensate for the decelerating effect of low temperatures on metabolic processes.

Mitochondrial proliferation has been found in cold adapted Southern and Northern hemisphere species (Londraville and Sidell, 1990; Johnston et al., 1998; Sommer and Pörtner, 2002) as well as in cold acclimated eurythermal species (Campbell and Davies, 1978; Egginton and Sidell, 1989). Furthermore, enhanced enzyme activities are found in cold adapted as well as in cold acclimated animals (Crockett and Sidell, 1990; Sokolova and Pörtner, 2001; Kawall et al., 2002; Lannig et al., 2003). Besides enhanced mitochondrial densities, an increase in total organ size has also been reported to compensate for the effects of low temperatures (Kent et al., 1988 (liver and heart); Seddon and Prosser, 1997 (liver); Lannig et al., 2003 (liver)).

Despite compensatory increase in tissue aerobic capacities, Antarctic animals show uncompensated resting metabolic rates (Clarke, 1991; van Dijk et al., 1999; for review see Pörtner et al., 2000; Peck, 2002) and uncompensated specific maximal respiration rates of mitochondria (Johnston et al., 1994; Johnston et al., 1998; Pörtner et al., 1999b; Hardewig et al., 1999). Pörtner et al. (2000) suggested that mitochondrial and whole animal aerobic capacities remain uncompensated only in cold-stenothermal organisms, as opposed to cold adapted eurytherms. This may also be associated with the inability of cold-stenotherms to acclimate to warmer temperatures (e.g. Weinstein and Somero, 1998).

The adjustment of aerobic scope by setting mitochondrial densities and capacities is seen as a crucial step in shifting thermal tolerance windows (cf. Pörtner, 2002a). Therefore,

the present study was designed to investigate the ability to adjust aerobic metabolism to changing temperature in Antarctic fish. We acclimated cold adapted Antarctic eelpout, *Pachycara brachycephalum*, from 0 to 5°C. Since different patterns of temperature compensation have been suggested for stenothermal and eurythermal ectotherms, the same parameters were compared in North Sea eelpout, *Zoarces viviparus* maintained at 5°C and 10°C. We investigated mitochondrial respiration rates and among enzyme activities, citrate synthase (CS) and $NADP^+$ -dependent isocitrate dehydrogenase (IDH) in liver mitochondria at different temperatures.

CS was chosen as an indicator of citric acid cycle activity and IDH to represent a key role in a mitochondrial substrate cycle, which may be relevant in the control of mitochondrial respiration, redox status and proton leakage (Sazanov and Jackson, 1994; Pörtner et al., 1999b).

Materials & Methods

Animals

Antarctic eelpout, *Pachycara brachycephalum* were caught by baited traps close to King George Island (depth 397-455 m) during the RV Polarstern Antarctic expedition XV/3 1998. The fish were kept in aquaria onboard RV Polarstern and later at the Alfred Wegener Institute (Bremerhaven) at ambient temperatures of 0°C ± 0.5°C and a salinity of 34‰. North Sea eelpout, *Zoarces viviparus* were caught in the German Bight near Helgoland (august 2001 and march 2002) and kept in aquaria at the Alfred Wegener Institute at temperatures of 10°C ± 1°C and a salinity of 34‰. Acclimation to 5°C started in spring 2002, with Antarctic and North Sea eelpouts placed in the same aquarium at 5°C ± 0.5°C and a salinity of 34‰. Further animals remained at control temperatures. All animals were kept under a 12:12-h light-dark cycle and fed life shrimp once per week. Experiments were carried out in the beginning of 2003, after an acclimation period at 5°C of at least 10 months.

Preparation of mitochondria

The isolation of mitochondria was modified after Hardewig et al. (1999). The fish were killed by a blow to the head, followed by a cut of the spine. The liver was removed immediately, chopped finely with scissors in an ice-cold glass dish, and extracted in 10 - 25 ml of homogenisation buffer, depending on liver wet mass, approximately 0.5 g per 10 ml (50 mM Hepes, 80 mM sucrose, 85 mM KCl, 5 mM EDTA, 5 mM EGTA, pH 7.1, 20°C with freshly added 1% BSA and 1µl ml^{-1} aprotinin) with a Potter-Elvejhem homogeniser. After

centrifugation (12 min at 400 g and 0°C) the pellet was re-homogenised in the same volume of homogenisation buffer and centrifuged again. The combined supernatants were centrifuged for 8 min at 10000 g and 0°C. The mitochondrial pellet was re-suspended in 1 - 2 ml assay medium (50 mM Hepes, 85 mM KCl, 80 mM sucrose, 5 mM KH_2PO_4, pH 7.1 (20°C) with freshly added 1% BSA and 1µl ml^{-1} aprotinin). In some cases the livers of two animals were pooled to enhance the volume of mitochondrial suspension but counted as one in the statistical analyses and results.

Mitochondrial respiration and enzyme assays

Mitochondrial oxygen consumption was measured at different temperatures (5, 8, 10, 15 and 20°C) using a Clarke-type oxygen electrode in a thermostatted respiration chamber (Eschweiler, Kiel). A total of 100 µl of the mitochondrial suspension were combined with assay medium, without BSA and aprotinin, to a total volume of 1 ml, containing 0.5 – 1.2 mg mitochondrial protein ml^{-1}, 5 µM di-adenosine pentaphosphate (Ap_5A, an inhibitor of myokinase) and 3.3 mM succinate as a substrate since respiration showed lower rates using pyruvate and malate. State III respiration was recorded after addition of 0.3 mM ADP and state VI respiration was determined after all ADP had been phosphorylated. Finally, the respiration induced by proton leakage was recorded after adding 2.5 µg ml^{-1} oligomycin, an inhibitor of mitochondrial F_0F_1-ATPase. P/O ratios were calculated by classical determination according to Chance and Williams (1956). Mitochondrial oxygen consumption was calculated as nmol O min^{-1} mg $protein^{-1}$ by adopting values of oxygen solubility in the assay medium from Johnston et al. (1994). Protein concentration was determined by the Biuret method after Gornall et al. (1949) using 5% deoxycholate to solubilize membrane proteins.

Since the high lipid content in liver disturbed the photometric measurements, 3 M TCA was used to precipitate and separate the protein by centrifugation for 15 min at 16000 g and 15°C. The pellet was resuspended with 1 ml diethylether and ethanol (4:1) and, after re-centrifugation the pellet was resuspended with 0.2 ml H_2O and 1 ml Biuret reagent. Since the extraction of mitochondria was still incomplete, mitochondrial protein content per g liver was calculated from comparisons of CS activities measured in the mitochondrial suspension and in liver tissue extracts (100% CS).

CS activity was quantified in the mitochondrial suspension, specified in units per mg mitochondrial protein, and in liver tissue (units per g wet wt tissue) using a modified procedure after Sidell et al. (1987). Mitochondrial suspension was added to assay medium (2:1) and, after 15 min of ultrasonic treatment at 0°C (output control 8, duty cycle 50%,

Branson Sonifier 450, Heinemann Ultraschalltechnik, Germany), was centrifuged for 5 min at 7400 g and 0°C.

For the determination of CS activity in liver tissue (100% CS) approximately 0.02 g of tissue, taken after chopping the liver during the preparation of isolated mitochondria isolation, were added to 1 ml of assay medium without BSA and aprotinin, subjected to ultrasonic treatement (15 min, output control 8, duty cycle 50% at 0°C) and centrifuged for 5 min at 7400 g and 0°C. The supernatants were kept on ice and enzyme activities remained stable for at least 24 hours. CS activity was determined in 100 mM Tris-HCl, pH 8 (20°C), 5 mM DTNB, 20 mM Acetyl-CoA, 20 mM oxaloacetate. The increase in absorbance at 412 nm was monitored in a thermostatted spectrophotometer at 5, 10, 15 and 20 °C, respectively. Enzyme activity was calculated using an extinction coefficient (ε_{412}) for DTNB of 13.61 l $mmol^{-1}$ cm^{-1}.

For the determination of $NADP^+$-dependent isocitrate dehydrogenase activity, mitochondrial suspension was added to stabilisation buffer (2:1; containing 50 mM Hepes, 85 mM KCl, 80 mM sucrose, 5 mM KH_2PO_4, pH 7.1 (20°C), 2 mM DTT, 20 mM MgCl, 20 mM isocitrate and 5 mM EDTA), treated for 5 min at 0°C in an ultrasonic bath (output control 2, duty cycle 50%) and centrifuged for 9 min at 7400 g and 0°C. The enzyme remained stable for more than 24 hours after this treatment. IDH activity was measured after Alp et al. (1976), by monitoring the appearance of NADPH at 339 nm in a temperature controlled spectrophotometer at 5, 10, 15 and 20 °C, respectively. The assay contained 70 mM Tris-HCl pH 7.1 (20°C), 8 mM $MgSO_4$, 2.5 mM $NADP^+$, 1 mM $MnCl_2$, 3 mM D/L-isocitrate and 20 mM citrate. Enzyme activity was calculated using an extinction coefficient (ε_{339}) for NAD(P)H of 6.3 l $mmol^{-1}$ cm^{-1}.

Allometric scaling

Since liver size and lipid content influence liver aerobic capacity available to the animal, we calculated total protein content, mitochondrial respiration rates and enzyme activities for total liver, normalized to a 40 g eelpout after Lannig et al. (2003). Total protein content and total CS activities were calculated with the following formula:

normalized protein (resp. CS activity) = total protein (resp. total CS) in liver x $(40/\text{body mass})^m$, where the body mass exponent m was determined from the slope of the linear regression in a double logarithmic plot of protein (resp. CS activity) in total liver versus body mass in gram.

Mitochondrial respiration rates and IDH activity in total liver were calculated from the normalized protein content. No differences were found in total CS activity between direct normalization, described above, and calculation from normalized protein content.

Calculation of the hepatosomatic index as:

I_H = liver mass (g) / body mass (g) x 100,

was carried out with normalized liver mass for a 40 g eelpout by the following formula:

normalized liver mass = exact liver mass x $(40/\text{body mass})^m$, where the body mass exponent m was determined from the slope of the linear regression in a double logarithmic plot of liver mass versus body mass in grams. Data of animals and mass exponents for the different allometric scalings are summarized in table 1.

Statistical analyses

All values are given as means (± standard deviation). Statistical significance was tested at the P=0.05 level using analysis of variance (ANOVA, SigmaStat) and post hoc procedures (Tukey test). Before statistical testing, log transformations were used, whenever necessary to meet statistical assumptions of the homogeneity of variances. However, non-transformed data are given in text and figures.

Results

Hepatosomatic index and mitochondrial liver protein content

Normalized hepatosomatic indices (I_H, Fig.1) were significantly higher in cold- than in warm-exposed North Sea and Antarctic eelpout. I_H was significantly higher in both control (0°C) and 5°C acclimated groups of *P. brachycephalum* compared to control groups (10°C) of *Z. viviparus*. However, no differences were found between *Z. viviparus* acclimated to 5°C and *P. brachycephalum* under control conditions (0°C). *P. brachycephalum* acclimated to 5°C showed a significantly lower I_H than *Z. viviparus* at 5°C.

Specific mitochondrial protein contents (mg g $liver^{-1}$) were more or less similar in control and acclimation groups of one species as well as in the two species (Fig. 2A). Normalized protein content in total liver showed acclimation effects in both species, with higher values in cold compared to warm acclimated boreal and polar eelpout (Fig. 2B). Species comparison revealed significantly higher mitochondrial liver protein in *P. brachycephalum* (0°C and 5°C) compared to control (10°C) *Z. viviparus* but similar total protein contents compared to 5°C acclimated *Z. viviparus*.

Mitochondrial respiration

Mitochondrial coupling of all groups was significantly higher when assayed at 5°C than at 20°C. At 5°C respiratory control ratios (RCR_{ol}, ratio of state III and state IV_{ol}) were 4.85 ± 1.29 (North Sea, 10°C), 8.37 ± 1.13 (North Sea, 5°C), 4.39 ± 0.93 (Antarctic, 5°C) and 4.87 ± 1.08 (Antarctic, 0°C) (Fig. 3A). The traditional RCR (ratio of state III and state IV without oligomycin, data not shown) was found to be only slightly lower than RCR_{ol}. Comparison of RCR_{ol} between groups of *Z. viviparus* revealed significantly higher values in animals acclimated at 5°C than in controls (10°C), whereas no acclimation effect was found in RCR_{ol} of Antarctic eelpout. Coupling was significantly higher at all temperatures in North Sea eelpout acclimated to 5°C than in control and in 5°C acclimated Antarctic eelpout.

Determination of classical P/O ratios yielded numbers between 1.5 and 2.5 for all acclimation groups assayed in the temperature range between 5°C and 10°C. Above 10°C P/O values of mitochondria from warm acclimated *P. brachycephalum* and *Z. viviparus* (at 20°C only) decreased significantly, resulting in significantly lower values compared to the more or less unchanged and temperature independent P/O ratios of cold acclimated Antarctic and North Sea eelpout (Fig. 3B).

Mitochondrial respiration rates (nmol O min^{-1} mg $protein^{-1}$; state III, state IV and state IV_{ol}) increased significantly with rising temperatures in all groups (Fig. 4), whereas acclimation had no effect on specific mitochondrial respiration within both species. When measured at 5°C specific state III respiration rates of North Sea eelpout were 11.5 ± 3.7 (10°C) and 14.2 ± 3.5 (5°C) and those of Antarctic eelpout were 6.8 ± 2.1 (0°C) and 9.7 ± 1.4 (5°C). Respiration rates were significantly lower in polar eelpout acclimated to 0°C than in boreal eelpout acclimated to 5°C and 10°C, for state III at all temperatures and for state IV and state IV_{ol} at 20°C only. Warm acclimated *P. brachycephalum* showed significant differences only in state III respiration, which was lower than in *Z. viviparus* controls (10°C) at assay temperatures between 8°C and 15°C. At investigated temperatures, no significant discontinuity in Arrhenius plots was found for all groups (data not shown). Q_{10} values and Arrhenius activation energies (E_A) were similar in all groups: *P. brachycephalum*: state III: Q_{10} = 2.0 ± 0.4, E_A = 49.2 ± 11.9 kJ mol^{-1}; state IV: Q_{10} = 2.9 ± 0.7, E_A = 67.1 ± 12.9 kJ mol^{-1} and state IV_{ol}: Q_{10} = 3.2 ± 0.8, E_A = 74.3 ± 14.7 kJ mol^{-1} and *Z. viviparus*: state III: Q_{10} = 2.2 ± 0.4, E_A = 53.4 ± 10.5 kJ mol^{-1}; state IV: Q_{10} = 3.2 ± 0.8, E_A = 76.9 ± 17.5 kJ mol^{-1} and state IV_{ol}: Q_{10} = 3.2 ± 0.5, E_A = 79.8 ± 8.6 kJ mol^{-1} (means ± standard deviation for control and acclimation groups of each species).

Mitochondrial state III respiration normalized for total liver of a 40 g animal showed significant acclimation effects in both species (Fig. 5A). Respiration was higher in total liver of cold- versus warm-exposed *Z. viviparus* and *P. brachycephalum*. Liver respiration capacities (state III) in 5°C acclimated boreal eelpout were significantly higher than in control (0°C) and 5°C acclimated polar eelpout at all temperatures. However, total liver mitochondria of control *P. brachycephalum* (0°C) respired at higher rates than total liver mitochondria of control *Z. viviparus* (10°C). State IV respiration in normalized liver was also higher in cold- than in warm-exposed *Z. viviparus* and *P. brachycephalum* (Fig. 5B). *Z. viviparus* at 10°C had significantly lower state IV values than controls and 5°C acclimated *P. brachycephalum*. State IV respiration under oligomycin, assumed to quantify oxygen consumption due to proton leakage through the inner mitochondrial membrane (IV_{ol}, Fig. 5C) showed higher rates after cold-acclimation in total liver of *Z. viviparus* only, whereas state IV_{ol} capacity remained similar in total liver of warm acclimated (5°C) and control (0°C) *P. brachycephalum*. State IV_{ol} rates were again higher in liver of 0°C and 5°C exposed Antarctic than of North Sea eelpout at 10°C.

Enzyme activities

Citrate synthase activities (CS) in the mitochondrial suspensions (U mg mitochondrial protein^{-1}) were similar in acclimated and control groups of both species (Fig. 6A). Control groups (0°C) of Antarctic eelpout displayed significantly higher specific CS activities than both control and acclimation groups of North Sea eelpout. However, this difference became less with warm acclimation and specific CS activity of liver mitochondria from 5°C acclimated *P. brachycephalum* was significantly higher only compared to those from *Z. viviparus* acclimated to 5°C. No discontinuities of Arrhenius plots were found in the investigated temperature range (data not shown) and Q_{10} values and Arrhenius activation energies (E_A) were similar in all groups, with $Q_{10} = 1.6 \pm 0.1$ and $E_A = 32.5 \pm 5.3$ kJ mol^{-1} for *P. brachycephalum* and with $Q_{10} = 1.6 \pm 0.1$ and $E_A = 33.5 \pm 4.2$ kJ mol^{-1} for *Z. viviparus* (means ± standard deviation for control and acclimation groups of each species). CS activities in total liver of a 40 g animal showed again no differences between groups of *Z. viviparus*, whereas higher total CS activities were found in control (0°C) compared to 5°C acclimated *P. brachycephalum* (Fig. 6B). The normalized liver of Antarctic eelpout (0°C and 5°C) displayed significantly higher total CS capacity compared to North Sea (5°C and 10°C) eelpout.

For $NADP^+$-dependent isocitrate dehydrogenase activities (IDH) in the mitochondrial suspension (U mg mitochondrial protein^{-1}), activities were higher in control (0°C) Antarctic

eelpout than in control (10°C) and cold acclimated (5°C) North Sea eelpout at all temperatures (Fig. 7A). Evaluation of IDH activity in total liver of a 40 g animal showed no differences between cold acclimated and control *Z. viviparus*, but again higher IDH activities in control (0°C) compared to 5°C acclimated Antarctic eelpout (Fig. 7B). Total IDH capacity was significantly higher in liver of control 0°C Antarctic than of 5°C and 10°C exposed North Sea eelpout, whereas this difference fell at warmer temperatures and IDH capacity in liver of 5°C acclimated *P. brachycephalum* was significantly higher than in control (10°C) *Z. viviparus* only. As for CS activities, no discontinuities were found in Arrhenius plots of IDH activities in the investigated temperature range (5°C – 20°C, data not shown) and Q_{10} values and Arrhenius activation energies (E_A) did not differ between groups, $Q_{10} = 2.9 \pm 0.3$ and $E_A = 72.4 \pm 6.3$ kJ mol^{-1} for *P. brachycephalum* and with $Q_{10} = 3.1 \pm 0.3$ and $E_A = 76.5 \pm 5.4$ kJ mol^{-1} for *Z. viviparus* (means ± standard deviation for control and acclimation groups of each species).

Discussion

Respiratory properties of liver mitochondria

Liver mitochondria of eelpout, *Z. viviparus* (North Sea, at 10°C and 5°C) and *P. brachycephalum* (Antarctica, at 0°C and 5°C) showed clear temperature dependence of coupling ratios. In the investigated temperature range between 5 and 20°C, mitochondria of all groups were more strongly coupled at low than at high temperatures (Fig. 3A). The decrease of coupling ratios with temperature was elicited by different thermal sensitivities of oxidative phosphorylation capacity (state III) and proton leakage capacity (state IV_{ol}) showing lower Arrhenius activation energies for state III than for state IV_{ol}. Progressive uncoupling with rising temperature was also found in liver mitochondria of the Antarctic Nothothenioid, *Lepidonotothen nudifrons* (Hardewig et al., 1999) and in gill mitochondria of the Antarctic bivalve, *Laternula elliptica* (Pörtner et al., 1999b). In contrast, coupling ratios of red muscle mitochondria of Arctic charr, *Salvelinus fontinalis* (Blier et al., 2001) and of liver mitochondria of North Sea, Norwegian and North Eastern Arctic cod, *Gadus morhua* (Fischer, 2002) were stable and independent of temperature. In other fish species like trout, carp or goldfish (van den Thillart and Modderkolk, 1978; Moyes et al., 1988; Blier and Guderley, 1993) coupling ratios were also fairly insensitive to temperature.

These findings suggest that there is no general pattern of changing mitochondrial coupling ratios with temperature and that patterns are species specific. Despite decreasing coupling ratios with increasing temperature in all groups, only mitochondria of the warm

acclimated North Sea and Antarctic eelpout showed the expected concomitant decrease in phosphorylation efficiency (ADP/O ratio) at high temperatures (Fig. 3B). Pörtner et al. (1999b) found no change in P/O ratio with temperature by classical determination (from changes in respiration slopes) but significantly lower P/O ratios at high temperatures by biochemical P/O ratio determinations. These differences associated with different methods might indicate that the classical determination is not sensitive enough for small changes in P/O ratios. Hardewig et al. (1999) discussed that unchanged P/O ratios despite progressive uncoupling might become explainable in classical analyses of P/O ratios when maximal phosphorylation rates are determined at low proton motive force and low proton leak and compared with the subsequent transition to maximum proton leakage at maximum proton motive force.

Acclimation had no effect on specific maximal phosphorylation capacities (state III in nmol O min^{-1} mg mitochondrial $protein^{-1}$, Fig. 4A) of liver mitochondria of *Z. viviparus*, 5°C compared to 10°C acclimation and of *P. brachycephalum*, 0°C compared to 5°C acclimation. Specific mitochondrial liver respiration rates were lower in Antarctic than in North Sea eelpout. Fischer (2002) also found no effect of cold acclimation on specific state III respiration rates of liver mitochondria from different cod populations, *Gadus morhua* (North Sea versus North Eastern Arctic), however, liver mitochondria of North Eastern Arctic cod respirated at higher rates than those of North Sea cod. Pörtner et al. (1999b) found temperature specific aerobic capacity of gill mitochondria of the Antarctic bivalve *L. elliptica* in the same order of magnitude as that of other bivalve mitochondria, and Johnston et al. (1994, 1998) and Guderley (1998) concluded that cold compensation of specific state III respiration does not occur in muscle of various fish from a latitudinal cline (largely Southern hemisphere species). In eurythermal fish from Northern latitudes, however, cold acclimation and adaptation are reflected in increased specific aerobic capacities of mitochondria and it was suggested as an explanation that an increase in the specific phosphorylation capacity typically occurs in cold adapted eurytherms living at variable temperatures but may be negligible in stenothermal animals living at permanently low temperatures (cf. Pörtner et al., 2000). In that respect cold acclimated *Z. viviparus* are more eurythermal than *P. brachycephalum*.

Cold compensation of muscle aerobic capacities of marine ectotherms is linked to an increase in mitochondrial densities (Egginton and Sidell, 1989; Johnston et al., 1998) or cristae densities (St.-Pierre et al., 1998). Since mitochondrial protein per g liver did not differ significantly between acclimation groups, and was only slightly higher in cold compared to

warm acclimated *Z. viviparus* and *P. brachycephalum*, mitochondrial proliferation did not seem to occur during cold compensation in liver (Fig. 2A). As found in previous studies, metabolic cold compensation in liver goes along with an increase in organ size (Kent et al., 1988; Seddon and Prosser, 1997; Lannig et al., 2003). This is confirmed by the present paper, which demonstrates higher hepatosomatic indices (I_H, Fig. 1), normalized to a 40 g animal, in cold- versus warm acclimated boreal and polar eelpout. *Z. viviparus* at 10°C showed the lowest I_H, compared to the other groups. Mitochondrial protein contents in total liver resulted higher in cold- versus warm acclimated *Z. viviparus* and *P. brachycephalum*. Moreover, Antarctic eelpout acclimated to 0°C and 5°C showed higher protein contents than North Sea eelpout acclimated to 10°C but similar to those acclimated to 5°C (Fig. 2B).

Due to different mitochondrial protein contents in total liver, the picture of mitochondrial respiration rates changed after calculation for total liver of a 40 g animal. Mitochondrial respiration in total liver clearly showed cold compensation. State III capacity was significantly higher in total liver of cold- versus warm acclimated *Z. viviparus* as well as *P. brachycephalum* (Fig. 5A). Respiration rates resulted higher in liver of Antarctic eelpout acclimated to 0°C and 5°C than in North Sea eelpout acclimated to 10°C but lower than in North Sea eelpout acclimated to 5°C. These findings are in line with data by van Dijk et al. (1999) who found higher metabolic rates (nmol O min^{-1} 100g $eelpout^{-1}$) in both cold adapted *P. brachycephalum* (0°C) and cold acclimated *Z. viviparus* (3°C) than in warm acclimated *Z. viviparus* (12°C). However, van Dijk et al. found similar standard metabolic rates in cold acclimated North Sea and cold-adapted Antarctic eelpout, therefore, the overcompensation in state III respiration capacities of liver mitochondria of cold acclimated *Z. viviparus* found in the present work does not necessarily imply a concomitant rise in whole animal metabolic rate.

Since mitochondrial proton leakage rates correlate with the standard metabolic rate of an animal (Brookes et al., 1998) and comprise 20 - 50% of standard metabolism in both endotherms and most likely ectotherms (Brand, 1990; Brand et al., 1994; Brookes et al., 1998) it is conceivable that it is not maximum mitochondrial oxygen consumption capacity during oxidative phosphorylation (state III) but rather oxygen consumption due to proton leakage through the inner mitochondrial membrane (state IV_{ol}) which mirrors elevated metabolic rates in the cold. State IV_{ol} respiration in total liver of polar versus cold acclimated boreal eelpouts revealed a similar pattern as found for the standard metabolism of eelpouts by van Dijk et al. (1999). State IV_{ol} rates in warm acclimated *Z. viviparus* were significantly lower than in cold acclimated *Z. viviparus* and in both 0°C and 5°C acclimated *P.*

brachycephalum, but did not differ between polar and cold acclimated boreal eelpout (Fig. 5C). Investigation of whole animal respiration rates also revealed similar values in *P. brachycephalum* acclimated to 0°C and 5°C (data not shown) despite different state III respiration capacities in the liver.

According to our earlier hypothesis proton leakage is down regulated in parallel with standard metabolism in cold-adapted stenothemal Antarctic ectotherms (Pörtner et al., 2000), a conclusion supported by proton leakage rates found in bivalves and nototheniods (Pörtner et al., 1999b; Hardewig et al., 1999). However, in the case of the zoarcids, no differences were found in mitochondrial liver state IV_{ol} capacity and standard metabolic rate between cold acclimated and cold-adapted zoarcids.It is conceivable be that the Antarctic zoarcid does not follow the trend found in invertebrates and notothenioids and shows no relative down regulation of standard metabolism as is also mirrored in the lacking down-regulation of the mitochondrial proton leak.

As a corollary, the present results suggest that liver metabolism mirrors the level of cold compensation at the whole animal level. Nonetheless, similar Q_{10} and E_A values of mitochondrial respiration rates but higher Q_{10} and E_A values of in vivo standard metabolic rates of *P. brachycephalum* compared to cold- and warm acclimated *Z. viviparus* (van Dijk et al., 1999) suggest that mitochondrial functional properties do not fully translate to the pattern of whole animal respiration at various temperatures. In this context, recent evidence strongly suggests that the rising cost of ventilation and circulation strongly determines the change in standard metabolic rate with temperature, as seen in the Antarctic eelpout (Mark et al., 2002).

Effects of temperature on mitochondrial enzymes

In ectothermic animals long-term temperature shifts may cause compensatory changes in glycolytic and mitochondrial enzyme levels and capacities. Permanent cold compensation by 1.5 to 5-fold higher citrate synthase and cytochrome c oxidase activities in muscles from Antarctic species (*Notothenia gibberifrons*, *Trematomus newnesi*) compared to their temperate counterparts (*Myoxocephalus octodecimspinosus*, *Tautoga onitis*) was described by Crockett and Sidell (1990). Torres and Somero (1988) observed enhanced CS activities in white muscle of Antarctic compared to California mesopelagic fish and, in a within species comparison, Lannig et al. (2003) found higher specific CS activities in liver of North Eastern Arctic compared to North Sea cod, *G. morhua*. These findings are consistent with our present data since liver mitochondria of *P. brachycephalum* (0°C) showed elevated specific CS activities compared to *Z. viviparus* acclimated to 10°C and 5°C, and, to a lesser extent,

elevated specific IDH activities (compared to 5°C acclimated *Z. viviparus* only) (Fig. 6A & 7A). Only a slight reduction in specific CS and IDH activities was found in warm versus cold acclimated *P. brachycephalum*. Due to the high mitochondrial protein content in liver of 0°C acclimated *P. brachycephalum* enzyme capacities were significantly higher than in *P. brachycephalum* at 5°C (by a factor of 1.43 for CS and 1.69 for IDH), than in *Z. viviparus* at 10°C (by a factor of 2.16 for CS and 3.72 for IDH) and than in *Z. viviparus* at 5°C (by a factor of 2.24 for CS and 2.66 for IDH) when calculated for total liver and measured at 5°C (Fig. 6B & 7B). Kent et al. (1988) also found a positive compensation of CS activity in liver of cold acclimated channel catfish, *Ictalurus punctatus*, after calculation for total liver. Their findings were related to a twofold increase in liver mass in the cold, accompanied by an increase of total protein, leaving protein concentrations per g liver unchanged. In North Sea cod, *G. morhua*, cold acclimation had no effect on CS activities per g liver, although positively compensated CS activities were found in total liver (Lannig et al., 2003). Slightly lower specific enzyme (CS and IDH) activities in liver mitochondria of cold compared to warm acclimated North Sea eelpout, remained uncompensated for despite the higher mitochondrial protein content in 5°C acclimated *Z. viviparus*. Somewhat unexpected, total liver capacities in both acclimation groups also resulted similar. In contrast to the present findings, Lucassen et al. (2003) found higher CS activity per g liver during cold acclimation (3.5°C) in North Sea eelpout, however, it might be that cold compensation of CS activities only starts when temperature falls below 5°C. Lucassen et al. found no differences in hepatosomatic indices either, whereas, in our study, cold-acclimation led to elevated I_H in *Z. viviparus*. It may well be that long-term cold acclimation (10 months in this study versus 25 days in the study by Lucassen et al.) led to a down regulation of specific CS capacities associated with an increase in I_H. In general, the highly positive cold compensation of CS activities, especially in polar ectotherms, is likely due to the role of mitochondria not only in energy metabolism but also in anabolic processes, with CS providing excess citrate for example for enhanced lipid synthesis in the cold (for review cf. Pörtner, 2002a; Pörtner, 2002b).

IDH displayed similar Q_{10} and E_A values as proton leakage rates (state IV_{ol}) and might be involved in setting the mitochondrial proton leakage rate as discussed by Pörtner et al. (2000). High Q_{10} and E_A values indicate that proton leakage might involve an enzyme catalyzed process (Ellory and Hall, 1987; Sazanov and Jackson, 1994; Pörtner et al., 1999b). However, further investigations of the mechanism of proton leakage are required. The present data indicate that IDH and proton leakage capacities are, if at all, only loosely correlated. Similar IDH activities but higher state IV_{ol} respiration rates were found in total liver of *Z.*

viviparus acclimated to 5°C compared to 10°C, whereas *P. brachycephalum* at 0°C had significantly higher IDH activities but only slightly higher state IV_{ol} rates than 5°C acclimated *P. brachycephalum* and *Z. viviparus*.

Role of Arrhenius activation energies

A mechanism involved in temperature dependent capacity changes is the minimization or maximization of Arrhenius activation energies (E_A) (Hochachka and Somero, 1984; Pörtner et al., 2000; Pörtner, 2002a). The traditional understanding is that E_A falls in the cold, indicated by a drop in E_A values of a wide range of enzymes (Hazel and Prosser, 1974). This picture has recently been questioned, and it has been argued that a rise in E_A of non-equilibrium enzymes like isocitrate dehydrogenase, of mitochondrial proton leakage and, in consequence, mitochondrial and whole animal oxygen demand plays a role in setting energy turnover low in polar, especially Antarctic, species and contributes to the level of extreme stenothermy found (Pörtner et al., 2000; Pörtner, 2002a). Mitochondrial IDHs from temperate eurythermal ectotherms generally show lower E_A values than those from stenothermal polar or deep sea organisms: E_A values for NADP-dependent IDH were 76.3 – 78.4 kJ mol^{-1} in rainbow trout (Moon and Hochachka, 1971), 55.2 kJ mol^{-1} in killifish *Fundulus heteroclitus* (Gonzales-Villasenor and Powers, 1986), and 55 kJ mol^{-1} in temperate populations of the polychaete *Arenicola marina* (Pörtner et al., 2000). These values are low compared to the E_A values found in stenothermal species, for example, 91.4 kJ mol^{-1} in the Notothenioid *L. nudifrons* (Hardewig et al., 1999), 138 kJ mol^{-1} in the Antarctic clam *L. elliptica* (Pörtner et al., 1999b) and 184.5 kJ mol^{-1} in the deep sea fish *Antimora rostrata* (Moon and Storey, 1975). In the present study E_A of $NADP^+$ -dependent IDH did not differ between thermal acclimation groups or between species and was found at 72.4 ± 6.3 kJ mol^{-1} for *P. brachycephalum* and at 76.5 ± 5.4 kJ mol^{-1} for *Z. viviparus* (means ± standard deviation for control and acclimation groups of each species), thus closer to the range of E_A values found in temperate fish. These findings also support the view developed above that *P. brachycephalum* is not as highly cold stenothermal as other Antarctic species.

Effect of warm acclimation on Antarctic eelpout

To our knowledge this is the first long-term warm acclimation experiment for a fish species from the cold-stable Antarctic ecosystem (Eastman, 1993). In former studies *Trematomus bernachii* and *T. newnesi*, both species belonging to the Antarctic endemic Notothenioidae, were acclimated to 4°C during 2 to 5 weeks only and this temperature is the

highest to which these fish can be warm acclimated for several weeks (cf. Weinstein and Somero, 1998). Some adjustments in metabolism due to temperature changes can occur in notothenioids. Gonzalez-Cabrera et al. (1995) found temperature-induced capacity changes for osmoregulation in Antarctic Notothenioids, *T. bernachii* and *T. newnesi*. Warm acclimation (4°C) led to decreased serum osmolality, which resulted from the positive compensation of Na^+/K^+-ATPase in osmoregulatory tissues. In our study, *P. brachycephalum* was acclimated to 5°C for at least 10 months and during this time none of the fishes died. They even showed positive growth (data not shown). Furthermore, the lack of significant differences in condition factors and gonadosomatic indices (Table 1) between the different species and acclimation groups, indicate that acclimation to 5°C does not stress *P. brachycephalum*. Evidently, *P. brachycephalum* is still able to undergo temperature compensation despite its life at stable cold temperatures. As in the study of Weinstein and Somero (1998), no temperature-induced changes were found in specific mitochondrial respiration rates but warm acclimation led to decreased aerobic capacities in total liver. Mitochondrial oxidative phosphorylation (state III) and enzyme activities, citrate synthase and $NADP^+$ -dependend isocitrate dehydrogenase, were lower in total liver of warm- versus cold acclimated *P. brachycephalum* due to decreased liver size and mitochondrial protein content in total liver. Since no data are available on liver size and mitochondrial liver protein of *T. bernachii*, it remains unclear whether Notothenioids also show adjustments in aerobic capacities at the whole organ level after warm acclimation.

Zoarcids are most abundant in North Pacific and North Atlantic as well as in the polar regions, Arctica and Antarctica. They are generally characterized by very low levels of standard metabolic rate and a benthic sluggish lifestyle. It might therefore be that in this group, the pressure is less to adjust aerobic metabolism to the permanent cold than in the more active notothenioids. Furthermore, it might be that *P. brachycephalum* has retained some features of a cold adapted but hypometabolic eurytherm and is at an intermediate stage between full cold-stenothermy and cold-eurythermy. Further investigations are required to investigate to what extent aerobic metabolism can be adjusted in other Antarctic ectotherms or whether down-regulation of aerobic capacities after warm acclimation only exists in the Zoarcidea.

The authors would like to thank M. Lucassen for assistance in developing the stabilisation buffer for analysis of $NADP^+$-dependent isocitrate dehydrogenase activity, T. Hirse for support in protein determinations as well as D. Storch for her help during tissue preparations.

References

Alp, R. P., Newshole, E. A., Zammit, V. A. (1976) Activities of citrate synthetase and NAD^+ -linked and $NADP^+$ -linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem. J.* **154**: 689-700

Blier, P. U. and Guderley, H. (1993) Mitochondrial activity in rainbow trout red muscle: the effect of temperature on the ADP-dependence of ATP synthesis. *J. Exp. Biol.* **176**: 145-57

Blier, P. U. and Lemieux, H. (2001) The impact of the thermal sensitivity of cytochrome c oxidase on the respiration rate of Arctic charr red muscle mitochondria. *J. Comp. Physiol. B* **171**: 247-253

Brand, M. D. (1990) The contribution of the leak of protons across the mitochondrial inner membrane to standard metabolic rate. *J. Theor. Biol.* **145**: 267-86

Brand, M. D., Chien, L.-F., Ainscow, E. K., Rolfe, D. F. S., Porter, R. K. (1994) The causes and functions of mitochondrial proton leak. *Biochem. Biophys. Acta* **1187**: 132-139

Brookes, P. S., Buckingham, J. A., Tenreiro, A. M., Hulbert, A. J., Brand, M. D. (1998) The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty acid composition. *Comp. Biochem. Physiol. B* **119**: 325-334

Campbell, C. and Davies, P. (1978) Temperature acclimation in the teleost, *Blennius pholis*: changes in enzyme activity and cell structure. *Comp. Biochem. Physiol. B* **61**: 165-167

Chance, B. and Williams, G. R. (1956) The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.* **17**: 65-134

Clarke, A. (1991) What is cold compensation and how should we measure it? *Am. Zool.* **31**: 81-92

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

Eastman, J. (1993) Antarctic fish biology: evolution in a unique environment. Academic Press, San Diego

Egginton, S. and Sidell, B. (1989) Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* **256**: R1-R10

Ellory, J. C. and Hall, A. C. (1987) Temperature effects on red cell membrane transport processes. In *Temperature and Animal Cells* (ed. K. Bowler and B. J. Fuller), *Symp. Soc. Exp. Biol.* **31**: 53-66

Fischer, T. (2002) The effects of climate induced temperature changes on cod (*Gadus morhua* L.): Linking ecological and physiological investigations. Ph.D. thesis, University Bremen

Gonzales-Villasenor, L. I. and Powers, D. A. (1986) A multilocus system for studying tissue and subcellular specialization. *J. Biol. Chem.* **261**: 11471-11477

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

Gornall, A. G., Bardawill, C. J., David, M. M. (1949) Determination of serum proteins by means of biuret reaction. *J. Biol. Chem.* **117**: 751-66

Guderley, H. (1998) Temperature and growth rates as modulators of the metabolic capacities of fish muscle. In *Cold ocean physiology*. (ed. H. O. Pörtner and R. Playle), pp. 58-87. Cambridge University Press

Hardewig, I., Peck, L. S., Pörtner, H. O. (1999) Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid *Lepidonotothen nudifrons*. *J. Comp. Physiol. B* **169**: 597-604

Hazel, J. R. and Prosser, C. L. (1974) Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**: 620-676

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

Johnston, I. A., Guderley, H., Franklin, C. E., Crockford, T., Kamunde, C. (1994) Are mitochondria subject to evolutionary temperature adaptations? *J. Exp. Biol.* **195**: 293-306

Johnston, I. A., Calvo, J., Guderley, H., Fernandez, D., Palmer, L. (1998) Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *J. Exp. Biol.* **201**: 1-12

Kawall, H. G., Torres, J. J., Sidell, B. D., Somero, G. N. (2002) Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. *Mar. Biol.* **140**: 279-286

Kent, J., Koban, M., Prosser, C. (1988) Cold-acclimation induced protein hypertrophy in channel catfish and green sunfish. *J. Comp. Physiol. B* **158**: 185-198

Lannig, G., Eckerle, L. G., Serendero, I., Sartoris, F. J., Fischer, T., Knust, R., Johansen, T., Pörtner, H. O. (2003) Temperature adaptation in eurythermal cod (*Gadus morhua*): a comparison of mitochondrial enzyme capacities in boreal and Arctic populations. *Mar. Biol.* **142**: 589-599

Londraville, R. and Sidell, B. (1990) Ultrastructure of aerobic muscle in Antarctic fishes may contribute to maintenance of diffusion fluxes. *J. Exp. Biol.* **150**: 205-220

Lucassen, M., Schmidt, A., Eckerle, L. G., Pörtner, H. O. (2003) Mitochondrial proliferation in the permanent versus temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* DOI: 10.1152/ajpregu.00111.2003

Mark, F. C., Bock, C., Pörtner, H. O. (2002) Oxygen-limited thermal tolerance in Antarctic fish investigated by MRI and 31P-MRS. *Am. J. Physiol.* **283**(5): R1254-R1262.

Moon, T. W. and Hochachka, P. W. (1971) Temperature and enzyme activity in poikilotherms: isocitrate dehydrogenases in rainbow trout liver. *Biochem. J.* **123**: 695-705

Moon, T. W. and Storey, K. B. (1975) The effects of temperature and hydrostatic pressure on enzymes of an abyssal fish, *Antimora rostrata*: liver NADP-linked isocitrate dehydrogenase. *Comp. Biochem. Physiol. B* **52**: 51-57

Moyes, C. D., Buck, L. T., Hochachka, P. W. (1988) Temperature effects on pH of mitochondria isolated from carp red muscle. *Am. J. Physiol.* **254**(4): R611-R615

Peck, L. S. (1989) Temperature and basal metabolism of two Antarctic marine herbivores. *J. Exp. Mar. Biol. Ecol.* **125**: 1-12

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

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) Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol. A* **132**: 739-761

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

Pörtner, H. O., Peck, L. S., Zielinski, S., Conway, L. Z. (1999a) Intracellular pH and energy metabolism in the highly stenothermal Antarctic bivalve *Limopsis marionensis* as a function of ambient temperature. *Polar. Biol.* **22**: 17-30

Pörtner, H. O., Hardewig, I., Peck, L. S. (1999b) Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternula elliptica*. *Comp. Biochem. Physiol. A* **124**: 179-189

Pörtner, H. O., van Dijk, P. L. M., Hardewig, I., Sommer, A. (2000) Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In *Antarctic Ecosystems: Models for Wider Ecological Understanding*. (ed. W. Davison and C. H. Williams), pp. 109-122. Christchurch, New Zealand, Caxton Press

Sazanov, L. A. and Jackson, J. B. (1994) Proton-translocating transhydrogenase and NAD- and NADP-linked isocitrate dehydrogenases operate in a substrate cycle which contributes to fine regulation of the tricarboxylic acid cycle activity in mitochondria. *FEBS Lett.* **344**: 109-116

Seddon, W. and Prosser, C. (1997) Seasonal variations in the temperature acclimation response of the channel catfish, *Ictalurus punctatus*. *Physiol. Zool.* **70**: 33-44

Sidell, W., Driedzic, W. R., Stowe, D. B., Johnston, I. A. (1987) Biomedical correlations of power development and metabolic fuel preferenda in fish hearts. *Physiol. Zool.* **60**: 221-232

Sokolova, I. M. and Pörtner, H. O. (2001) Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. obtusata* from different latitudes and shore levels. *Mar. Biol.* **139**: 113-126

Somero, G. N. and de Vries, A. L. (1967) Temperature tolerance of some Antarctic fishes. *Science* **156**: 257-258

Sommer, A. M. and Pörtner, H. O. (2002) Metabolic cold adaptation in the lugworm *Arenicola marina* (L.): comparison of White Sea and a North Sea population. *Mar. Ecol. Prog. Ser.* **240**: 171-182

St.-Pierre, J., Charest, P.-M., Guderley, H. (1998) Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimation of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **201**: 2961-2970

Torres, J. and Somero, G. (1988) Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. *Mar. Biol.* **98**: 169-180

van den Thillart, G. and Modderkolk, J. (1978) The effect of acclimation temperature on the activation energies of state III respiration and on the unsaturation of membrane

lipids of goldfish mitochondria. *Biochem. Biophys. Acta.* **510**: 38-51

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**: 3611-3621

Weinstein, R. B. and Somero, G. N. (1998) Effects of temperature on mitochondrial function in the Antarctic fish *Trematomus bernachii*. *J. Comp. Physiol.* **168**: 190-196

Table 1

Body and liver masses, condition factors (k-factor), gonadosomatic indices (I_G) and mass exponents (m) of regression equations (with p values) for the normalization to a 40g specimen of the two eelpout species, *Z. viviparus* (North Sea) and *P. brachycephalum* (Antarctica) from the different acclimation groups. Data are means ± SD.

specie	***Zoarces viviparus*** North Sea		***Pachycara brachycephalum*** Antarctica	
acclimation temperature (°C)	10 °C (control)	5 °C	5 °C	0 °C (control)
n	6 - 13	3 - 5	17 - 19	14 - 20
body mass (g)	42.88 ± 22.29	63.34 ± 17.34	39.19 ± 8.11	35.25 ± 11.62
liver mass (g)	0.85 ± 0.70	2.43 ± 1.01	1.18 ± 0.57	1.16 ± 0.58
condition factor	0.37 ± 0.08	0.41 ± 0.02	0.36 ± 0.03	0.40 ± 0.04
gonadosomatic index	0.54 ± 0.15	0.39 ± 0.19	0.42 ± 0.12	0.33 ± 0.24
m for normalization of hepatosomatic index	1.36 p<0.0001	1.01 p=0.01	2.29 p<0.0001	1.37 p<0.0001
m for normalization of mitochondrial protein content	1.91 p=0.043	1.52 p=0.021	1.38 p=0.019	0.90 p=0.035
m for normalization of CS activity	1.11 p<0.0001	1.39 p=0.0008	1.20 p<0.0001	0.98 p<0.0001

Figure legends

Fig. 1

Hepatosomatic indices, normalized to a 40 g animal, in Antarctic and North Sea Zoarcidae, *P. brachycephalum* (Antarctica) under control conditions (0°C) (n=20) and acclimated to 5°C (n=19), *Z. viviparus* (North Sea) acclimated to 5°C (n=5) and under control conditions (10°C) (n=13). Data marked with the same letters are significantly different.

Fig. 2

Mitochondrial protein content, specific (**A**) and total (**B**, normalized to a 40 g animal), in liver from Antarctic and North Sea Zoarcidae, *P. brachycephalum* (Antarctica) under control conditions (0°C) (n=8) and acclimated to 5°C (n=7-8), *Z. viviparus* (North Sea) acclimated to 5°C (n=4) and under control conditions (10°C) (n=4-6). Data marked with the same letters are significantly different.

Fig. 3

Temperature dependence of respiratory coupling ratios under oligomycin (RCR_{ol}, **A**) and of P/O ratios (**B**) of liver mitochondria from Antarctic and North Sea Zoarcidae, *P. brachycephalum* (Antarctica) under control conditions (0°C) (filled circles, n=4-8) and acclimated to 5°C (open circles, n=4-9) and *Z. viviparus* (North Sea) acclimated to 5°C (open squares, n=4-5) and under control conditions (10°C) (filled squares, n=4-7). For better viewing squares were shifted to the right.

* indicates values significantly different from those at 5°C.

a indicates RCR_{ol} of *Z. viviparus* 5°C significantly different to data from the other acclimation groups, when compared at the same temperature, at 8°C to *P. brachycephalum* (0°C and 5°C) only.

b indicates P/O ratios significantly different to data from the other acclimation groups, when compared at the same temperature

Fig. 4

Temperature dependence of specific respiration rates, state III (**A**), state IV (**B**) and state IV_{ol} (**C**) of liver mitochondria from Antarctic and North Sea Zoarcidae,

P. brachycephalum (Antarctica) under control conditions (0°C) (filled circles, n=5-7) and acclimated to 5°C (open circles, n=4-8) and *Z. viviparus* (North Sea) acclimated to 5°C (open

squares, n=4-5) and under control conditions (10°C) (filled squares, n=4-8). For better viewing squares were shifted to the right.

* indicates values significantly different from those at 5°C and 8°C.

a indicates data from *P. brachycephalum* 0°C significantly different to data from *Z. viviparus* at 5°C and 10°C, when compared at the same temperature.

b indicates data from *P. brachycephalum* at 5°C significantly different to data from *Z. viviparus* at 10°C, when compared at the same temperature.

Fig. 5

Temperature dependence of total mitochondrial respiration rates, state III (**A**), state IV (**B**) and state IV_{ol} (**C**) in liver (normalized to a 40 g animal) from Antarctic and North Sea Zoarcidae, *P. brachycephalum* (Antarctica) under control conditions (0°C) (filled circles, n=4-6) and acclimated to 5°C (open circles, n=4-8) and *Z. viviparus* (North Sea) acclimated to 5°C (open squares, n=4-5) and under control conditions (10°C) (filled squares, n=3-5). For better viewing squares were shifted to the right.

a indicates data from *Z. viviparus* 5°C significantly different to data from the other acclimation groups, when compared at the same temperature.

b indicates data from *P. brachycephalum* 0°C significantly different to data from *P. brachycephalum* 5°C (not at 5°C) and from *Z. viviparus* 10°C, when compared at the same temperature.

c indicates data from *Z. viviparus* 10°C significantly different to data from the other acclimation groups, when compared at the same temperature (for state IV (B) at 5°C not to *Z. viviparus* 5°C).

Fig. 6

Temperature dependence of citrate synthase activity (CS), specific (**A**) and total (**B**, normalized to a 40 g animal), in liver from Antarctic and North Sea Zoarcidae, *P. brachycephalum* (Antarctica) under control conditions (0°C) (filled circles, n=6-9) and acclimated to 5°C (open circles, n=7-8) and *Z. viviparus* (North Sea) acclimated to 5°C (open squares, n=4-5) and under control conditions (10°C) (filled squares, n=5-6). For better viewing squares were shifted to the right.

a indicates data significantly different to data from *Z. viviparus* 5°C and 10°C, when compared at the same temperature.

b indicates data from *P. brachycephalum* 5°C significantly different to data from *Z. viviparus* 5°C, when compared at the same temperature.
c indicates data from *P. brachycephalum* 0°C significantly different to data from the other acclimation groups, when compared at the same temperature.

Fig. 7
Temperature dependence of $NADP^+$ -dependent isocitratedehydrogenase activity (IDH), specific (**A**) and total (**B,** normalized to a 40 g animal), in liver from Antarctic and North Sea Zoarcidae, *P. brachycephalum* (Antarctica) under control conditions (0°C) (filled circles, n=6-8) and acclimated to 5°C (open circles, n=5-6) and *Z. viviparus* (North Sea) acclimated to 5°C (open squares, n=3-5) and under control conditions (10°C) (filled squares, n=4-6). For better viewing squares were shifted to the right.
a indicates data from *P. brachycephalum* 0°C significantly different to data from *Z. viviparus* 5°C and 10°C, when compared at the same temperature.
b indicates data from *P. brachycephalum* 5°C significantly different to data from *Z. viviparus* 10°C, when compared at the same temperature.
c indicates data from *P. brachycephalum* 0°C significantly different to data from the other acclimation groups, when compared at the same temperature.

figure 1

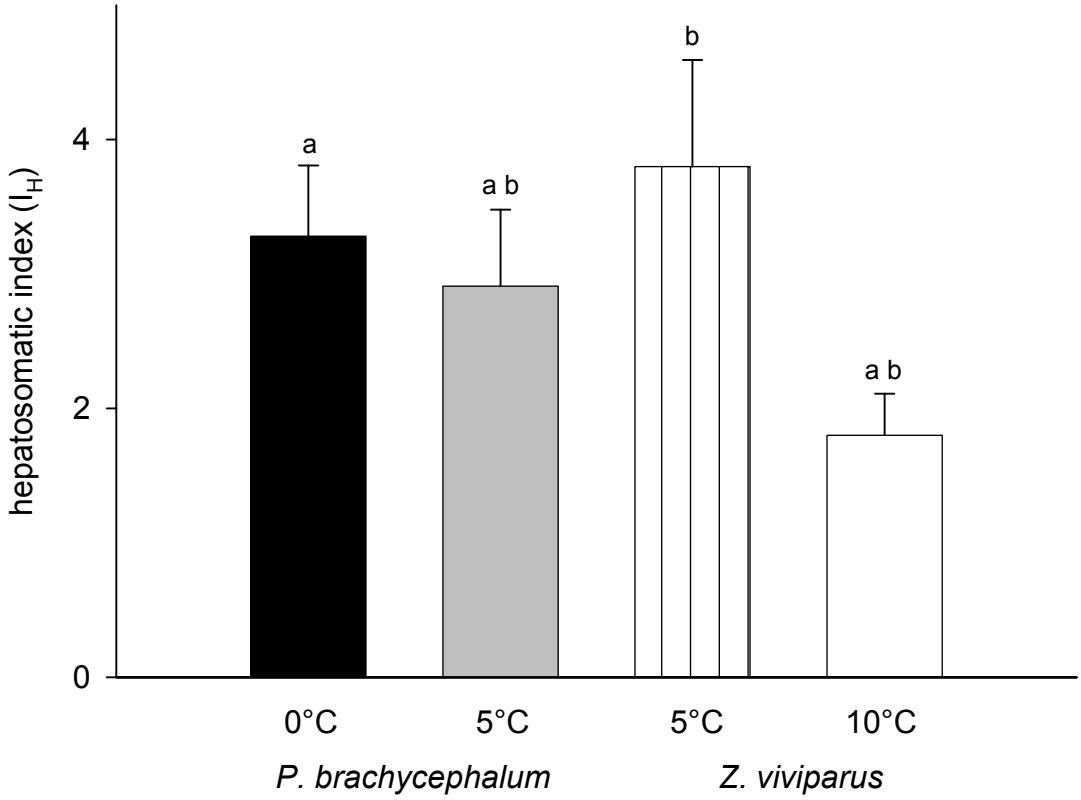

hepatosomatic index (I_H)
4
2
0
a
a b
b
a b
0°C
5°C
5°C
10°C
P. brachycephalum
Z. viviparus

figure 2

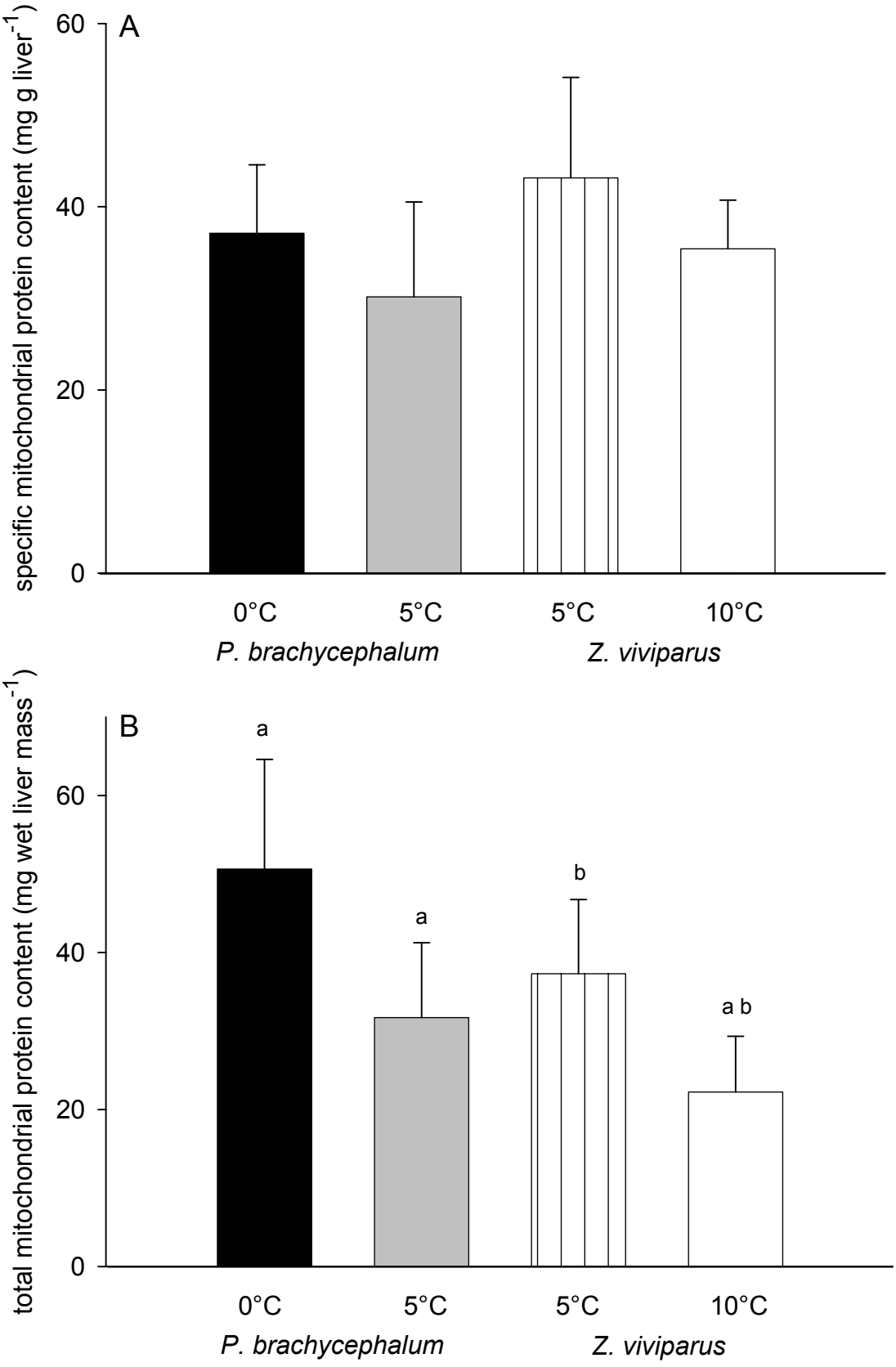

A
specific mitochondrial protein content (mg g liver-1)
60
40
20
0
0°C
5°C
5°C
10°C
P. brachycephalum
Z. viviparus
B
total mitochondrial protein content (mg wet liver mass-1)
a
a
b
a b
60
40
20
0
0°C
5°C
5°C
10°C
P. brachycephalum
Z. viviparus

figure 3

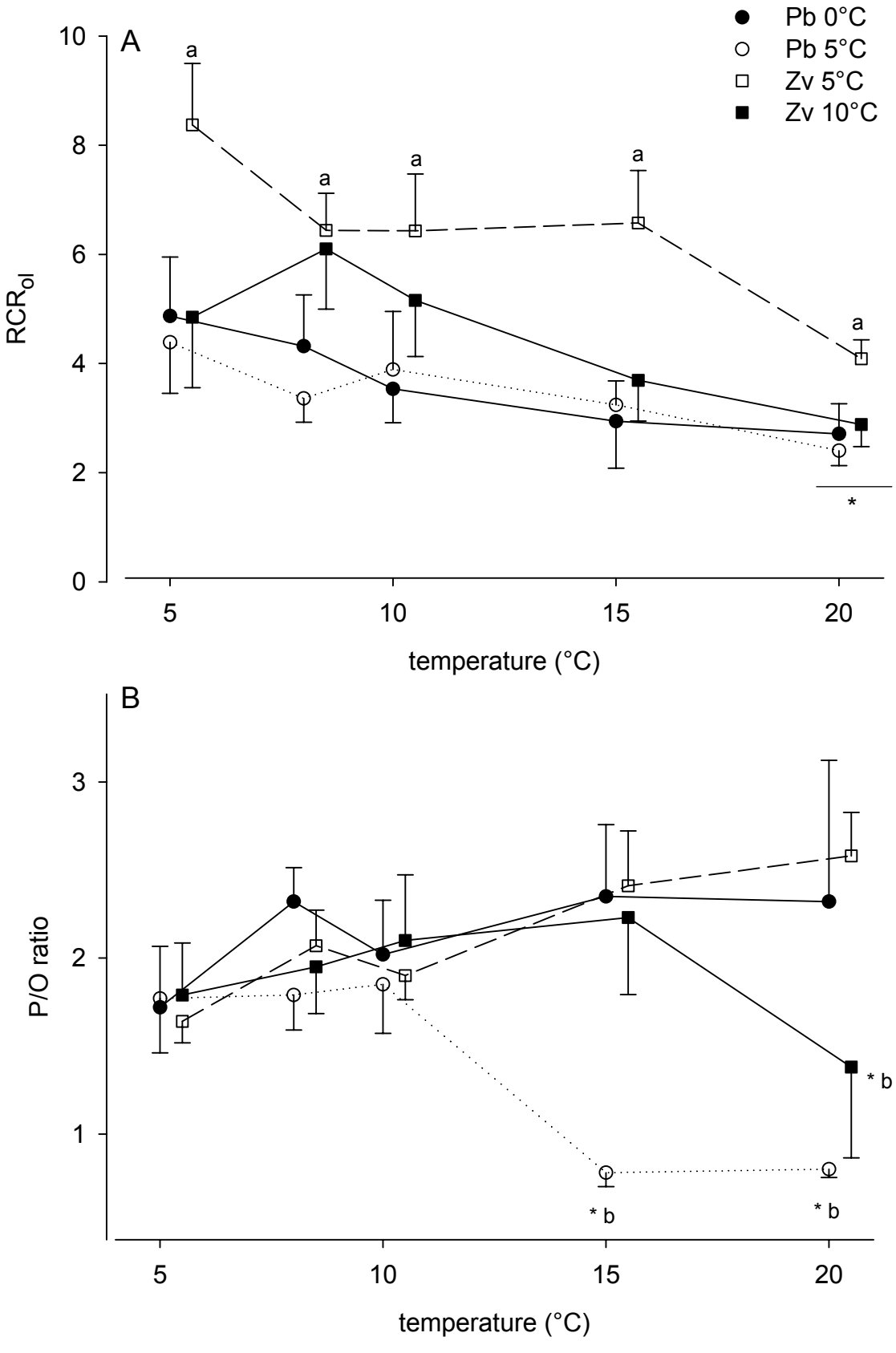

A
Pb 0°C
Pb 5°C
Zv 5°C
Zv 10°C
RCR$_{ol}$
temperature (°C)
a
*
B
P/O ratio
temperature (°C)
* b

figure 4

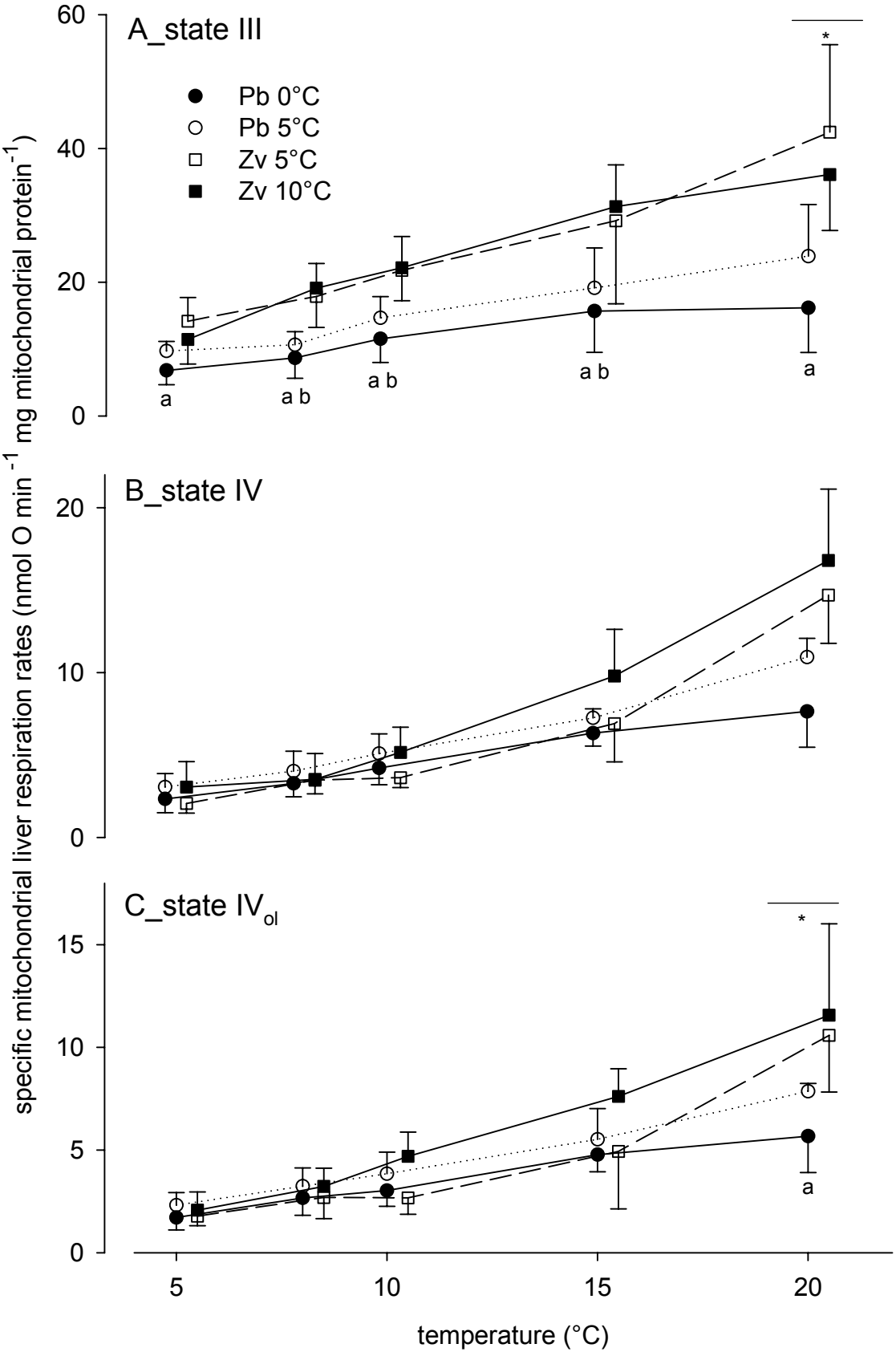

A_state III
Pb 0°C
Pb 5°C
Zv 5°C
Zv 10°C
B_state IV
C_state IV$_{ol}$
specific mitochondrial liver respiration rates (nmol O min^{-1} mg mitochondrial protein^{-1})
temperature (°C)
a
a b
a b
a b
a
*
*
0
20
40
60
0
10
20
0
5
10
15
5
10
15
20

figure 5

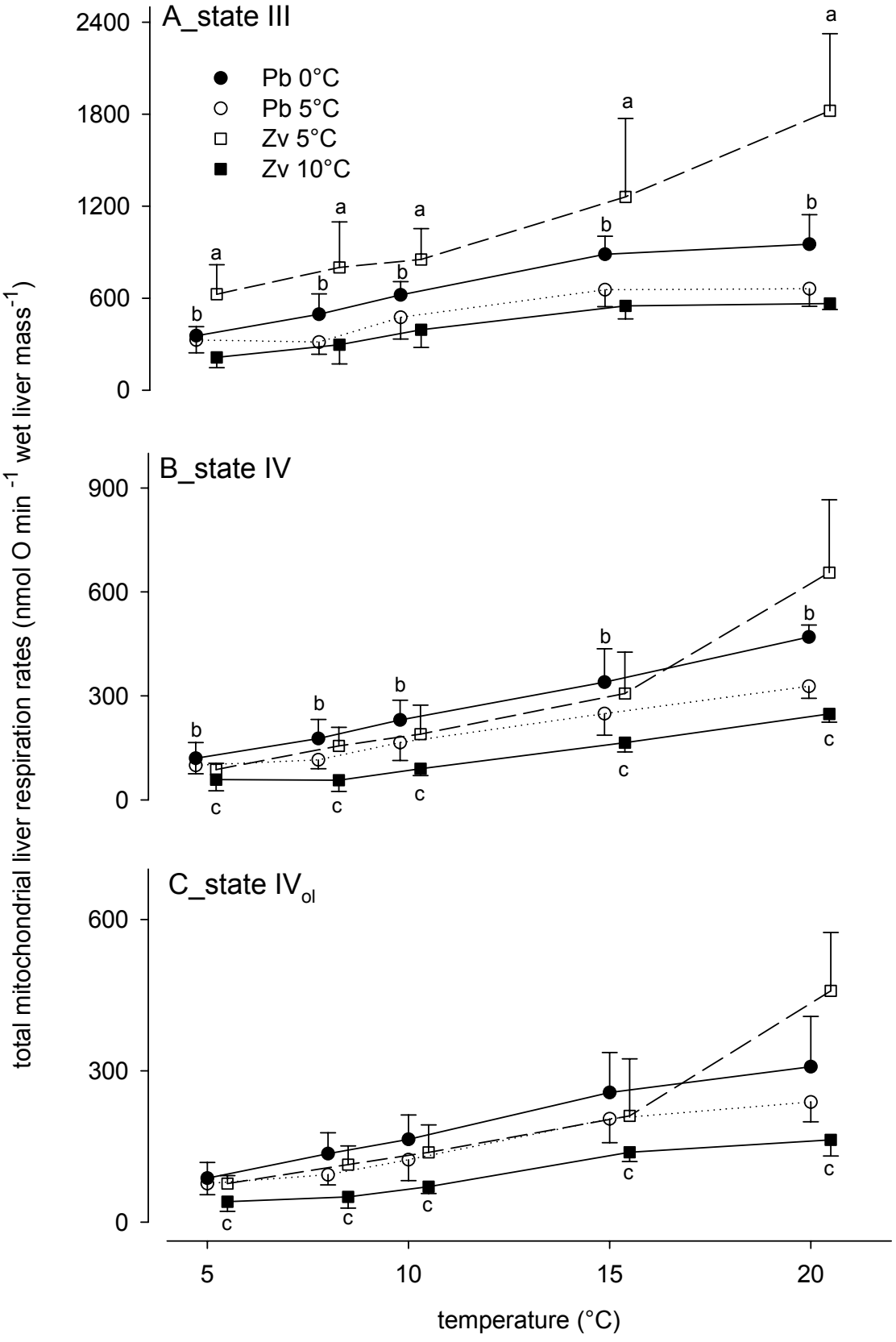

A_state III
B_state IV
C_state IV$_{ol}$
Pb 0°C
Pb 5°C
Zv 5°C
Zv 10°C
total mitochondrial liver respiration rates (nmol O min^{-1} wet liver mass^{-1})
temperature (°C)
2400
1800
1200
600
0
900
600
300
0
600
300
0
5
10
15
20

figure 6

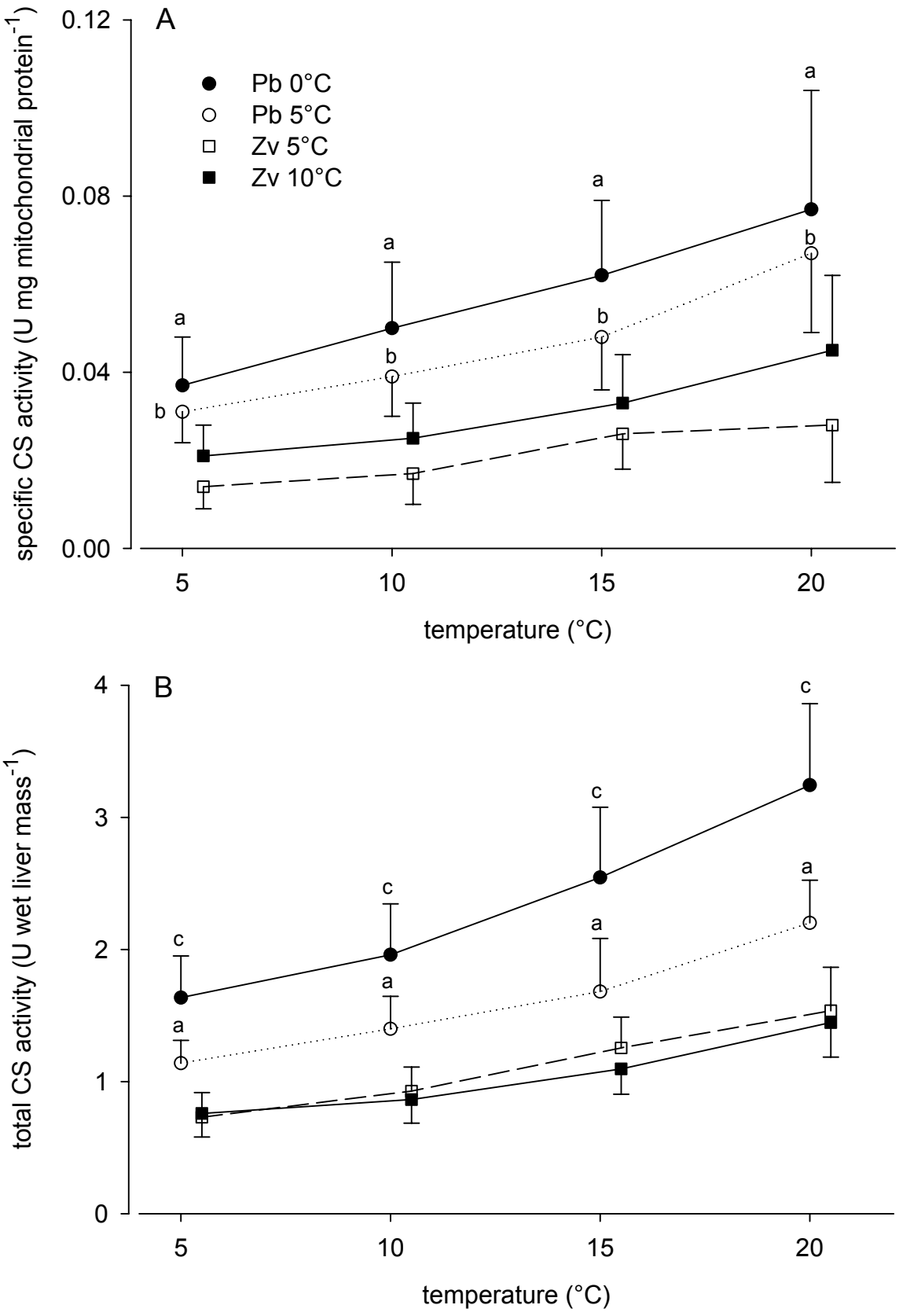
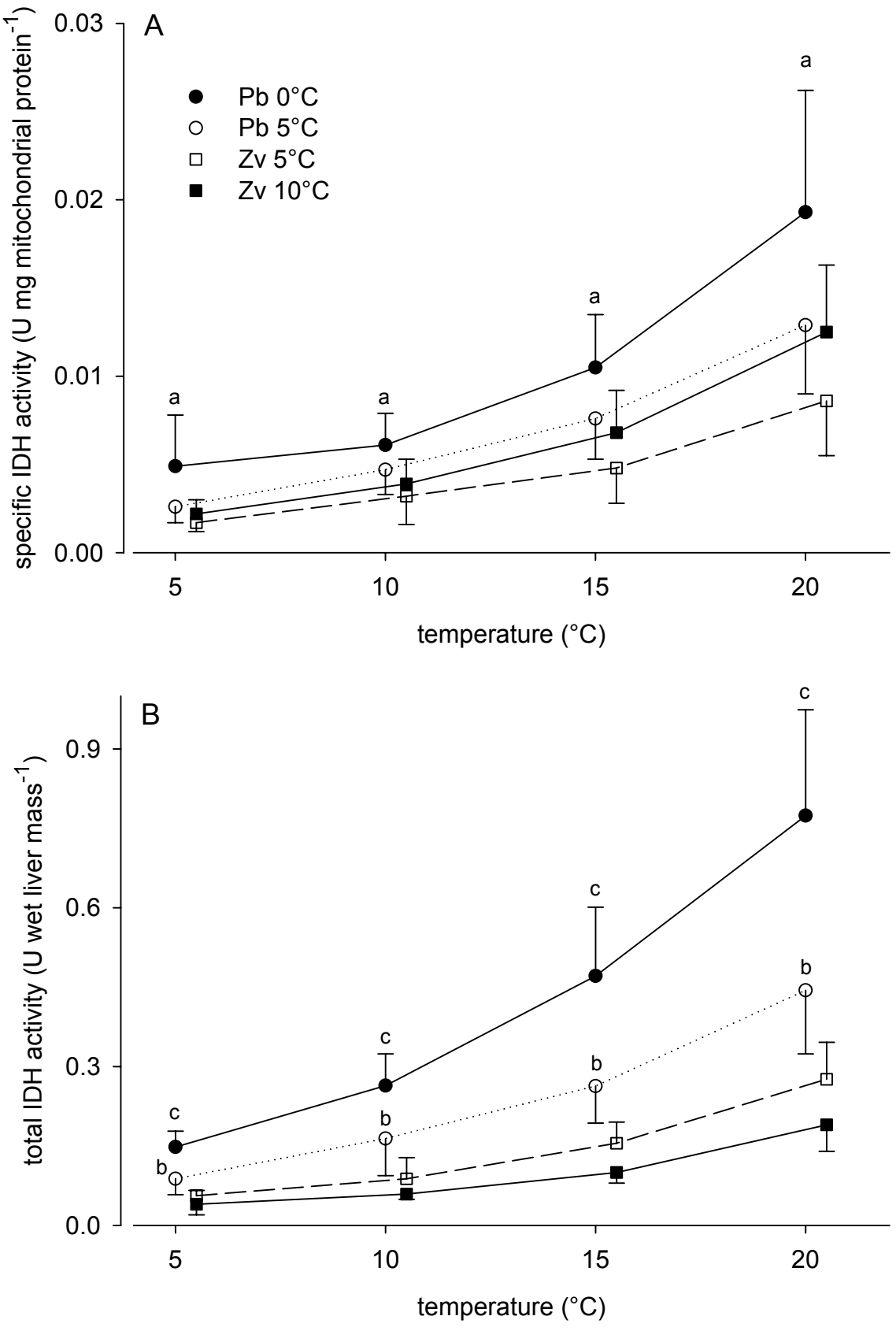

A
Pb 0°C
Pb 5°C
Zv 5°C
Zv 10°C
specific CS activity (U mg mitochondrial protein-1)
0.12
0.08
0.04
0.00
5
10
15
20
temperature (°C)
B
total CS activity (U wet liver mass-1)
4
3
2
1
0
5
10
15
20
temperature (°C)

figure 7


A
Pb 0°C
Pb 5°C
Zv 5°C
Zv 10°C
specific IDH activity (U mg mitochondrial protein-1)
0.03
0.02
0.01
0.00
5
10
15
20
temperature (°C)
B
total IDH activity (U wet liver mass-1)
0.9
0.6
0.3
0.0
5
10
15
20
temperature (°C)

Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L. studied by magnetic resonance imaging (MRI) and on-line venous oxygen monitoring

Gisela Lannig, Christian Bock, Franz-Josef Sartoris and Hans O. Pörtner*

Alfred Wegener Institute for Marine and Polar Research, Columbusstrasse,
27568 Bremerhaven, Germany

running head: Temperature dependent circulation and venous oxygen tensions in cod

* Author to whom correspondence should be addressed:
phone: +49-471-4831-1307; fax: +49-471-4831-1149; email: hpoertner@awi-bremerhaven.de

Keywords: *in vivo* MRI, cod, venous PO_2, heart rate, temperature, blood flow

Abbreviations: MRI: magnetic resonance imaging; f_H: heart rate; P_{VO2}: venous oxygen partial pressure; T_c: critical temperature; T_o: optimum temperature; T_p: pejus temperature; ROI: region of interest

Abstract

The hypothesis of an oxygen limited thermal tolerance due to limitations in cardiovascular performance was tested in Atlantic cod, *Gadus morhua* from the North Sea. Temperature dependent heart rate was recorded via a catheter implanted in the last gill arch and changes in arterial and venous blood flow were simultaneously determined by flow weighted Magnetic resonance imaging (MRI). In additional experiments, changes in venous oxygen tensions were monitored on-line using an oxygen sensor implanted into the *ductus Cuvier*. Experiments were performed starting at 10°C (acclimation temperature) with an acute temperature change of 1°C per hour during cooling to 1°C or warming to 19°C.

An exponential increase in heart rate occurred between 2 and 16°C (Q_{10} = 2.38 ± 0.35). Thermal sensitivity was reduced beyond 16°C when cardiac arrhythmia became visible indicating onset of a dysfunction in cardiovascular performance. Concomitant flow weighted MRI measurements of temperature dependent blood flow in *Aorta dorsalis* and *Vena caudalis* revealed an overall hyperbolic increase in blood flow during warming: Cooling from 10 to 1°C caused an overproportional drop in blood flow below 4°C, whereas warming from 10 to 19°C elicited a moderate increase in blood flow. Therefore, the slope of temperature dependent heart rate is not mirrored by a concomitant slope of blood flow changes. Venous P_{O2} changes with temperature followed an inverse U-shaped curve. Highest P_{VO2} levels were obtained at 5.0 ± 0.2°C with a narrow optimum temperature range for oxygen supply to tissues in cod. Thermal limitation may already set in below 2°C and beyond 7°C, respectively, characterised by decreased venous P_{O2} owing to onset of a progressive mismatch between oxygen demand and circulatory performance. Further warming led to a sharp drop in P_{VO2} beyond 16.1 ± 1.2°C °C in accordance with the onset of cardiac arrhythmia and likely, the critical temperature.

In conclusion, progressive cooling or warming brings cod from a temperature range of optimum cardiac performance into a pejus range, when aerobic scope falls before critical temperatures are reached. The pejus range appears very wide on the warm side. These findings are discussed in the light of geographical distribution limits found for *G. morhua* in nature and might be the reason for the predicted northern shift in the distribution of North Sea cod with global warming.

Introduction

Impacts of decadal-scale climate variations on marine communities and populations have been documented by Cushing (1982), Beamish (1995) and Bakun (1996), in line with the consideration that the geographical distribution of ectotherms is mainly correlated with ambient temperature. Temperature related trends in stocks of *G. morhua* in the North Sea were reported by Dippner (1997) and O`Brien et al. (2000), however, cause and effect relationships were not clear. Recently, a northward shift of North Sea cod populations due to warming was suggested to be linked to the thermal physiology of the animals (Pörtner et al. 2001), with a species and population specific pattern of oxygen limited thermal tolerance that has been elaborated as a general feature in animal organisms (Pörtner 2001). Among marine invertebrates and fish evidence for an oxygen limited thermal tolerance firstly arose from the observations of low and high temperature thresholds defined as critical temperatures (T_c), which are associated with the transition to anaerobic mitochondrial metabolism. Secondly and within the envelope of critical temperatures, early thermal limitation occurs by the onset of a limitation in whole animal aerobic scope at low and high pejus temperatures (pejus = getting worse). Pejus values are set by limited capacities of oxygen supply mechanisms, first seen in crustaceans as the onset of a decrease in arterial oxygen partial pressure (Frederich and Pörtner 2000, for review see Pörtner et al. 1998, 2000, Pörtner 2001).

Consecutively, thermal limitation in fish was also interpreted as caused firstly by limited oxygen supply capacity and secondly transition to anaerobic metabolism (e.g. van Dijk et al. 1999, Mark et al. 2002). The processes of thermal adaptation cause an optimisation of whole animal aerobic scope and capacity to a limited thermal window (Pörtner et al. 2001, Pörtner 2002). Such optimisation may be linked to behavioural preferences. For instance, Atlantic cod (*Gadus morhua*) from the north-west Atlantic react very sensitively to ambient temperature and follow a migration highway between 2°C and 3°C as shown by Rose (1993).

While the limitation of both circulatory and ventilatory performance appears responsible for thermal limitation in crustaceans (Frederich and Pörtner 2000) ventilation was affected later than circulation in fish (Mark et al. 2002). In rainbow trout, *Salmo gairdneri*, Heath and Hughes (1973) found that heart rate decreased at temperatures above 24°C whereas ventilation remained virtually unchanged until death of the animals occurred. More recently Sartoris et al. (2003) suggested that oxygen uptake via ventilation did not decrease with acute warming in fish, therefore, limited cardiac rather than ventilatory performance might be more crucial in the thermal limitation of fish. This discrepancy might be due to insufficient oxygen supply to the myocardium which is perfused by venous blood in those fish without coronary

circulation (Farrell, 1996, 2002). As summarised by Farrell (2002) for exercised salmonid fish, cardiac output during exercise reaches its maximum within an optimal temperature range. A further increase in acclimation temperature leads to increased oxygen demand by baseline metabolism leaving less oxygen for cardiac scope. With cardiac circulation finally being deficient in oxygen the heart becomes hypoxic and a reduction in functional capacity or scope results. Similar relationships may explain the drop in venous oxygen tensions observed in gill blood of cod during warming (Sartoris et al. 2003). Moreover, insufficient capacity of the circulatory system in fish would not only be the cause of unbalanced oxygen delivery and demand but vice versa, circulatory organs may also be the first affected by limited aerobic scope, oxygen deficiency, anaerobiosis and energetic failure at extreme temperatures (for review see Pörtner 2002).

Consequently, we set out to investigate whether and how temperature dependent cardiovascular performance might contribute to thermal limitation in cod. We investigated heart rate (f_H), arterial and venous blood flow and venous P_{O2} (P_{VO2}) in the *ductus Cuvier* of cod, *G. morhua* from the North Sea during acute temperature change.

Materials and methods

Animals and experimental procedure

Cod*, G. morhua* of the southern North Sea (mean size: 48.3 ± 5.3 cm, mean mass: 847.2 ± 251.1 g) were caught by bottom drawling in the German Bight in April 2001. Fish were kept in natural sea water (32‰ salinity) of 10 ± 1°C for at least 4 weeks under a controlled photoperiod (12h:12h L:D). Cod were fed a diet of frozen mussels, *Mytilus edulis*, twice per week. Feeding was terminated one week prior to the start of experimentation.

Two experimental series were carried out, firstly temperature dependent heart rate frequency (f_H) was recorded in fish catheterised in the afferent branchial artery, paralleled by concomitant measurements of blood flow with MR imaging. In a second set of experiments temperature induced changes in venous oxygen partial pressure (P_{VO2}) in the *ductus Cuvier* were investigated using micro-optodes (for surgery and data collection procedures see below). After surgery the fish was placed in a Plexiglas flow-through chamber of approx. 6 litre volume (length: 63 cm, diameter: 12 cm). The fish was oriented towards the incoming water with a constant flow of aerated seawater at 2-3 l min^{-1} and 10°C for recovery. Since MR imaging techniques applicable to marine animals like RARE and FLASH could not be used for pelagic fish without movement artefacts (Bock et al. 2001, 2002) the fish was restrained by bails in a way that the animal was still able to move its tail and pectoral fins but large

horizontal movements were prevented. This set-up was also used for P_{VO2} measurements in the second experimental series. In both series temperature was changed by 1°C h^{-1}. To monitor temperature, fluoroptic temperature sensors (Polytec, Germany) were installed in the tank reservoir as well as in the experimental chamber. Temperature was controlled to ± 0.1°C by means of cryostats.

Prior to experimentation, fish were left inside the experimental set-up at 10°C for at least 24 hours to recover from handling stress (Sartoris et al. 2003). Data (heart rate, blood flow and P_{VO2}) were collected immediately after surgery. After control measurements at 10°C (acclimation temperature) water temperature was decreased towards 1°C by 1°C h^{-1}. Afterwards, the water was warmed to 10°C within 30 min and the cod were left to recover over night. Temperature was increased to 19°C by 1°C h^{-1} on the next day, when heart rates in all cod had returned to control values. In contrast to heart rate and blood flow analyses, measurements of venous P_{O2} were performed in different individuals during either temperature decrease or increase since the lifetime of the micro-optodes was limited to 48 h after implantation. At the end of the experiments, animals were anaesthetized by use of 0.08 g l^{-1} tricaine methanesulphonate (MS222). The catheter or micro-optode was removed (see surgery procedures) and the fish were brought back into the aquarium. None of the fishes died and after 12 hours (next morning) all animals resumed feeding.

Surgery and data collection procedures

For all surgeries, cod were anaesthetized with 0.08 g l^{-1} MS222 and weighed. Their body length was determined and they were then transferred to an operating table. During surgery (approx. 30 min), gills were continuously perfused with aerated seawater (8 – 10°C) containing 0.04 g l^{-1} MS222.

Heart rate

A polyethylene catheter (PE50, 30 cm long), filled with heparinized (50 IU ml^{-1}) 0.9% NaCl solution was inserted into the afferent branchial artery of the last gill arch (approx. 0.5 cm), secured with skin suture and fixed to the gill arch with cyanoacrylate glue. For better fixation the catheter was also secured on the fish skin. After placing the cod into the chamber the catheter was connected to an approx. 1.5 m long PE 50 catheter, attached to a pressure transducer (UFI, Motro, Canada) that was connected to a bridge amplifier of a MacLab system (AD Instruments, Australia) and recordings were stored on a computer. Sampling rate

was $0.1s^{-1}$. Prior to the start of experimentation daily calibration of the pressure transducer was performed against a static water column.

Venous partial pressure of oxygen

For P_{VO2} measurements an optode was implanted into the *ductus Cuvier* following a procedure modified from Farrell and Clutterham (2003). Prior to insertion, the optode was calibrated in oxygen-free and air-saturated seawater and the optode tip was soaked in a 100 IU ml^{-1} heparin solution. A dorso-ventral incision was made in the left cleithrum to expose the *ductus Cuvier*. A PE50 catheter (approx. 2 cm long) was placed right in front of the *ductus Cuvier*, secured with a skin suture and fixed with cyanoacrylate glue. Afterwards, a small incision was made into the vein. The optode was passed through the fixed PE50 and advanced approx. 1 cm retrograde into the ductus. The optode was then fixed to the PE50 tubing with glue and sutures were used to secure the optode in the cleithrum and on the fish skin in front of the dorsal fin. In this way changes in position of the optode despite fish movements could be prevented.

The measurements were done following the principles described by Sartoris et al. (2003) using fibre optic micro-optodes (Pre Sens GmbH, Germany) connected to a computer (sampling rate: 1 min^{-1}). Temperature corrections as noted by Sartoris et al. (2003), were not necessary, since extended Tx-type optodes with integrated temperature compensation were used instead of Microx I-type optodes.

Blood flow

MR imaging for the determination of relative blood flow changes in different vessels was performed with a Snapshot FLASH sequence (Haase 1990). Imaging parameters were as follows: Matrix size: 128x128, TR= 9ms, TE= 3.1ms, 45°-60° hermite pulses, pulse length 2000 µs, 1 slice, slice thickness 5 mm, field of view (FOV): 6 x 6 cm, distance of centre 40 mm, number of averages 32, 10 dummy scans, total acquisition time 32s. The analysis yields a relative parameter of flow as units of distance per time.

Figure 1 presents a typical axial view of a flow weighted MR image from North Sea cod under control conditions. When compared to classical flow weighted Flash sequences the faster Snapshot Flash method in combination with the restriction by the bails (visible on the left side of the figure) allowed the acquisition of MR images without movement artefacts and sufficient signal to noise ratio. The *Aorta dorsalis* as well as the *Vena caudalis* can be clearly

identified as bright spots (see arrows) beneath the spinal cord of the fish. The black area under the vessels displays a cross section of the swim bladder.

Data analysis

Heart rate (f_H) was derived from the pulsatile changes in ventral aortic blood pressure and counted for each temperature and cod. Values of venous P_{O2} recorded as % air saturation were converted to P_{VO2} (torr; 1 torr=1 mm Hg=133.3 Pa) using the following formula: $P_{VO2} = (P_{atm} - P_{H2O}) * 0.2095 * (\%\text{ air saturation} / 100)$, with P_{atm}: atmospheric pressure (mm Hg), P_{H2O}: water vapour pressure (mm Hg) dependent on temperature calculated after Dejours (1975) and proportionate oxygen content in air: 0.2095.

Mean signal intensities were calculated by an operator controlled pixel by pixel analysis of various regions of interest (ROIs) for the determination of relative blood flow changes. ROIs were obtained from the *Aorta dorsalis*, the *Vena caudalis* and from muscle tissue. Ratios of vessel ROIs versus muscle tissue were generated to correct for other possible effects on image contrast than flow (e.g. T1, T2, temperature). Blood flow changes are given in arbitrary units.

Statistics

Data are presented as means ± standard deviation for each temperature. Statistical significance was tested at the $p<0.05$ level using a one-way analysis of variance and post-hoc Turkey test (Sigma Stat 2.0; SPSS, USA). Discontinuities in slopes were calculated in Arrhenius plots after Yeager and Ultsch (1989) followed by an F-test for slope comparisons. Regressions were calculated using Sigma Plot 8.0 (SPSS, USA).

Results

Acute temperature changes induced an immediate response of heart rate of cod resulting in an exponential rise with increasing temperature over the total investigated temperature range (Fig.2A). Heart rate (f_H) of 36.6 ± 3.6 beats min^{-1} under control conditions (10°C) decreased significantly during progressive cooling (1°C h^{-1}) to a minimum value of 17.5 ± 3.2 beats min^{-1} at 2°C. In response to warming (1°C h^{-1}) f_H rose to a maximum of 65.9 ± 4.1 beats min^{-1} at 19°C. At temperatures below 7°C and above 12°C f_H was significantly different to control values at 10°C. Over the total temperature range from 2 to 19°C best curve fit for temperature dependent heart rate yielded a polynomial quadratic regression since at around 16°C the Arrhenius analysis revealed a discontinuity (Fig. 2B). From 2°C to 16°C

heart rate displayed an exponential rise with temperature following a mean Q_{10} of 2.38 ± 0.35. When cod started to show cardiac failure indicated by arrhythmia at high temperatures (see Fig.3B), the water was cooled immediately; therefore, the number of data points in the high temperature range is not sufficient for the comparison of regression slopes. Nonetheless, linear regression analysis indicates a change in slope from –6.96 in the lower temperature range (16.28°C to 1.7°C; $p<0.0001$) to –2.50 in the high temperature range (18.8°C to 16.6°C; $p=0.2123$) (Fig. 2B). One cod already failed to show an increase in f_H above 15°C, these values were not included in the analysis and are shown as squares in Fig 2B.

Mean values of arterial and venous blood flow derived from flow weighted MR imaging data obtained in cod close to the anterior end of the pectoral fin are shown in Fig. 4. A drop in blood flow was observed when temperature fell below 4°C, whereas no significant break point in flow was evident at temperatures beyond 4°C. In general blood flow increased in both, *Aorta dorsalis* and *Vena caudalis* with temperature, an observation corresponding to the changes in heart rate on the first sight. However, best curve fit for both vessels yielded a hyperbolic regression resulting in a strong increase in blood flow during warming in the lower and a moderate increase in the upper temperature range.

Changes in venous P_{O2} (P_{VO2}) with temperature are shown in Fig. 5 for 3 individual cod, since control values at 10°C differed largely between individuals and ranged from 18 torr to 70 torr. However, in response to cooling all cod showed an inverse U-shaped curve of temperature dependent venous P_{O2} values with significant discontinuities in slopes at 4.7°C, 5.1°C and 5.2°C, respectively (Fig. 5A). As a general pattern, decreasing temperature from 10°C by 1°C h^{-1} led to an increase in venous P_{O2} with maximum values at a mean temperature of 5.0 ± 0.2°C. Further cooling then caused a progressive decrease in venous P_{O2}.

When increasing temperature from 10°C to approximately 19°C all cod investigated showed a decrease in venous P_{O2} (Fig. 5B). An overproportional drop in venous P_{O2} became visible at temperatures around 16°C with some variability between individual cod. Significant discontinuities in slopes were found at 16.4°C, 17°C and at 14.7°C, resulting in a mean temperature at 16.0 ± 1.2°C. Above these temperatures the slope of the P_{VO2} decrement was significantly increased (see figure legend for regression analysis).

Figure 6 summarises the observed changes in venous P_{O2}, with values normalized to % of the highest P_{VO2} values found in the whole temperature range. Temperature-dependent measurements of venous P_{O2} revealed the highest values at 5.0 ± 0.2°C. The figure contains assigned tolerance ranges and threshold temperatures using the terminology used by Frederich and Pörtner (2000, see discussion). The optimum temperature range (T_o) was interpreted to be

limited by pejus values at temperatures (T_p) when P_{VO2} values fell significantly below the maximum values at 5°C. The lower T_p (I) was set to 1.6°C and the upper T_p (II) to 7.3°C, respectively. A critical temperature (T_c) is indicated at 16.0 ± 1.2°C when P_{VO2} decreased more strongly during warming (only seen in Fig. 5B in each individual pattern of P_{VO2} development), in line with the onset of cardiac arrhythmia.

Discussion

Methodology

The aim of this study was to investigate the relationship between temperature tolerance and limited oxygen delivery in *G. morhua* from the North Sea by determination of temperature dependent heart rate (f_H), arterial and venous blood flow and venous oxygen partial pressure in the *ductus Cuvier* (P_{VO2}).

The use of the last gill arch for recording heart rate via the afferent branchial artery made catheter fixation easier than via use of the second or third gill arches. Strong ventilation especially at high temperatures did not affect the preparation and thus the quality of recordings. Since electronic elements are affected by strong magnetic fields, it was necessary to use a long catheter (approx. 2 m) between the centre of the NMR system (location of the fish chamber) and the pressure transducer. Nevertheless, the reliability of the approach was supported by the quality of the recordings (Fig. 3).

In a recent study MR imaging could not be performed in un-anaesthetized pelagic fish since even small fish movements led to image artefacts with classical MRI techniques (Bock et al. 2002). Minimising the elbowroom of the fish with bails as well as the use of a faster MRI technique (Snapshot FLASH, Haase 1990) solved this problem and revealed flow weighted MR images in similarly good quality as obtained in immobilized crustacea (Bock et al. 2001) and in benthic (inactive) marine fish (Bock et al. 2002, Mark et al. 2002). An effect of stress factors on the animals during individual experiments can be assumed minimal, since the cod showed no fight or flight reactions after recovery from anaesthesia.

Low venous P_{O2} values were recorded right after surgery and increased during recovery to constant values, a pattern similar to the one recently described by Farrell and Clutterham (2003). The slope of P_{VO2} change during recovery is a good indicator for a favorable position of the optode in the *ductus Cuvier*. Overall, our findings further support the suitability of the optode system as a tool for P_{O2} measurements in the circulatory and ventilatory system of aquatic animals (Frederich and Pörtner, 2000; Sartoris et al. 2003, Farrell and Clutterham 2003). Using classical blood measuring systems Davie and Farrell

(1991) reported venous P_{O2} in resting fish near 30-40 torr, which corresponds to the mean of our data. Moreover, P_{VO2} recordings during temperature change yielded similar slopes despite different starting values.

The rather rapid and progressive temperature change used in this study does not represent a situation, which cod would likely experience in its natural environment. The experimental protocol was chosen such that cod had no time to acclimate to the different temperatures; therefore, the onset and effect of acclimation phenomena that may involve adaptive shifts in thresholds of thermal tolerance are likely avoided.

Cardiovascular performance

Mean control heart rate (f_H) was 36.6 ± 3.6 beats min^{-1} at 10°C and is in good agreement with those reported for Atlantic cod by Pettersson and Nilsson (1980), Axelsson and Nilsson (1986) and Webber et al. (1998). Heart rates obtained at 2°C, 5°C and at 7°C with 17.5 ± 3.2 beats min^{-1}, 22.7 ± 3.0 beats min^{-1} and 25.9 ± 3.6 beats min^{-1}, respectively, are similar to the 18 beats min^{-1} (2.5°C), 22 beats min^{-1} (5°C) and to the 27 beats min^{-1} (7.5°C) obtained in 5°C acclimated Nova Scotia cod swimming at a low speed of 7 cm s^{-1} (Claireaux et al. 1995). Heart beat frequency of cod responded quickly to changes in water temperature and displayed a more or less exponential change (Fig. 1). The Q_{10} of 2.38 ± 0.35 fits well within the range of Q_{10} values found for routine heart rates in fish with reported Q_{10} values between 1.3 and 3 (see Farrell 1984, 1996).

However, a trend (see results) was obvious that cod could not enhance heart rate much beyond 16°C and cardiac arrhythmia became visible (see Fig 2B and 3) indicating dysfunction in cardiovascular performance at higher temperatures. Farrell (1996) assumed a temperature induced upper frequency limit for heart rate of around 120 beats min^{-1} for most fish species depending, however, on acclimation temperature. In cold acclimated animals a drop in pacemaker rate upon warming occurs at a lower temperature limit, whereas warm acclimation increases the temperature at which heart rate as well as pacemaker rate drop. Temperature affects pacemaker discharge by decreasing the action potential duration with increasing temperature (Harper et al. 1995). Cold acclimation led to a drop in heart rate of isolated hearts of goldfish, *Carassius auratus*. A maximum of 38 beats min^{-1} at 16.3°C in hearts from lower acclimation temperatures (10°C) compares to a maximum of 51 beats min^{-1} at 24°C in isolated hearts of warm (25°C) acclimated animals (Tsukuda et al. 1985). With further warming heart rate decreased, became irregular and stopped. During acute temperature increase (1.5°C h^{-1}) heart rate of 15°C acclimated rainbow trout, *S. gairdneri* reached a

maximum of approximately 110 beats per min between 22°C and 24°C (Heath and Hughes 1973).

In our experiments with cod acclimated to 10°C, the upper limit in heart rate seemed to be reached at 65.6 ± 3.89 beats min^{-1} for temperatures above 16°C. This finding is in line with observations by Rinne et al. (2002), who reported that temperature dependent heart rate of Norwegian cod, *G. morhua* never exceeded 70 beats per min. In order to minimize thermal stress, temperature was not increased above approx. 19°C and reduced immediately, when cardiac arrhythmia became visible. Therefore, a potential decrease in heart rate at even higher temperatures likely occurs outside the range of experimental temperatures of the present study. Only one cod displayed a decrease in heart rate beyond 16°C (see open squares in Fig. 2) emphasising that a high tolerance limit is reached in cod at ≥ 16°C with some variability between individuals. These findings are in line with those reported by Sartoris et al. (2003) who defined a critical temperature (T_c) close to 16°C when cod showed a sudden drop in intracellular pH and energetic collapse in white muscle. All of these findings match the definition of the critical temperature as the temperature where aerobic scope becomes nil and anaerobic metabolism sets in (cf. Pörtner 2002). In our previous study the fish died within 1 hour despite immediate cooling. Cooling upon cardiac arrhythmia rescued the fish in our present study which indicates that onset of dysfunction in cardiac performance is an early marker for the upper critical temperatures in fish.

Concomitant measurements of blood flow in *A. dorsalis* and *V. caudalis* by flow weighted MRI revealed a very moderate increase in blood flow with rising temperature (Fig. 4). A drop in blood flow was observed when temperature fell below 4°C, whereas no significant break point in flow was evident at temperatures beyond 4°C. A similar pattern was found in Antarctic eelpout, *Pachycara brachycephalum* by Mark et al. (2002) when temperature was increased by 1°C once every 12h. They reported blood flow in the *A. dorsalis* to rise steadily up to 6°C, when no further increase occurred. It was suggested that a pejus temperature was reached close to 7°C in the Antarctic eelpout when blood flow became limiting likely due to insufficient cardiovascular performance.

Our findings indicate that a temperature induced increase in heart rate is not reflected by a similar factorial increment in relative blood flow. This is in line with Farrell's suggestion that cardiac contractility, which is an important determinant of arterial blood pressure *in vivo*, and stroke volume decreased with rising temperature (Farrell 1996, 2002). In line with our findings, Heath and Hughes (1973) found only a slight increase in blood pressure with temperature in rainbow trout, *S. gairdneri*. This might explain the mismatch between elevated

heart rates and blood flow at higher temperatures. Enhanced arterial and venous vasodilatation could also explain the blood flow pattern seen (measured as relative distance per unit time) at higher temperatures such that a somewhat stronger increase in volume flow is conceivable. However, apparent changes in the diameter of blood vessels were not visible and therefore volume changes were most likely smaller than 20%.

The measurements of venous P_{O2} in the *ductus Cuvier* at different temperatures support the conclusion that a mismatch between rising organismic oxygen demand and oxygen supply by the cardiovascular system sets in during warming (Fig. 5). Highest P_{VO2} in cod was obtained at 5.0 ± 0.2°C indicating an optimum temperature for energy efficient blood oxygen transport in cod. This finding is in good agreement with a suggested smaller difference between arterial and venous oxygen contents in Nova Scotia cod at 5°C acclimated animals compared to animals acclimated to 10°C as reported by Webber et al. (1998). This pattern is explained by the observed lower Q_{10} value for cardiac output than for oxygen consumption. However, cod from the North Sea is considered to be genetically distinct from the Scotian Shelf cod used by Pogson et al. (1995) and Webber et al. (1998) such that comparative investigations in North Sea cod acclimated to 5°C appear warranted.

Below and above 5°C in our North Sea cod P_{VO2} decreased steadily, in line with the suggested onset of a mismatch between oxygen transport via the blood and oxygen demand and P_{VO2} became significantly different to P_{VO2} at 5°C at 1.6°C and 7.3°C. With rising temperature an elevated difference between arterial and venous P_{O2} was also found by Heath and Hughes (1973) and Sartoris et al. (2003). Below 5°C the lower P_{VO2} values likely resulted from a larger effect of cooling on circulatory oxygen supply than on oxygen demand. Above 5°C the drop in P_{VO2} may indicate that increased tissue oxygen consumption with rising temperature was not fully compensated for by increased oxygen supply through blood flow. Venous P_{O2} results as a result of finalized oxygen diffusion to tissues and in the case of the fish heart (without coronary circulation) it is in fact the "arterial" pressure head of oxygen diffusion to the cardiac tissue. Therefore, the statement that the pattern of P_{VO2} change is an indicator of a progressive mismatch between circulatory oxygen supply and elevated metabolic rate is valid for the heart of the fish itself.

In the first study that elaborated the relationship between temperature dependent critical and pejus thresholds in an animal, pejus temperatures were identified on both sides of a wide plateau of high arterial $P_{O2,}$ in the spider crab, *Maja squinado* (Frederich and Pörtner, 2000). The respective threshold values were in fact those found to delineate the geographical distribution range of these animals. Such a clear analysis may not yet be possible for cod.

Arterial P_{O2} does not display a temperature limited plateau in cod, likely due to the non-limited capacity of ventilation within the thermal tolerance range (cf. Sartoris et al. 2003). Our present observations in venous blood indicate a lower T_p at 1.6°C (T_p I) and an upper T_p (T_p II) at temperatures beyond 7°C. This would be lower than our previous estimate (Sartoris et al. 2003) with an upper T_p close to 16°C, just below the critical temperature (T_c). Considering the pattern of whole organism oxygen demand (T. Fischer, R. Knust, H.O. Pörtner, unpubl.), venous oxygen tension and blood flow in North Sea cod indicates, however, that the decrement in aerobic scope starts early on beyond 7°C but may be much shallower in North Sea cod than seen in Antarctic eelpout by Mark et al. (2002) such that the range of thermal tolerance may be widened and the pejus range may not become limiting until closer to 16°C (Sartoris et al. 2003). Thus, the loss in aerobic scope in the pejus range may occur more slowly in eurythermal cod with moderate effects on functional scope and flexibility.

A sharp drop in P_{VO2} was only reached at around 16°C, differing between individual cod as shown in Fig. 5. This may be a fatal drop at T_c when energetic deficiency sets in (Sartoris et al. 2003). The mean value of T_c at 16.0 ± 1.2°C found here is in good agreement with data by Sartoris et al. (2003). The T_c might also reflect onset of insufficient oxygen supply to the heart when oxygen tension in the *ductus Cuvier* decreased to 25.4 ± 8.9 torr. This low pressure head may elicit the observed dysfunction in heart performance at higher temperatures. This value is higher than the suggested limiting 8-10 torr by Steffensen and Farrell (1998) and Sartoris et al. (2003) and might be explained by the location of the optode in the *ductus Cuvier* which lacks venous blood from the sinus venosus of the liver.

Sartoris et al. (2003) found arterial P_{O2} largely constant during warming and thus arterial oxygen uptake (*i.e.* ventilation) did not become limiting in cod within the range of thermal tolerance. Our present findings indicate that circulatory rather than ventilatory performance sets the limit of thermal tolerance in cod as also concluded by Mark et al. (2002) for Antarctic eelpout. This contrasts the situation in crustaceans (Frederich and Pörtner 2000) where breakpoints in ventilation, heart rate and arterial haemolymph P_{O2} coincided in the spider crab *M. squinado*. In cod myocardial oxygen supply is provided by venous blood. The warming induced right shift in O_2-haemoglobin dissociation curve would favour oxygen supply to the myocardium (Farrell and Clutherham 2003), however, less oxygen may remain for the myocardium due to rising oxygen uptake by other tissues. The suggestion that the upper lethal temperature is set by dysfunction in cardiac muscle due to insufficient oxygen supply matches the respective conclusions for exercising fish by Farrell (2002). Maximal exercise in fish acclimated at temperatures above its optimum leads to a decrease in venous

P_{O2} below the venous oxygen threshold required to support maximum cardiac performance. Therefore, heart becomes hypoxic and looses scope, when temperature approaches the upper limit (Farrell 2002). These considerations are in line with the progressive loss in whole organism aerobic scope suggested by the present study.

Ecological implications

The present findings indicate that oxygen supply to tissues is optimal, with probably lowest energetic costs in a relatively narrow temperature range around 5°C in North Sea cod acclimated to 10°C. Such patterns may cast some light on explaining the preferred thermal environment and the geographical distribution of cod.

Cod, *Gadus morhua* is found in all of the North Atlantic with a distribution limit at the Barents Sea in the North. Its southernmost limit is found in the Bay of Biscay. Larger cod are found in relatively cold water, from –0.5°C up to 12-14°C, but are most abundant in water layers of 1°C to 5°C; closer to its lower than to its higher lethal temperatures of around –2°C and 20°C, respectively (Jones 1968). These values may differ between populations or body size. Newfoundland cod, for example, traverse the cold waters of the shelf along a deep highway of warm oceanic water between 2°C and 3°C (Rose 1993). This might in fact be close to the optimum temperature for that population, somewhat lower than the optimum seen here in 1 kg North Sea cod at 5°C. Righton et al. (2001) proposed decreased vertical movements in North Sea cod during summer in response to varied abundance of prey species. However, the likely reason for cod to spend much or most of their time on the sea floor during summer and to return back to vertical migrations only during October and November might also lie in the higher summer temperatures of surface waters which in the North Sea vary between monthly averages of 7°C to 16°C (Laevastu 1993). During May-June a seasonal thermocline can develop which deepens throughout the summer and breaks down by November. Bottom water temperatures remain between 6°C and 8°C (Brander 1994, Dippner pers. commun). Furthermore, no cod are found in the southern North Sea during warm summers despite sufficient abundance of prey (R. Knust, pers. commun.). A decrease in cod recruitment observed during the last decade has also been associated with higher average temperatures of the North Sea. With respect to temperature North Sea cod live near the southern distribution limits of the species (O`Brian et al. 2000) and according to the present study, close to the upper limits of thermal tolerance.

All of these in situ observations of the natural thermal range of cod and its thermal preference and limitation match the present finding of an optimum and likely most energy

efficient pattern of oxygen supply between 2°C and 7°C for North Sea cod. In this population low and high pejus ranges start at 1.6°C and 7.3°C, respectively. Within pejus ranges survival is still possible but performance becomes progressively restricted. In the upper range of temperatures the progressive performance decrement would be slow until closer to the observed critical temperature at 16.0 ± 1.2°C when the drop in venous P_{O2} becomes fatal. Such limitation may also explain the 88% mortality in Iceland cod when acclimated to 15.6°C (Björnsson et al. 2001).

Preferred temperatures of cod observed in the laboratory vary depending on acclimation temperature, body size and time. Schurmann and Steffensen (1992) found it at 13.9°C for North Sea cod (Kattegat) between 80 and 200g acclimated to 12°C but during periods of inactivity they selected the lowest possible temperature in the experimental set-up. Nova Scotia cod of 1.2 to 1.5 kg and acclimated to 5°C displayed marked daily cycles in their thermal distribution, deeper in the water tank during daytime between 5°C and 6°C and swimming up into warmer water of 7°C during the night (Claireaux et al. 1995). Sogard and Olla (1996) assumed migration to cold temperatures as an energy-conserving response in the walleye pollock, *Theragra chalcogramma*. Thus around 5°C, metabolic costs might be lowest in North Sea cod due to efficient oxygen supply (cardiovascular performance and blood flow) at low oxygen demand (tissue and myocardial oxygen consumption)

Present findings of an optimal oxygen supply and energy efficient aerobic scope between 2°C and 7°C would, at first sight, contrast a high temperature of optimum growth (around 10°C, regardless of the population) and higher growth rates found in cod (120 to 462 g) acclimated to 12°C compared to 4°C (Pörtner et al. 2001). However, optimal temperature for growth and feed conversion depends on fish size in a way that the temperature for optimal growth and feed conversion decreases with body size. As reported by Björnsson et al. (2001) the temperature for optimum growth changed from 17-15°C for 2 g cod to 7°C for 2 kg cod from the southwest coast of Iceland. Furthermore, growth rates are usually obtained under conditions of unlimited food availability. Sogard and Olla (1996) showed that occupancy of cold water increased as food ration decreased from high to intermediate levels. Brett et al. (1969) and Jobling (1994) found a decrease in optimal growth temperature with increased food restriction.

However, North Sea cod avoid higher temperatures in their natural environment despite sufficient prey availability. Acclimation at temperatures beyond its optimum (above 7°C) are tolerated by North Sea cod under laboratory conditions since the limitation in aerobic scope does not seem to be critical. In contrast to laboratory maintained cod, animals in nature

hunt their prey and the limited aerobic scope at higher temperatures may hamper active hunting. According to Farrell (2002) maximum cardiac scope in exercised salmonids was found in fish acclimated to its preferred temperature, whereas acclimation below and beyond this temperature led to decreased aerobic scope, when measured at the respective acclimation temperature. In fish cardiovascular performance likely sets the optimum temperature range for aerobic scope. Again, it would be worth to investigate temperature dependent venous P_{O2} in North Sea cod acclimated to 5°C as it is unclear whether an acclimation dependent shift of the optimum occurs. Genetic differences have been found between populations of cod such that preference temperatures and temperatures of optimum oxygen supply may differ between cod populations in similar ways as found for the composition of haemoglobin isoforms and for metabolic organisation (Brix et al. 1998; Pörtner et al. 2001, Lannig et al. 2003). Even in the same population different haemoglobin genotypes were held responsible for different preferred temperatures between individuals. Cod from the northern part of the Oresund (Denmark) preferred a temperature of 15.4 ± 1.1 °C when possessing HbI-1 whereas the chose 8.2 ± 1.5 °C when possessing Hb1-2 (Petersen and Steffensen 2003). The potential strengthening of such differences between populations also warrant further investigation.

Summary

During acute temperature change oxygen supply through cardiovascular performance of resting 1 kg cod North Sea cod, *G. morhua* acclimated to 10°C appears optimal at temperatures around 5°C indicated by maximum values of venous P_{O2} and moderate heart rates. Below and above 5°C venous P_{O2} decreased indicating onset of a progressive mismatch between oxygen delivery and demand due to insufficient up-regulation of blood flow. Thermal limitation may, therefore, already set in at the transition from optimum to pejus range starting at temperatures below 1.6°C and beyond 7°C. At temperatures above 16°C a sharp drop in venous oxygen tension was observed paralleled by the onset of cardiac arrhythmia towards higher temperatures, indicating the loss of aerobic scope at the T_c. These findings reveal a wide shallow pejus range between 7 and 16°C. The finding of a relatively early onset of reduced performance is in good agreement with the general knowledge of thermal preference and distribution of cod. Although significant aerobic scope is still present at the acclimation temperature of 10°C this temperature is already beyond the thermal optimum of adult North Sea cod. This might be the reason why cod avoid higher temperatures in their natural environment when the limited aerobic scope hampers the expression of full performance during active hunting for prey. These considerations underline why global

warming causes a northward shift of the geographical distribution of *G. morhua* from the North Sea as predicted by Pörtner et al. (2001).

Acknowledgement

A contribution to the ELOISE project: "Effects of climate induced temperature change on marine coastal fishes (CLICOFI)", funded by the European Union program "Climate and environment", contract no. ENV4-CT97-0596. The authors wish to thank R. M. Wittig for technical support as well as F. Melzner for assistance during some of the surgeries. The authors hereby declare that experiments performed during this study comply with current legislation.

References

Axelsson M and Nilsson S (1986) Blood pressure control during exercise in the Atlantic cod, *Gadus morhua*. J Exp Biol 126: 225-236

Bakun A (1996) Patterns in the Ocean-Ocean Processes and Marine Population Dynamics. California Sea Grant College System, La Jolla, 323pp

Beamish RJ (1995) Climate Change and Northern Fish Populations. Canadian Special Publication of Fisheries and Aquatic Science 121: 739pp

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

Bock C, Frederich M, Wittig RM, Pörtner HO (2001) Simultaneous observations of haemolymph flow and ventilation in marine spider crabs at different temperatures: a flow weighted MRI study. Magnetic Resonance Imaging 19: 1113-1124

Bock C, Sartoris FJ, Pörtner HO (2002) In vivo MR spectroscopy and MR imaging on non-anaesthetized marine fish: techniques and first results. Magnetic Resonance Imaging 20: 165-172

Brander K (1994) Spawning and life history information for North Atlantic cod stocks. ICES Cooperative Research Report No. 205

Brett JR, Shelbourn JE, Shoop CT (1969) Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J Fish Res Board Can 26: 2363-2394

Brix O, Foras E, Strand I (1998) Genetic Variation and Functional Properties of Atlantic cod Hemoglobins: Introduction a Modified Tonometric Method for Studying Fragile Hemoglobins. Comp Biochem Physiol A 119(2): 575-583

Claireaux G, Webber DM, Kerr SR, Boutilier RG (1995) Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. J Exp Biol 198: 49-60

Cushing DH (1982) Climate and Fisheries. Academic Press, London, 373pp

Davie PS and Farrell AP (1991) The coronary and luminal circulations of the myocardium of fishes. Can J Zool 69: 1993-2001

Dejours P (1975) Principles of comparative respiratory physiology. Elsevier, New York.

Dippner JW (1997) Recruitment success of different fish stocks in the North Sea in relation to climate variability. Deutsche Hydrogr Zeitschr 49: 277-293

Farrell AP (1984) A review of cardiac performance in teleost heart: intrinsic and humoral regulation. Can J Zool 62: 523-536

Farrell AP (1996) Effects of temperature on cardiovascular performance. In: Global Warming: Implications for fresh water and marine fish, ed. Wood CM and McDonald DG. Cambridge University Press 1996: 135-158

Farrell AP (2002) Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. Comp Biochem Physiol A 132: 797-810

Farrell, AP and Clutterham, SM (2003) On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. J Exp Biol 206: 487-496

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

Haase A (1990) Snapshot FLASH MRI. Applications to T1, T2 and chemical-shift imaging. Magn Reson Med 13(1): 77-89

Harper AA, Newton IP, Watt PW (1995) The effect of temperature on spontaneous action potential discharge of the isolated sinus venosus from winter and summer plaice (*Pleuronectes platessa*). J Exp Biol 198: 137-140

Heath AG and Hughes GM (1973) Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). J Exp Biol 59: 323-338

Jobling M (1994) Fish bioenergetics. Chapman and Hall, London. 309 pp

Jones FRH (1968) Fish Migration. Spottiswood, Ballantyne & Co. Ltd., London and Colchester. 144 pp

Laevastu T (1993) Marine climate, weather and fisheries. The Effects of Weather and Climate Changes On Fisheries and Ocean Resources. Blackwell Scientific Publications Ltd., Oxford. 67 pp

Lannig G, Eckerle LG, Serendero I, Sartoris FJ, Fischer T, Knust R, Johansen T, Pörtner HO (2003) Temperature adaptation in eurythermal cod (*Gadus morhua*): a comparison of mitochondrial enzyme capacities in boreal and Arctic populations. Mar Biol 142: 589-599

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

O`Brien CM, Fox CJ, Planque B, Casey J (2000) Climate variability and North Sea cod. Nature 404: 142

Petersen, MF and Steffensen JF (2003) Preferred temperature of juvenile Atlantic cod Gadus morhua with different haemoglobin genotypes at normoxia and moderate hypoxia. J Exp Biol 206: 359-364

Pettersson, K and Nilsson, S (1980) Drug induced changes in cardio-vascular parameters in the Atlantic Cod, *Gadus morhua*. J Comp Physiol 137: 131-138

Pogson GH, Mesa KA, Boutilier RG (1995) Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and RFLP loci. Genetics 139: 375-385

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88: 137-146

Pörtner HO (2002) Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarcy of thermal tolerance in animals. Comp Biochem Physiol A 132: 739-761

Pörtner HO, Hardewig I, Sartoris FJ, van Dijk PLM (1998) Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? *in: Cold Ocean Physiology; eds.: Pörtner HO, Playle R*; Cambridge University Press, Cambridge, 88-120

Pörtner HO, van Dijk PLM, Hardewig I, Sommer A (2000) Levelos of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. *in: Antarctic Ecosystems: Models for a wider unterstanding; eds.: Davison W, Williams CW*; Caxton Press, Christchurch, New Zealand, 109-222

Pörtner HO, 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 FJ, 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

Righton D, Metcalfe J, Connolly P (2001) Different behaviour of North and Irish Sea cod. Nature 411: 156

Rinne JN, Holand B, Sundnes G (2002) Comparison of heart rate in fishes: cold, temperate sea water versus warm, desert rivers. http://www-heb.pac.dfo-mpo.gc.ca/congress/2002/Cardiovas/Rinne.pdf

Rose GA (1993) Cod spawning on a migration highway in the north-west Atlantic. Nature 366: 458-461

Sartoris FJ, Bock C, Serendero I, Lannig G, Pörtner HO (2003) Temperature dependent changes in energy metabolism, intracellular pH and blood oxygen tension in the Atlantic cod, *Gadus morhua*. J Fish Biol 62: 1-15

Schurmann H and Steffensen JF (1992) Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod, *Gadus morhua* L.. J Fish Biol 41: 927-934

Sogard SM and Olla BL (1996) Food deprivation affects vertical distribution and activity of a marine fish in a thermal gradient: potential energy-conserving mechanisms. Mar Ecol Prog Ser 133: 43-55

Steffensen JF and Farrell AP (1998) Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to hypoxia. Comp Biochem Physiol A 119: 585-592

Tsukuda H, Liu B, Fujii KI (1985) Pulsation rate and oxygen consumption of isolated hearts of the goldfish, Carassius auratus, acclimated to different temperatures. Comp Biochem Physiol A 82(2): 281-283

van Dijk PLM, Hardewig I, Tesch C, Pörtner HO (1999) Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae) J Exp Biol 202: 3611-3621

Webber DM, Boutilier RG, Kerr SR (1998) Cardiac output as a predictor of metabolic rate in cod *Gadus morhua*. J Exp Biol 201: 2779-2789

Yeager DP and Ultsch GR (1989) Physiological regulation and conformation: a Basic program for the determination of critical points. Physiol Zool 62(4): 888-907.

Figure legends

Fig. 1

Typical flow weighted MR image of an axial view of cod, *G. morhua* obtained at the anterior end of the pectoral fin under control conditions (10°C). See text for explanations.

Fig. 2

Heart rate frequency (f_H, beats min^{-1}) of cod, *G. morhua* during an acute temperature change (1°C h^{-1}; n=3-4) depicted in a linear (A) and in an Arrhenius plot (B). Open squares are data of one cod, whose f_H failed to increase at around 15°C. As indicated by the asterisks f_H values between 2°C and 7°C and between 12°C and 19°C were significantly different to control f_H at 10°C. Best curve fit for temperature dependent heart rate yielded a polynomial quadratic regression: $f(x) = (15.2\pm1.5)+(0.9\pm0.3) * x + (0.1\pm0.01) * x^2$; $R^2=0.94$; $p<0.0001$, with an Arrhenius Break temperature (ABT) at around 16.5°C and a decreased temperature dependence of heart rate (dotted line-fit indicates an overall trend within the data).

Fig. 3

Typical heart rate recordings of an individual cod, *G. morhua* at different temperatures. Note the onset of cardiac arrhythmia at 17°C.

Fig. 4

Relative blood flow changes in the *Aorta dorsalis* and *Vena caudalis* of cod, *G. morhua* with acute temperature change of 1°C per hour starting at control temperature 10°C. Best curve fit for both vessels yield a hyperbolic regression with a strong increase in blood flow in the lower and a moderate increase in the upper temperature range;

A. dorsalis: $f(x) = (2.3\pm0.05) * x / ((0.7\pm0.2) + x)$; $R^2=0.21$; $p<0.0001$

V. caudalis: $f(x) = (2.1\pm0.04) * x / ((1.2\pm0.2) + x)$; $R^2=0.45$; $p<0.0001$

Fig. 5

Venous P_{O2} measured in the *ductus Cuvier* of cod, *G. morhua* with acute temperature decrements (A) and increments (B) starting at a control temperature of 10°C. Graphs represent recordings of individual cod. Arrows indicate the temperature at which significant discontinuities in slopes of venous P_{O2} occurred during temperature changes.

A temperature decrease led to inverse U-shape curves with maximum values of venous P_{O2} at around 5°C;

I: $f(x) = (39.5\pm1.2) + (5.8\pm0.4)*x - (0.6\pm0.03)*x^2$; R2=0.56; p<0.0001

II: $f(x) = (32.04\pm0.7) + (4.3\pm0.3)*x - (0.6\pm0.03)*x^2$; R2=0.77; p<0.0001

III: $f(x) = (52.02\pm2.1) + (18.5\pm0.8)*x - (1.7\pm0.07)*x^2$; R2=0.59; p<0.0001

Temperature increase led to progressively decreasing values of venous P_{O2} turning into a significantly larger slope at temperatures of around 16°C;

I: f(10-16.4°C) = (80.1±1.2) – (3.8±0.09)*x; R^2=0.83; p<0.0001

f(16.6-17.3°C) = (195.3±31.7) – (10.6±1.9)*x; R^2=0.45; p<0.0001

II: f(10-16.9°C) = (45.8±0.8) – (0.09±0.06)*x; R^2=0.006; p=0.1145

f(17-19.6°C) = (159.8±6.9) – (6.8±0.4)*x; R^2=0.64; p<0.0001

III: f(10-14.6°C) = (47.9±0.7) – (1.7±0.06)*x; R^2=0.73; p<0.0001

f(14.7-19.2°C) = (58.5±1.01) – (2.4±0.06)*x; R^2=0.87; p<0.0001

Fig. 6

Summarised relative (%) changes in venous P_{O2} measured in the *ductus Cuvier* of cod, *G. morhua* during acute temperature changes starting from the control temperature of 10°C. Data are expressed as percent fractions of the highest P_{O2} values (n=3). Arrows indicate the optimum temperature range (T_o) with highest P_{VO2} values limited by pejus temperatures (T_p I and T_p II identify the temperatures at which P_{VO2} values fell significantly below the maximum) and the upper critical temperatures (T_c) identifies onset of a significantly stronger decrease in P_{VO2} during warming. The discontinuity in slope at the upper Tc is better seen in the measurements of individual cod (Fig. 5B), since the mean values revealed no obvious drop in P_{VO2} at around 16°C due to different temperature dependent patterns in P_{VO2} of individual cod.

figure 1

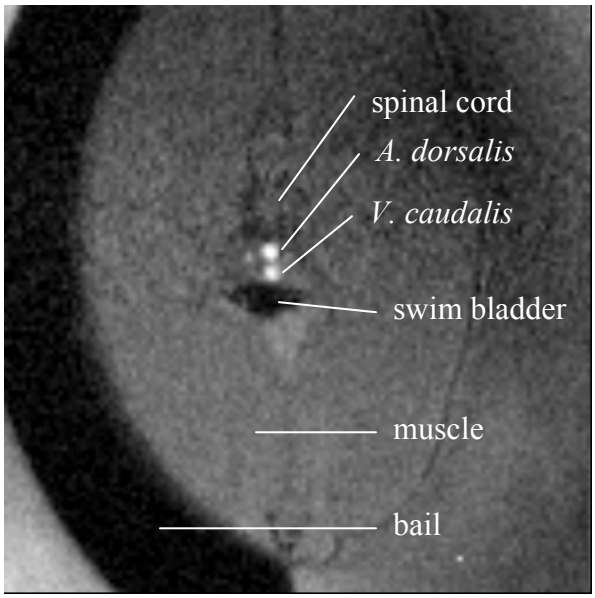


figure 2

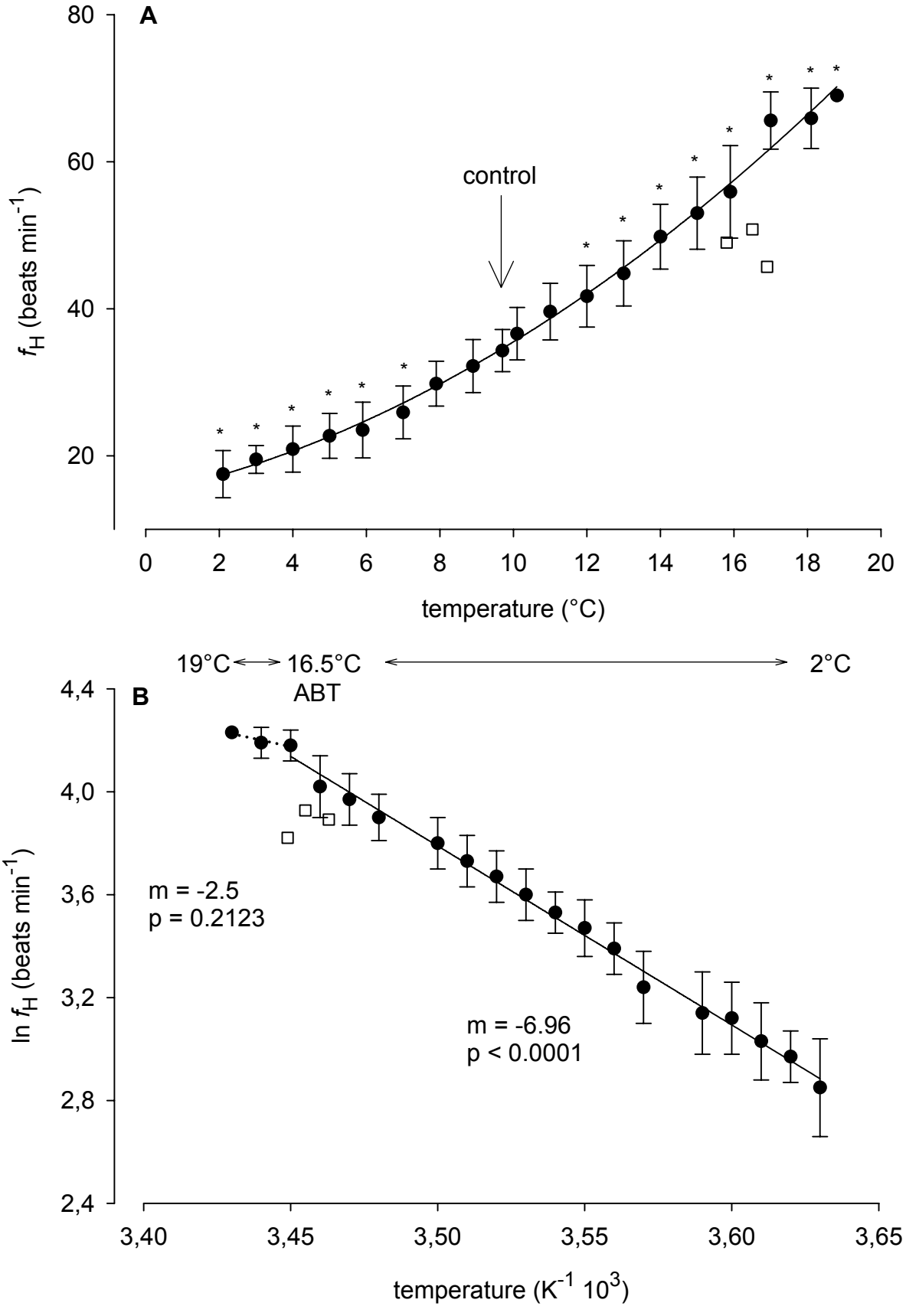

A
80
60
40
20
f_H (beats min^{-1})
control
0
2
4
6
8
10
12
14
16
18
20
temperature (°C)
19°C
16.5°C
ABT
2°C
B
4,4
4,0
3,6
3,2
2,8
2,4
ln f_H (beats min^{-1})
m = -2.5
p = 0.2123
m = -6.96
p < 0.0001
3,40
3,45
3,50
3,55
3,60
3,65
temperature (K^{-1} 10^3)

figure 3

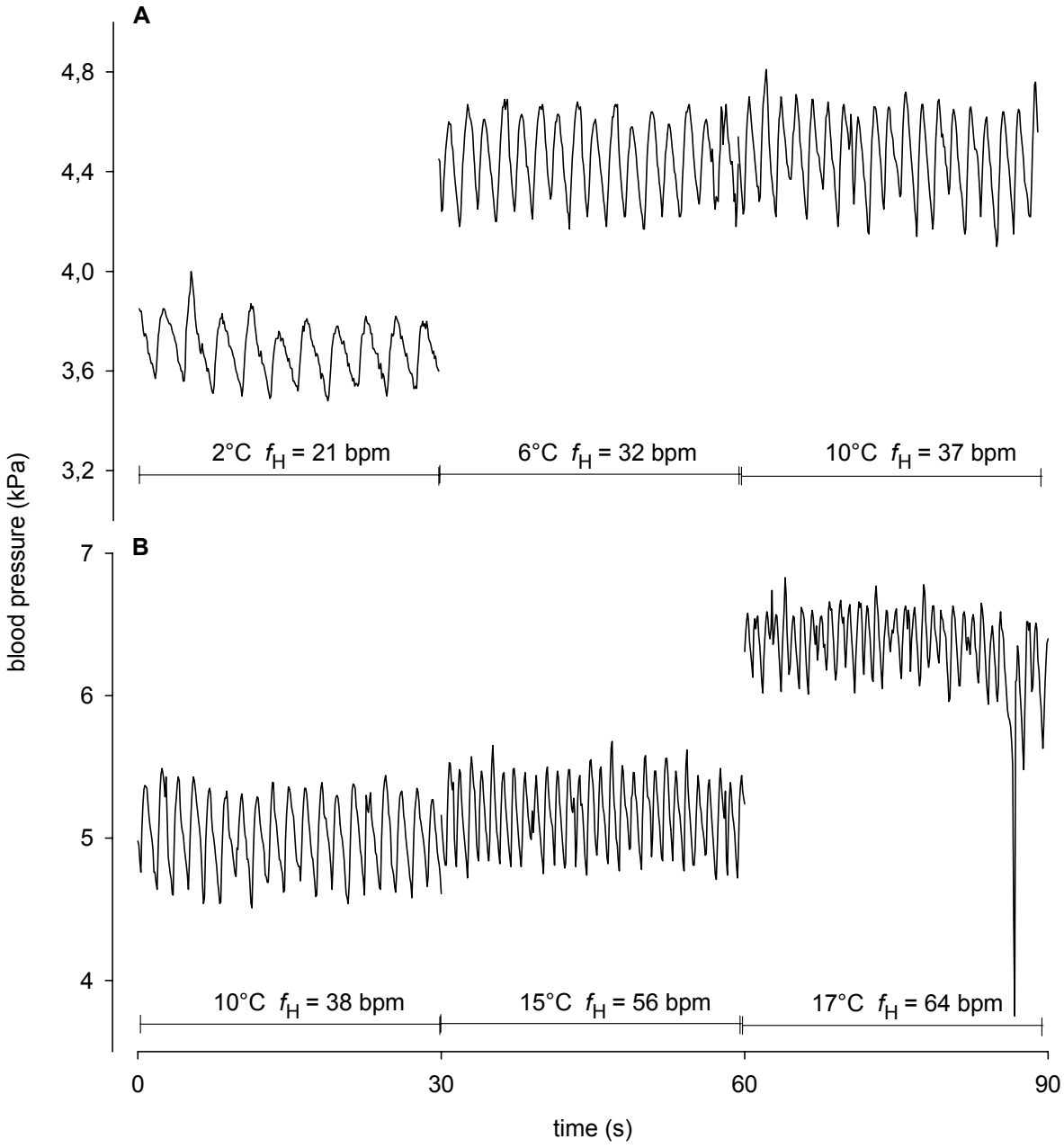

A
4,8
4,4
4,0
3,6
3,2
2°C f_H = 21 bpm
6°C f_H = 32 bpm
10°C f_H = 37 bpm
B
7
6
5
4
10°C f_H = 38 bpm
15°C f_H = 56 bpm
17°C f_H = 64 bpm
blood pressure (kPa)
0
30
60
90
time (s)

figure 4

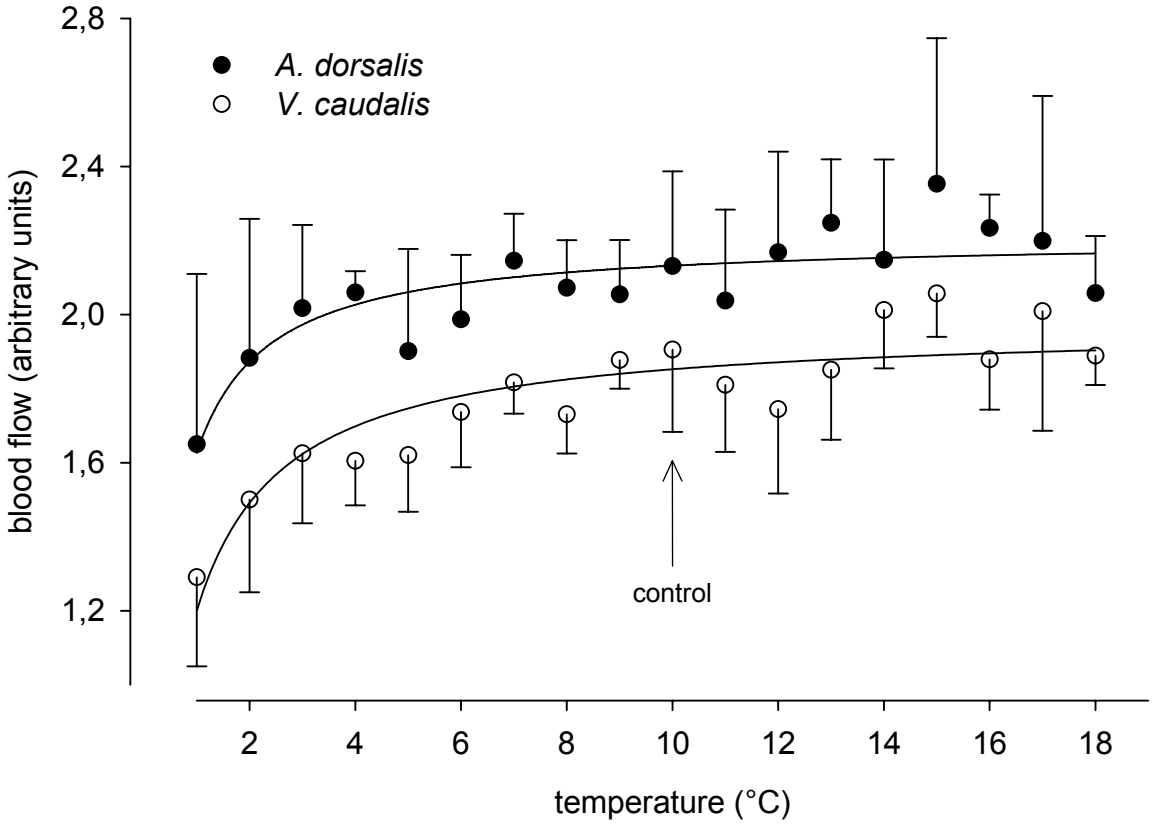

2,8
2,4
2,0
1,6
1,2
blood flow (arbitrary units)
A. dorsalis
V. caudalis
control
2
4
6
8
10
12
14
16
18
temperature (°C)

figure 5

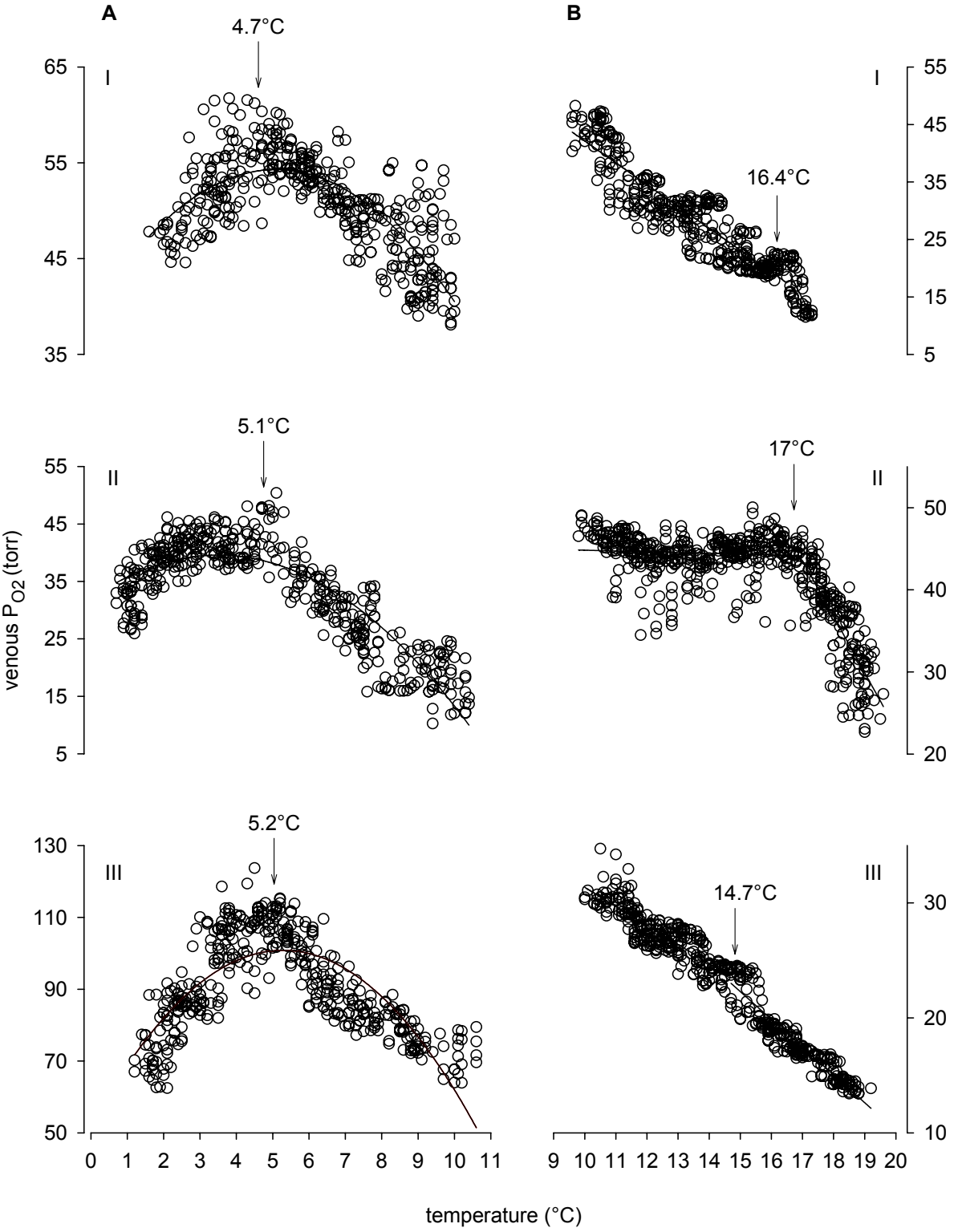
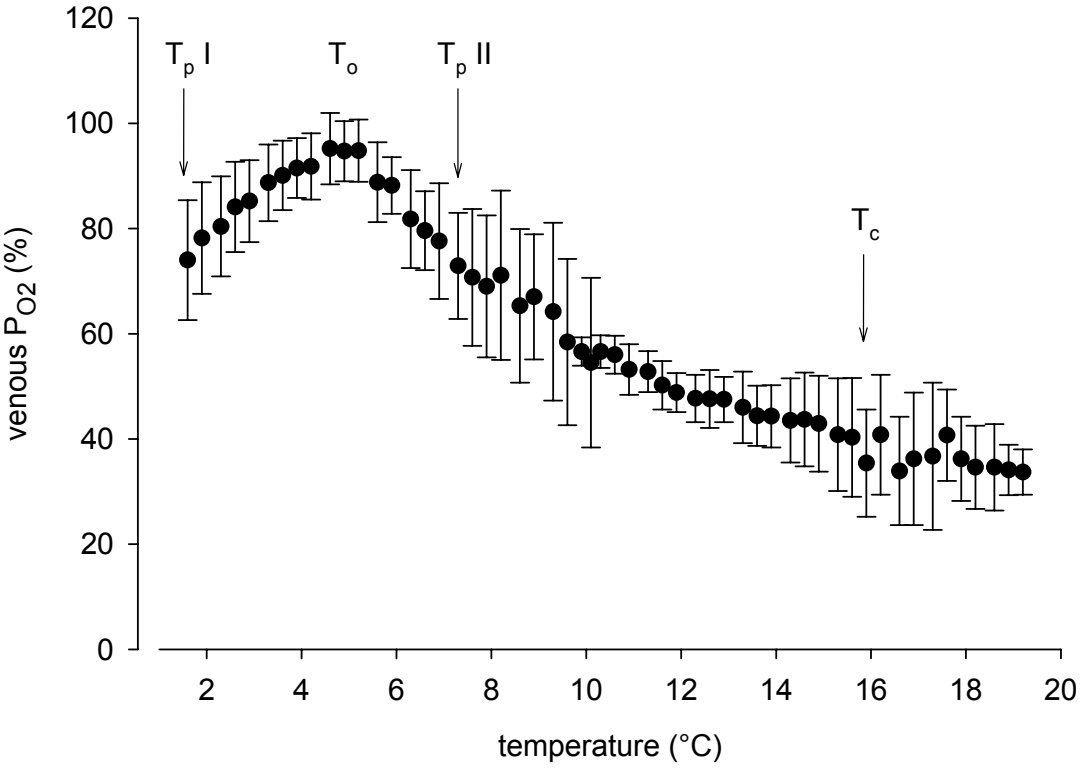

A
B
4.7°C
16.4°C
5.1°C
17°C
5.2°C
14.7°C
I
II
III
venous P_{O2} (torr)
temperature (°C)

figure 6


120
100
80
60
40
20
0
venous P_{O2} (%)
T_p I
T_o
T_p II
T_c
2
4
6
8
10
12
14
16
18
20
temperature (°C)

4. Diskussion

In der abschließenden Diskussion werden die Ergebnisse der Publikationen in ihrer Gesamtheit diskutiert und durch weitere, unveröffentlichte Daten ergänzt. Eine detaillierte Diskussion der Einzelergebnisse ist in den entsprechenden Publikationen zu finden.

Die Anpassungsmechanismen des aeroben Stoffwechsels während der Akklimatisation (Anpassung von Organismen an einen einzigen Faktor unter Laborbedingungen) und der Adaptation (evolutionsbedingte Anpassung) an unterschiedliche Temperaturen auf mitochondrialer und enzymatischer Ebene werden zusammenfassend dargestellt und im Hinblick auf Energiehaushalt und Verbreitungsgrenzen der untersuchten Tiere diskutiert. Weiterhin werden die Ergebnisse des temperaturabhängigen Herz-Kreislauf-Systems von *G. morhua* der südlichen Nordsee im Hinblick auf thermale Toleranzgrenzen und mögliche Konsequenzen der globalen Erwärmung auf seine Verbreitungsgebiete diskutiert. Abschließend wird das Toleranzmodell von Frederich und Pörtner (2000) zur Interpretation der Temperaturanpassung in marinen Fischen modifiziert.

4.1 Einfluss der Temperatur auf die aerobe Kapazität

Niedrige Umgebungstemperaturen führen bei ektothermen Organismen zu einer verminderten Stoffwechselrate und erfordern eine entsprechende Anpassung des Metabolismus. In ektothermen Organismen geht die Anpassung an niedrige Temperaturen mit einer Erhöhung der Kapazität zur aeroben Energiegewinnung einher (siehe Übersichtsartikel von Prosser 1991, Guderley 1998, Pörtner et al. 1998).

Im Muskelgewebe wurde eine spezifische Mitochondrienproliferation sowohl in kaltadaptierten Arten der südlichen und nördlichen Hemisphäre (Londraville und Sidell 1990; Johnston et al. 1998; Sommer und Pörtner 2002) als auch in kaltakklimatisierten, eurythermen Arten (Campbell und Davies 1978; Tylor und Sidell 1984; Egginton und Sidell 1989) nachgewiesen. Eine kälteinduzierte Steigerung der aeroben Kapazität in der Leber scheint hingegen über eine Größenzunahme des gesamten Organs zu erfolgen (Publikation I & III; Kent et al. 1988; Seddon und Prosser 1997).

4.1.1 Hepatosomatischer Index und Proteingehalt

Der hepatosomatische Index (I_H = Verhältnis von Leber- zu Tiergewicht * 100) von kalt gehälterten borealen und polaren Tieren war im Vergleich zu warm gehälterten Tieren bei moderater Fütterung erhöht (Abb. 9 A & B). Diese neueren Ergebnisse am Kabeljau scheinen die in Publikation I geäußerte Vermutung zu bestätigen, dass eine *ad libitum* Fütterung mit

fetthaltiger Nahrung wie z.B. Sprotte beim Nordseekabeljau während des Wachstumsexperiments einen Temperatureffekt auf den hepatosomatischen Index maskieren kann (Abb. 9 C).

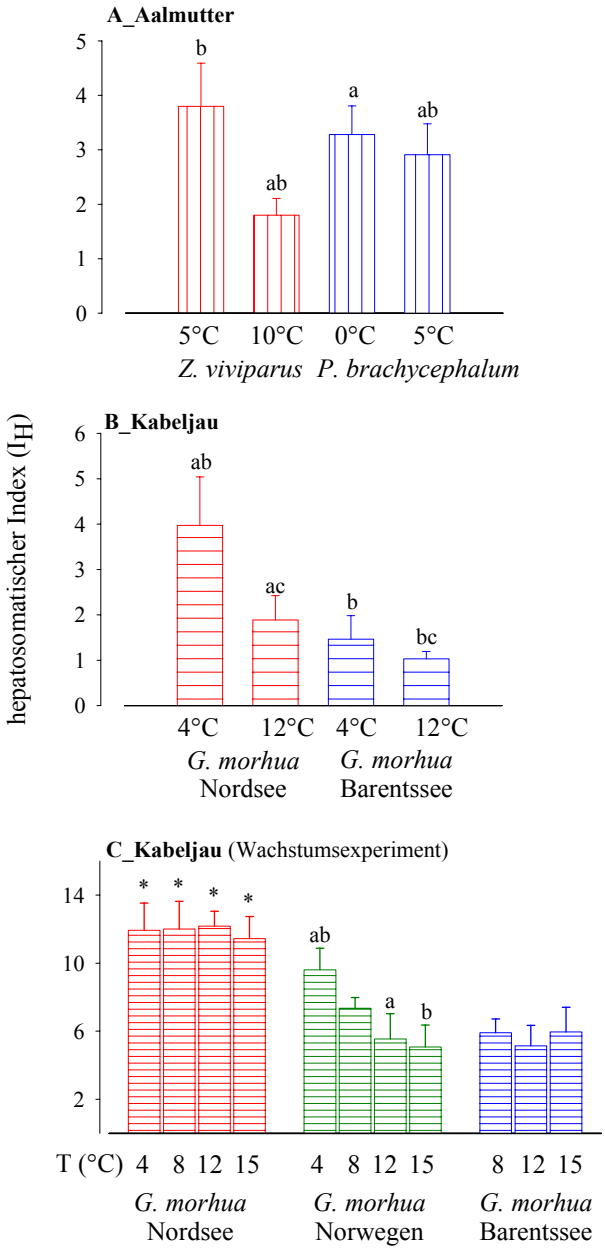


Abb. 9 Hepatosomatischer Index (I_H), normalisiert auf eine jeweils einheitliche Tiergröße von Aalmutter (**A**: *Z. viviparus* (n=5-13), *P. brachycephalum* (n=19-20) auf ein 40 g Tier) und Kabeljau (**B&C**: *G. morhua* der Nordsee (NC, n=4-8), der Norwegischen Küste (NCC, n=6-9) und der Barentssee (NEAC, n=4-7) auf ein 1,2 kg Tier) bei verschiedenen Hälterungstemperaturen. In der Kälte ergaben sich höhere Werte innerhalb einer Art bzw. Population. Bei gleicher Hälterungstemperatur lagen die I_H-Werte borealer jedoch oberhalb der von polaren Tieren. (Abb. 9 A & C verändert nach Publikation III bzw. I)
Gekennzeichnete Daten mit gleichem Buchstaben sind signifikant unterschiedlich.
* kennzeichnet signifikanten Unterschied zwischen den Daten von NC und denen von NCC und NEAC bei gleicher Akklimatisationstemperatur.

Die beim Wachstumsexperiment fehlende 4°C-Akklimatisation von Kabeljau aus der Barentssee wurde am AWI (Bremerhaven) nachgeholt und ergab einen Temperatureffekt auf

den hepatosomatischen Index der Tiere, der bei dem Wachstumsexperiment zwischen den 8°C, 12°C und 15°C akklimatisierten Tieren nicht gefunden wurde (siehe Abb. 9 B & C).

Die Zunahme der Lebergröße in der Kälte kann an einer erhöhten Durchschnittsgröße der Zellen liegen, wie sie in kaltakklimatisierten Goldfischen gefunden wurde (Das 1967) oder an einer Zunahme der Zellanzahl, wie in kaltakklimatisierten Aalen (Jankowsky et al. 1984). Welches Phänomen bei Aalmuttern und Kabeljau eintritt, bleibt zu untersuchen. Nach F. Mark scheint jedoch *P. brachycephalum* im Vergleich zu *Z. viviparus* größere Leberzellen zu besitzen (pers. Mitteilung).

Die kälteinduzierte Erhöhung des mitochondrialen Proteingehalts in der Leber aufgrund einer Größenzunahme des gesamten Organs ist in Abbildung 10 zu sehen. Es zeigte sich, dass trotz eines höheren hepatosomatischen Index von borealen Tieren die polaren Tiere bei gleicher Akklimatisationstemperatur einen höheren, zumindest aber einen vergleichbaren mitochondrialen Proteingehalt hatten. Da sich die Gesamtproteingehalte zwischen den Kabeljaupopulationen nicht unterschieden (Abb. 10 C), geht eine Kälteadaptation vermutlich mit einer Verschiebung des hepatischen Stoffwechsels zu einer erhöhten mitochondrialen Kapazität einher. Die kaltstenotherme antarktische Aalmutter *P. brachycephalum* war in der Lage ihren hepatischen mitochondrialen Proteingehalt im selben Maße wie die boreale eurytherme *Z. viviparus* an unterschiedliche Temperaturen anzupassen (Abb. 10 A). Im Gegensatz dazu hatte die kaltadaptierte eurytherme Kabeljaupopulation einen höheren mitochondrialen Proteingehalt im Vergleich zu den borealen Tieren und scheinen nur bis zu einem gewissen Grad ihre mitochondriale Kapazität an höhere Temperaturen anpassen zu können (Abb. 10 B).

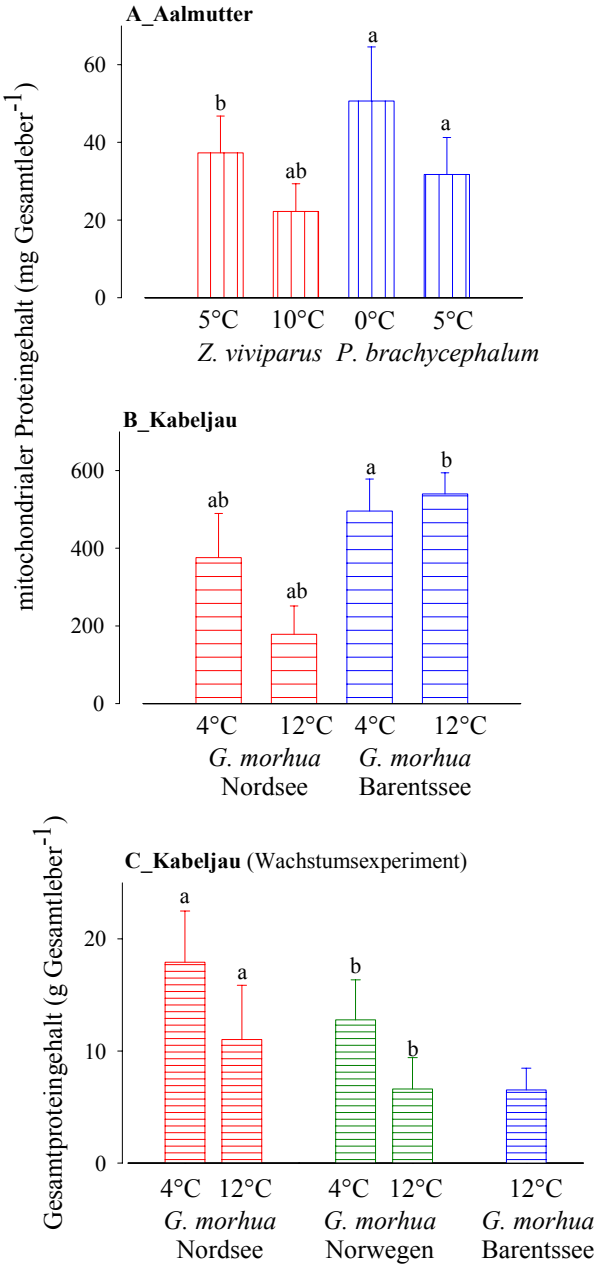


Abb. 10 Proteingehalt der Gesamtleber von Aalmutter (**A**: *Z. viviparus* (n=4), *P. brachycephalum* (n=7-8), normalisiert auf ein 40 g Tier) und Kabeljau (**B&C**: *G. morhua* der Nordsee (NC. n=4-8), der Norwegischen Küste (NCC, n=4-9) und der Barentssee (NEAC, n=3-7), normalisiert auf ein 1,2 kg Tier) bei verschiedenen Hälterungstemperaturen. In der Kälte ergaben sich höhere Proteingehalte in der Leber innerhalb einer Art bzw. Population. Die polaren Tiere hatten im Vergleich zu den borealen Tieren einen erhöhten mitochondrialen Proteingehalt (A&B), hingegen unterschied sich der Gesamtproteingehalt der verschiedenen Kabeljaupopulationen bei gleicher Akklimatisationstemperatur nicht (C). Dieser Befund deutet auf eine Verschiebung des hepatischen Stoffwechsels zu einer erhöhten mitochondrialen Kapazität hin. (Abb. 10 A & C verändert nach Publikation III bzw. I)
Gekennzeichnete Daten mit gleichem Buchstaben sind signifikant unterschiedlich.

4.1.2 Mitochondriale Respiration

Eine Erhöhung der Mitochondriendichte während der Kälteakklimatisation und -adaptation führt zu einer Kapazitätssteigerung der aeroben Energiegewinnung in der Gesamtleber (Abb. 11 A), allerdings stieg auch der Anteil des mitochondrialen

Sauerstoffverbrauchs, welcher durch Protonen-Leckströme (*proton leakage*) verursacht wird (Abb. 11 B). Dabei zeigten die kaltakklimatisierten eurythermen Tiere im Vergleich zu den kaltadaptierten stenothermen Tieren, bei vergleichbaren mitochondrialen Proteingehalten, eine höhere Kältekompensation der aeroben ATP-Synthese-Kapazität, jedoch vergleichbares *proton leakage*.

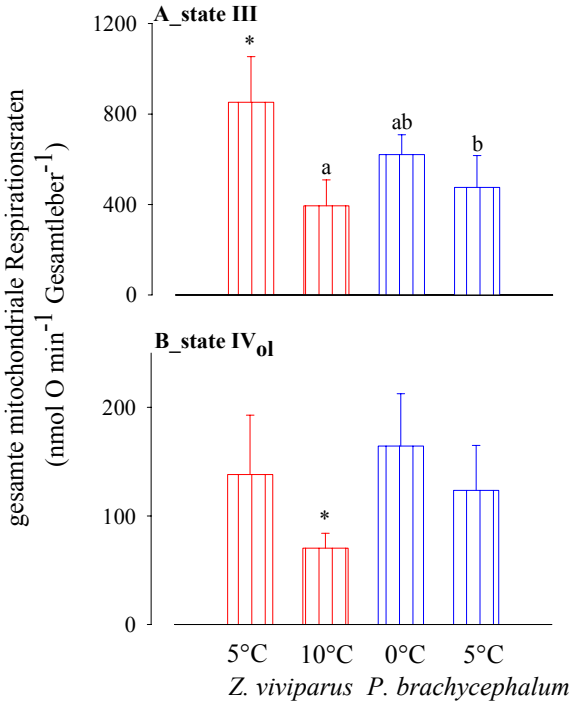


Abb. 11 Mitochondriale Respirationsraten, oxidative Phosphorylierung (state III, **A**) und *proton leakage* (state IV_{ol}, **B**) der Gesamtleber (normalisiert auf ein 40 g Tier und gemessen bei 10°C) von *Z. viviparus* (Nordsee) bei Kontroll- (10°C; n=3-5) und Akklimatisationstemperatur (5°C; n=4-5) und *P. brachycephalum* (Antarktis) bei Kontroll- (0°C; n=4-6) und Akklimatisationstemperatur (5°C; n=4-8). In der Kälte ergaben sich signifikant erhöhte Phosphorylierungsraten innerhalb beider Arten, wohingegen ein signifikanter Temperatureffekt nur auf die *proton leakage*-Raten von *Z. viviparus* zu erkennen ist. Die niedrigsten Werte wurden jeweils in der Kontrollgruppe der borealen Art gefunden. (Abbildung verändert nach Publikation III)
Gekennzeichnete Daten mit gleichem Buchstaben sind signifikant unterschiedlich. * kennzeichnet signifikanten Unterschied zu allen anderen Daten.

Dieser Unterschied kann mit der erhöhten spezifischen oxidativen Phosphorylierung auf mitochondrialer Ebene von *Z. viviparus* im Vergleich zu *P. brachycephalum* erklärt werden, wohingegen sich kein Unterschied im spezifischen *proton leakage* fand (Publikation III, Fig. 4). Das Fehlen einer kältekompensierten aeroben ATP-Synthese-Kapazität auf mitochondrialer Ebene wurde schon im Muskelgewebe von Fischen im latitudinalen Vergleich (größtenteils südliche Hemisphäre) nachgewiesen (Johnston et al. 1994, 1998; Guderley 1998).

Der gefundene Unterschied zwischen kaltadaptierten Stenothermen (Antarktis) und kaltadaptierten Eurythermen (Arktis und Subarktis) hinsichtlich einer Verminderung ihres

mitochondrialen Proteingehalts während einer Wärmeakklimatisation spiegelt sich auch in der Anpassungsfähigkeit ihrer mitochondrialen Respiration wider. Denn im Gegensatz zur antarktischen Aalmutter hatte kaltadaptierter Kabeljau sowohl eine erhöhte spezifische ATP-Synthese-Kapazität als auch ein erhöhtes spezifisches *proton leakage* im Vergleich zu den Tieren aus der Nordsee (Fischer 2002). Diese Befunde sind in Übereinstimmung mit der von Pörtner et al. (2000) getroffenen Vermutung, dass kaltadaptierte Eurytherme im Vergleich zu kaltadaptierten Stenothermen eine höhere Phosphorylierungskapazität als kaltakklimatisierte Eurytherme besitzen. Die variablen Habitattemperaturen der nördlichen Breiten führen möglicherweise zu einer erhöhten ATP-Synthese-Kapazität einhergehend mit einem höheren Energiebedarf bei kaltadaptierten Eurythermen.

Eine Überkompensation der ATP-Synthese, wie sie bei kaltakklimatisierter *Z. viviparus* gefunden wurde (Abb. 11 A) führt jedoch nicht unweigerlich zu einem höheren Standardstoffwechsel als bei *P. brachycephalum*. Dieser Schluss stützt sich auf Befunde von van Dijk et al. (1999), die zwischen kaltakklimatisierter *Z. viviparus* (0°C) und kaltadaptierter *P. brachycephalum* (0°C) keinen Unterschied im Standardstoffwechsel (µmol O_2 min^{-1} 100g $Aalmutter^{-1}$) fanden, wohingegen warmakklimatisierte *Z. viviparus* die niedrigsten Werte hatte. Unter der Berücksichtigung, dass der durch *proton leakage* verursachte Sauerstoffverbrauch zwischen 20 und 50% zum Standardstoffwechsel eines Tieres beitragen kann (Brand 1990; Brookes et al. 1998) und dieser in der Kälte aufgrund der gesteigerten Mitochondriendichte zunimmt (Abb. 11 B), könnte der erhöhte Standard-Metabolismus von Tieren in der Kälte größtenteils auf erhöhtes *proton leakage* zurückgeführt werden. Eine Annahme, die aufgrund der ebenfalls ähnlichen *proton leakage*-Raten zwischen den kaltakklimatisierten und kaltadaptierten Zoarciden getroffen wurde (Abb. 11 B). Weiterhin sprechen die Befunde von Fischer (2002) für diese Vermutung. In dessen Arbeit wurden sowohl erhöhtes *proton leakage* als auch erhöhter Standardstoffwechsel bei kaltadaptierten Kabeljau der Barentssee im Vergleich zu Kabeljau der Nordsee gefunden.

Es wird vermutet, dass kaltadaptierte Stenotherme der Antarktis im Gegensatz zu kaltadaptierten und kaltakklimatisierten Eurythermen den Sauerstoffverbrauch über *proton leakage* evolutionsbedingt vermindern konnten und deshalb keine Erhöhung des Standardstoffwechsels im Sinne der *metabolic cold adaptation* zeigen (Pörtner et al. 1998, 2000). Die vorliegende Untersuchungen an Zoarciden ergab keine Reduzierung des *proton leakage* in der antarktischen Aalmutter im Vergleich zur borealen Aalmutter und deutet möglicherweise darauf hin, dass *P. brachycephalum* aufgrund ihrer bodenlebenden trägen Lebensweise und eines damit einhergehenden Hypometabolismus, eine Minimierung des

Sauerstoffverbrauchs über *proton leakage* nicht benötigt, wie sie bei aktiveren Notothenioiden gefunden wurde (Hardewig et al. 1999b). Unter Berücksichtigung, dass Leben in warmen Gewässern vermutlich den evolutionären Ursprung darstellt (Arntz et al. 1994) kann nicht ausgeschlossen werden, dass sich *P. brachycephalum* einige Merkmale eines kaltadaptierten, aber hypometabolischen eurythermen Organismus bewahrt hat.

4.1.3 Mitochondriale Enzymaktivität

Ein erhöhter Mitochondriengehalt impliziert auch eine erhöhte Konzentration an mitochondrialen Enzymen und somit eine gesteigerte Enzymaktivität. Erhöhte Enzymaktivitäten im Muskel wurden in kaltakklimatisierten Fischen in Arbeiten von Foster et al. (1993), Cordiner und Egginton (1997), Hardewig et al. (1999a) und Guderley und Leroy (2001) sowie in kaltadaptierten Fischen bei Torres und Somero (1988) und Crockett und Sidell (1990) gefunden. Untersuchungen an Muskelgewebe von *G. morhua* verschiedener Populationen aus dem Wachstumsexperiment ergaben ebenfalls höhere Aktivitäten von Citrat-Synthase (CS), Cytochrom c-Oxidase (CCO) und von Enzymen des Elektronen-Transport-Systems (ETS) sowohl in den kalt- versus warmakklimatisierten Tieren einer Population als auch in den nördlichen Populationen im Vergleich zur borealen Population (Publikation I, Fig. 3).

Bei Untersuchungen zur Temperaturanpassung aerober Enzyme in der Leber ist im Gegensatz zum Muskelgewebe kein so eindeutiges Bild zu erkennen. In kalt- versus warmakklimatisierten Fischen wurde eine positive Kompensation der CS, jedoch unveränderte CCO-Aktivität in der Gesamtleber (Kent et al. 1988) und pro g Leber (Lucassen et al. 2003) gefunden. Unveränderte oder sogar eine verminderte spezifische CCO-Aktivität pro g Leber konnte nach einer Kälteakklimatisation beim Goldfisch *Carassius auratus* (van den Thillart und Modderkolk 1978), bei der Goldorfe *Leuciscus idus melanotus* (Rafael und Braunbeck 1988) und bei der Aalmutter *Z. viviparus* (Hardewig et al. 1999a) nachgewiesen werden. Ähnliche Ergebnisse fanden sich bei den Untersuchungen in der Leber der verschiedenen Kabeljaupopulationen. Bei allen Populationen wurden in der Leber eine kältekompensierte CS-, hingegen unveränderte CCO- (im Falle von NEAC sogar verminderte CCO) und unveränderte ETS-Aktivitäten gefunden (Publikation I, Fig. 5). Im Populationsvergleich hatten die Tiere der nördlichen Breitengrade im Vergleich zur borealen Population erhöhte CS-Aktivitäten bei allen Akklimatisationstemperaturen, wohingegen erhöhte CCO- und ETS-Aktivitäten nur bei den 15°C-akklimatisierten Tieren nachgewiesen werden konnte.

Eine kälteinduzierte Erhöhung der Citrat-Synthase-Aktivität zeigte sich ebenfalls bei der Aalmutter und bei den moderat gefütterten Kabeljaupopulationen (siehe Abb. 12).

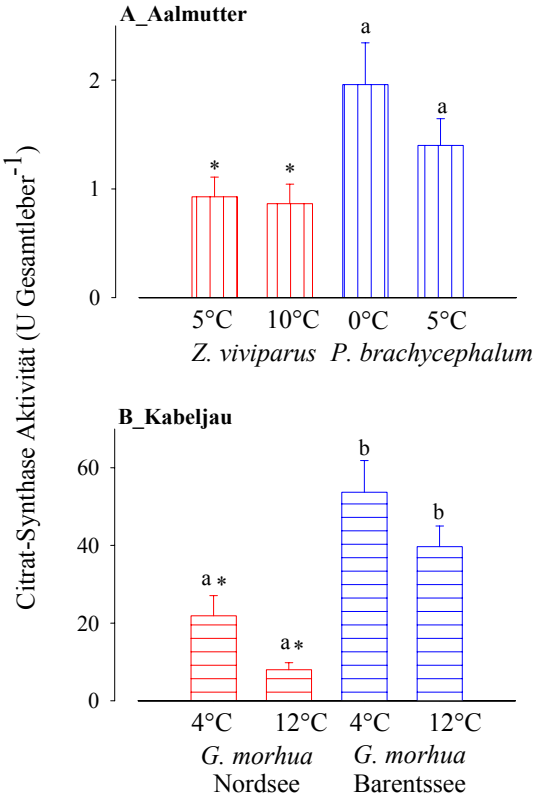


Abb. 12 Aktivität der Citrat-Synthase in der Gesamtleber von Aalmutter (**A**: *Z. viviparus* (n=4-6) und *P. brachycephalum* (n=6-9), normalisiert auf ein 40 g Tier und bei 10°C gemessen) und Kabeljau (**B**: *G. morhua* der Nordsee (NC, n=4-8) und der Barentssee (NEAC, n=3-8), normalisiert auf ein 1,2 kg Tier und bei 12°C gemessen) bei verschiedenen Hälterungstemperaturen. In der Kälte ergaben sich erhöhte CS-Aktivitäten innerhalb einer Art bzw. Population (nicht für *Z. viviparus*). Kaltadaptierte Tiere zeigten im Vergleich zu borealen Tieren eine höhere CS-Aktivität. (Abbildung 12 A verändert nach Publikation III).
Gekennzeichnete Daten mit gleichem Buchstaben sind signifikant unterschiedlich. * kennzeichnet signifikanten Unterscheid zwischen den Daten der borealen Tiere und denen der polaren Tiere.

Diese Ergebnisse lassen erkennen, dass eine Erhöhung der Enzymaktivität nicht allein auf einen kälteinduzierten, erhöhten mitochondrialen Proteingehalt der Leber zurückzuführen sein muss (zur Erinnerung: Kabeljau aus der Barentssee zeigte keinen Unterschied zwischen kalt- und warmakklimatisierten Tieren). Eine temperaturbedingte Anpassung der Enzymaktivität kann ebenso auf mitochondrialer Ebene geschehen (siehe Tabelle 2). Die bei 4°C gehälterte Kabeljaupopulation der Barentssee wie auch der Nordsee hatte eine höhere CS-Aktivität pro mg mitochondriales Protein als die bei 12°C gehälterten Tiere. Weiterhin zeigte sich, dass eine Temperaturanpassung der spezifischen Citrat-Synthase-Aktivität möglicherweise artspezifisch ist, da kaltakklimatisierte *Z. viviparus* keine höhere, sondern tendenziell sogar eine niedrigere spezifische CS-Aktivität hatte als die Tiere der Kontrollgruppe. Eine unveränderte spezifische Citrat-Synthase-Aktivität mit der Temperatur

wurde auch in isolierten Muskelmitochondrien der Regenbogenforelle, *Oncorhynchus mykiss* nachgewiesen (Bouchard und Guderley 2003). Hingegen hatte *P. brachycephalum* eine gesteigerte spezifische CS-Aktivität in der Kälte. Ebenso zeigten die polaren Tiere im Vergleich zu den borealen Tieren erhöhte Werte bei gleicher Akklimatisationstemperatur.

Tabelle 2 Spezifische Citrat-Synthase-Aktivität (units pro mg mitochondriales Protein) in der Leber verschiedener Kabeljaupopulationen, *G. morhua* und Aalmutterarten, *Z. viviparus* und *P. brachycephalum* bei unterschiedlichen Hälterungstemperaturen. (Daten wurden bei 12°C (Kabeljau) und 10°C (Aalmutter) bestimmt. Gekennzeichnete Daten mit gleichem Buchstaben sind signifikant unterschiedlich)

Population bzw. Art	Kälteakklimatisation	Wärmeakklimatisation
Kabeljau		
G. morhua (Nordsee, n=4)	**4°C**: 0,061 ± 0,011^{a}	**12°C**: 0,048 ± 0,012^{ab}
G. morhua (Barentssee, n=4)	**4°C**: 0,112 ± 0,035^{ab}	**12°C**: 0,069 ± 0,008^{b}
Aalmutter		
Z. viviparus (Nordsee, n=3-6)	**5°C**: 0,017 ± 0,007^{c}	**10°C**: 0,025 ± 0,008^{d} (Kontrolle)
P. brachycephalum (Antarktis, n=5-8)	**0°C**: 0,050 ± 0,015^{cd} (Kontrolle)	**5°C**: 0,039 ± 0,009^{c}

Eine gesteigerte spezifische Enzymaktivität kann durch eine Absenkung der Aktivierungsenergie für die Entstehung des Enzym-Substrat-Komplexes erfolgen. Da sich die Aktivierungsenergien weder zwischen den unterschiedlich gehälterten Tieren einer Art bzw. Population noch zwischen den Arten bzw. Populationen unterschieden (siehe Publikation I & III), könnte die gesteigerte CS-Aktivität in der Kälte an einer erhöhten Enzymmenge pro Mitochondrium liegen, was jedoch noch zu untersuchen wäre.

Überraschend waren die vergleichbaren Citrat-Synthase-Aktivitäten in der Gesamtleber zwischen kaltakklimatisierter (5°C) und bei 10°C gehälterter (Kontrolle) *Z. viviparus* (Abb. 12 A). Dieser Befund steht im Gegensatz zu den Ergebnissen von Lucassen et al. (2003), die höhere CS-Aktivität pro g Leber bei kalt- (3,5°C) gegenüber warmakklimatisierter (10°C) *Z. viviparus* nachwiesen. Möglicherweise tritt eine kältekompensierte CS-Aktivität erst bei einer Hälterung unter 5°C ein. Da Lucassen et al. (2003) im Gegensatz zu der vorliegenden Arbeit keine kälteinduzierte Erhöhung des hepatosomatischen Index (I_H) gefunden hatte, könnte eine Langzeit-Kälteakklimatisation (10 Monate in dieser Arbeit im Vergleich zu 25 Tagen in deren Arbeit) zu einer Reduktion der

spezifischen CS-Kapazität einhergehend mit einem Anstieg im I_H führen.

Bei der Untersuchung zur Temperaturanpassung der $NADP^+$-abhängigen Isocitrat-Dehydrogenase (IDH) ergab der Populations- bzw. Artenvergleich höhere IDH-Aktivitäten in polaren als in borealen Tieren, wobei zwischen den bei 5°C akklimatisierten Aalmutterarten nur ein tendenzieller Unterschied zu erkennen war (Abb. 13). Im Falle der IDH-Kapazität scheint es Unterschiede in der Kältekompensation zwischen eurythermen und stenothermen Tieren zu geben, da *G. morhua* und *Z. viviparus* im Vergleich zu *P. brachycephalum* keinen Unterschied in der IDH-Kapazität der Gesamtleber in kalt- versus warmakklimatisierten Exemplaren zeigten. Da sowohl *Z. viviparus* als auch *G. morhua* aus der Nordsee nach der Kälteakklimatisation erhöhte mitochondriale Proteingehalte in der Leber hatten, deuten die ähnlichen IDH-Aktivitäten auf eine spezifische Regulierung des Enzyms auf mitochondrialer Ebene hin.

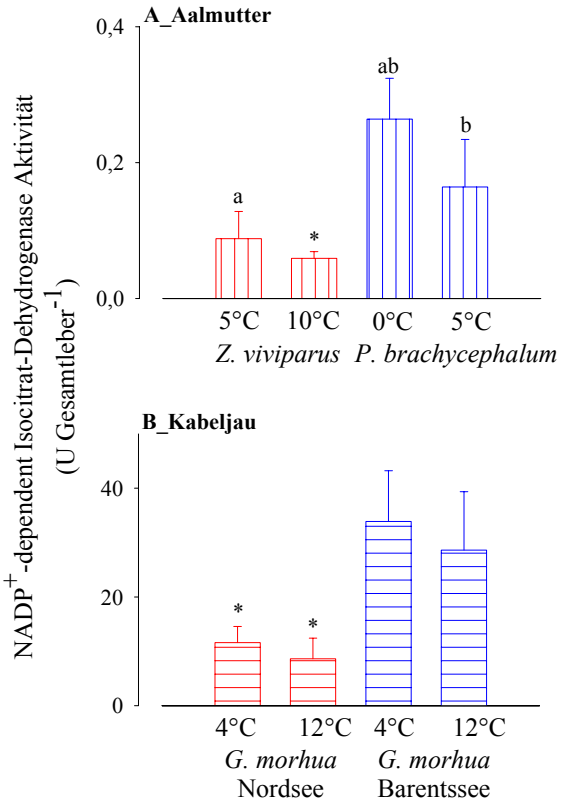


Abb. 13 Aktivitäten der $NADP^+$-abhängigen Isocitrat-Dehydrogenase in der Gesamtleber von Aalmutter (**A**: *Z. viviparus* (n=4) und *P. brachycephalum* (n=7-8), normalisiert auf ein 40 g Tier und bei 10°C gemessen) und Kabeljau (**B**: *G. morhua* der Nordsee (n=4) und der Barentssee (n=3-4), normalisiert auf ein 1,2 kg Tier und bei 12°C gemessen) bei verschiedenen Hälterungstemperaturen. Im Gegensatz zur stenothermen *P. brachycephalum* hatte die Temperatur bei den eurythermen Tieren, *Z. viviparus* und *G. morhua* keinen Effekt auf die IDH-Kapazität der Leber. Die polaren hatten im Vergleich zu den borealen Tieren eine erhöhte IDH-Kapazität. (Abbildung 13 A verändert nach Publikation III).
Gekennzeichnete Daten mit gleichem Buchstaben sind signifikant unterschiedlich. * kennzeichnet signifikanten Unterscheid zwischen Daten der borealen Tiere zu denen der polaren Tiere.

Im Gegensatz zu den polaren Tieren wurde bei den borealen Tieren eine tendenziell niedrigere IDH-Aktivität pro mg mitochondriales Protein bei den kaltakklimatisierten Exemplaren gefunden, die zu einer verminderten Kältekompensation auf der Ebene der Gesamtleber führten (siehe Tabelle 3). Wie schon bei der Citrat-Synthase wurde auch bei der IDH kein Unterschied in den Aktivierungsenergien gefunden (siehe Publikation III und CLICOFI-Zwischenbericht 2001). Eine zu untersuchende Erklärung für die erhöhte IDH-Aktivität von *P. brachycephalum* wäre, dass unterschiedliche Isoformen des Enzyms zwischen den stenothermen und den eurythermen Tieren existieren. So fanden Hummel et al. (1997) in ihrer Untersuchung an Populationen vom Wattwurm *Arenicola marina* im longitudinalen Gradienten eine Korrelation zwischen dem Vorkommen bestimmter IDH-Isoformen und der durchschnittlichen Jahrestemperatur im Lebensraum der jeweiligen Population.

Tabelle 3 Spezifische $NADP^+$-abhängige Isocitrat-Dehydrogenase Aktivität (units pro mg mitochondriales Protein) in der Leber verschiedener Kabeljaupopulationen (*G. morhua*) und Aalmutterarten (*Z. viviparus* und *P. brachycephalum*) bei unterschiedlichen Hälterungstemperaturen. (Daten wurden bei 12°C (Kabeljau) und 10°C (Aalmutter) ermittelt).

Population bzw. Art	Kälteakklimatisation	Wärmeakklimatisation
boreal		
G. morhua (Nordsee, n=4)	**4°C:** 0,029 ± 0,008	**12°C:** 0,048 ± 0,012
Z. viviparus (Nordsee, n=3-6)	**5°C:** 0,003 ± 0,001	**10°C:** 0,004 ± 0,001 (Kontrolle)
polar		
G. morhua (Barentssee, n=3-4)	**4°C:** 0,071 ± 0,032	**12°C:** 0,049 ± 0,014
P. brachycephalum (Antarktis, n=5-8)	**0°C:** 0,006 ± 0,001 (Kontrolle)	**5°C:** 0,005 ± 0,001

Vor allem polare Tiere haben im Vergleich zu kaltakklimatisierten borealen Exemplaren höhere Enzymaktivitäten pro mg mitochondriales Protein. Dies könnte ein Hinweis auf adaptive Mechanismen zur Kältekompensation auf enzymatischer Ebene sein. Nach einer These von Pogson (1988) läuft die biochemische Temperaturanpassung generell in zwei Schritten ab: 1. durch Änderung der Enzymmenge und 2. durch Modulation der katalytischen Eigenschaften von Enzymen. Letzteres kann durch Änderungen der näheren Umgebung des Enzyms hervorgerufen werden, z. B. durch die Modulation von

Membraneigenschaften (Prosser 1991; Miranda und Hazel 1996), durch temperaturbedingte Unterschiede der Enzymfaltung (Hochachka und Somero 1984) oder durch die Expression verschiedener Isoenzyme (Hochachka und Somero 1984; Prosser 1991; Hummel et al. 1997). Welcher Mechanismus bei den polaren Tieren die Aktivitätsänderung der Enzyme verursacht, muss jedoch noch geklärt werden.

Die Befunde zeigten weiterhin, dass während einer Temperaturänderung mitochondriale Matrixenzyme (Citrat-Synthase (CS) und $NADP^+$-abhängige Isocitrat-Dehydrogenase (IDH)) im Vergleich zu Membranenzymen (Cytochrom c-Oxidase (CCO) und Enzymen des Elektronen-Transport-Systems) stärker reguliert werden und möglicherweise eine funktionelle Änderung in den Lebermitochondrien eintritt. Lucassen et al. (2003) wiesen nach, dass das Verhältnis von CS-Aktivität zu CCO-Aktivität in kaltadaptierter *P. brachycephalum* und kaltakklimatisierter *Z. viviparus* größer war als in warmakklimatisierter *Z. viviparus*, wobei die antarktische Art den höchsten Wert zeigte. Die erhöhte CS-Kapazität in kaltakklimatisierten, vor allem jedoch in kaltadaptierten Tieren deutet möglicherweise auf eine Verschiebung des Stoffwechsels in Richtung der Lipidbiosynthese hin (siehe Übersichtsartikel Pörtner 2002a,b). Mitochondrien sind neben dem Energiestoffwechsel auch an anabolischen Prozessen beteiligt. Das über die Citrat-Synthase gebildete Citrat dient dann dem Transport von Acetylgruppen aus den Mitochondrien zur Fettsäuresynthese in das Cytosol. Durch eine Modifizierung der Lipidzusammensetzung kann die physiologische Effizienz biologischer Membranen bei verschiedenen Habitattemperaturen aufrechterhalten werden, wie von Cossins und Sinensky (1986) als homöoviskose Adaptation (*homeoviscous adaptation*) bezeichnet (siehe auch Übersichtsartikel Storelli et al. 1998). Es wird vermutet, dass der hohe Lipidgehalt polarer Organismen eine Anpassung an verminderte Diffusionsgeschwindigkeiten bei kalten Umgebungstemperaturen darstellt, da die Diffusionskonstante für Sauerstoff in Lipiden etwa 4,4-mal höher ist als im wässrigen Cytoplasma (Londraville und Sidell 1990). Eine neuere Arbeit von Pörtner et al. (2003 eingereicht) deutet hingegen an, dass die erleichterte Sauerstoffdiffusion aufgrund des erhöhten Lipidgehaltes ein Nebenprodukt des zur Lipidbiosynthese verschobenen Stoffwechsels bei polaren Tieren darstellt.

Die erhöhte IDH-Kapazität der polaren Tiere könnte ebenfalls an einer Kältekompensierung anderer Prozesse als der aeroben Kapazität liegen, da das gebildete α-Ketoglutarat neben seiner Funktion im Citratzyklus für die Bildung von Aminosäuren nötig ist, die wiederum Bausteine für Peptide und Proteine sind.

4.2 Kosten der Kältekompensation

Beim Kabeljau werden Energiereserven hauptsächlich in der Leber als Lipid gespeichert (Love 1970, Lambert und Dutil 1997). Messungen des Lipidgehalts der Leber könnten somit einen Hinweis liefern, ob eine Kälteakklimatisation bzw. –adaptation zu erhöhten Kosten im Tier führt. In der Tat hatte kaltakklimatisierter Nordseekabeljau einen geringeren hepatischen Lipidgehalt als die bei 12°C akklimatisierten Tiere (Publikation I, Fig. 7 A). Ein Populationsvergleich ist aufgrund der unterschiedlichen Fütterung der Tiere erschwert (siehe Publikation I) und daher nicht aussagekräftig.

Clarke und North (1991) und Clarke und Peck (1991) vermuteten, dass neben der Temperatur vor allem die Nahrungsverfügbarkeit das Wachstum limitiert. Im Wachstumsexperiment mit ausreichender Fütterung (*ad libitum*) wurden jedoch verminderte temperaturspezifische Wachstumsraten von *G. morhua* mit zunehmender geographischer Breite der Verbreitungsgebiete gefunden, und populationsunabhängiges maximales Wachstum lag zwischen 10°C und 11°C (Pörtner et al. 2001; Fischer 2002). Fonds et al. (1989) ermittelten für *Z. viviparus* maximale Längenzuwachsraten bei 15°C. Ober- und unterhalb dieser Temperatur nahm das Wachstum der Tiere ab. Dies deutet auf einen kostenintensiveren Stoffwechsel in der Kälte hin. Vor allem bei Organismen der nördlichen Breitengrade werden erhöhte Energiekosten aufgrund der Eurythermie vieler arktischer und subarktischer Arten vermutet (Pörtner et al. 2000, 2001; Pörtner 2002a,b). Eine Erhöhung der Mitochondriendichte und die Kosten erhöhter aerober Enzymkapazität in der Kälte führen zu einem Anstieg im Stoffwechsel. Solche Anpassungsmechanismen können den Energiehaushalt verschieben und somit die Kapazitäten von Energiespeicherung, Wachstum und Reproduktion verringern. Vor allem der kälteinduzierte erhöhte Sauerstoffverbrauch durch *proton leakage* trägt vermutlich zu einem Anstieg der energetischen Kosten bei.

4.3 Einfluss der Temperatur auf Herz-Kreislauf-Parameter

Die Kälteakklimatisation und –adaptation führt zu einer verminderten Temperaturtoleranz, und kritische Temperaturen werden bei kaltakklimatisierten und kaltadaptierten Tieren früher als bei warmakklimatisierten Tieren erreicht (Peck 1989; Pörtner et al. 1999; van Dijk et al. 1999; Hardewig et al. 1999b). Die Untersuchungen an isolierten Mitochondrien zeigten, dass die Funktion der Mitochondrien noch jenseits der oberen kritischen Temperatur des Ganztiers gewährleistet ist, sofern ausreichend Sauerstoff zur Verfügung steht. Das Eintreten von Anaerobiose mit dem Erreichen der oberen kritischen Temperatur ist also nicht auf eine verminderte mitochondriale Leistung zurückzuführen

(Hardewig et al. 1999b; Pörtner et al. 1999). Die temperaturbedingte Limitierung des Stoffwechsels des Ganztiers liegt eher an einer verminderten Sauerstoffversorgung der Gewebe. Bei Evertebraten wie zum Beispiel dem Spritzwurm *Sipunculus nudus* und der Seespinne *M. squinado* konnte eine Korrelation zwischen einem Abfall im PO_2 und einer reduzierten Ventilations- und Zirkulationsleistung nachgewiesen werden (Zielinski und Pörtner 1996; Frederich und Pörtner 2000). Bei Fischen wurden die Temperaturgrenzen ebenfalls in Hinblick auf eine verminderte Sauerstoffversorgungsleistung diskutiert (van Dijk et al. 1999; Mark et al. 2002); allerdings fehlen noch direkte Messungen. Die *in vivo*-Versuche an Nordseekabeljau aus dieser Arbeit sollten daher zur weiteren Klärung beitragen.

Mit einer Temperaturadaptation geht eine Optimierung der aeroben Leistung und Kapazität einher, die den jeweiligen Temperatur-Toleranzbereich des Tieres limitiert (siehe Übersichtsartikel Pörtner 2001, 2002a,b). Eine solche Optimierung führt bei Kabeljau möglicherweise zu einer Verhaltensanpassung, da temperaturabhängige Verbreitungsmuster von *G. morhua* in der Nordsee und im Atlantik beobachtet wurden (siehe Einleitung). Dies deutet darauf hin, dass Kabeljau extreme Temperaturen meidet. Obwohl die Tiere in der Lage sind, sich an unterschiedliche Umgebungstemperaturen anzupassen (siehe Kapitel 4.1), wandert Kabeljau bei Temperaturen oberhalb von 10°C in kältere Gebiete in Richtung der Norwegischen Küste ab (T. Fischer, pers. Mitteilung). Zudem beobachtete Rose (1993), dass neufundländischer Kabeljau bei seiner Wanderung im nordwestlichen Atlantik einer Wassersäule zwischen 2°C und 3°C folgt.

Die Veränderungen von Herz-Kreislauf-Parametern während akuter Temperaturänderung sind in Abbildung 14 dargestellt. Der Verlauf des venösen PO_2 bei progressiver Temperaturänderung zwischen 1 und 19°C erinnert in seiner Form an eine Optimumkurve, wie sie bei *M. squinado* für den temperaturabhängigen arteriellen PO_2 gefunden wurde (Frederich und Pörtner 2000). Die Kurve zeigt allerdings einen geringeren Abfall im venösen PO_2 von *G. morhua* bei höheren als bei niedrigeren Temperaturen. Höchste venöse PO_2-Werte wurden bei 5,0 ± 0,2°C gefunden. Ober- und unterhalb dieser Temperatur kam es zu einer progressiven Abnahme im venösen Sauerstoffpartialdruck, was auf eine Verschiebung des Gleichgewichtes zwischen Sauerstoffversorgung und –verbrauch hindeutet.

Aus einer Arbeit von Heath und Hughes (1973) an der Regenbogenforelle *Salmo gairdneri* geht hervor, dass mit zunehmender Temperatur die Differenz zwischen arteriellem und venösem Sauerstoffgehalt ansteigt. Einen ähnlichen Befund ergaben die Messungen des PO_2 in der Kieme von *G. morhua*. Mit zunehmender Temperatur (10 bis 19°C) wurde eine Abnahme im venösen PO_2 (afferente Branchialarterie) beobachtet, wohingegen der arterielle

PO_2 (efferente Branchialarterie) keine signifikanten Änderungen zeigte (Publikation II, Fig. 3). Die Erniedrigung im venösen PO_2 mit steigender Temperatur liegt folglich nicht an einer eingeschränkten Ventilation, wie bei der Seespinne zu beobachten war (Frederich und Pörtner 2000), sondern deutet eher auf eine verminderte Leistung des Herz-Kreislauf-Systems hin.

Die temperaturbedingte Steigerung oder Abnahme der Herzfrequenz spiegelte sich nicht in einer entsprechenden Änderung des Blutflusses wider. Flussgewichtete MRI-Untersuchungen zeigten zwar einen temperaturinduzierten Anstieg des Blutflusses in der *Aorta dorsalis* und der *Vena caudalis*, jedoch konnte oberhalb von 4°C nur noch eine geringfügige Steigerung mit der Temperatur beobachtet werden. Ein ähnlicher Befund ergab sich bei der Untersuchung an *P. brachycephalum*. Mit zunehmender Temperatur (1°C pro 12 hrs) fanden Mark et al. (2002) einen Anstieg im Blutfluss der *A. dorsalis* zwischen 0°C und 6°C, der sich trotz weiterer Temperaturerhöhung nicht mehr steigerte. Der vergleichsweise geringe Anstieg im Blutfluss mit Temperaturerhöhung oberhalb von 4°C gegenüber der exponentiell ansteigenden Herzrate könnte an einer verminderten Kapazität der Herzkontraktion und des Herzschlagvolumens (*stroke volume*) liegen wie von Farrell (1996, 2002) vorgeschlagen. Die Herzkontraktion ist ein wichtiger Parameter für den arteriellen Blutdruck und Heath und Hughes (1973) zeigten, dass sich der Blutdruck in der ventralen und dorsalen Aorta in der Regenbogenforelle *S. gairdneri* mit steigender Temperatur nur geringfügig erhöhte.

Es konnten Schwellenwerte identifiziert werden, bei denen der Optimumbereich mit hohem venösen PO_2 verlassen wird (Pejus-Temperaturen). Die im unteren und oberen Temperaturbereich identifizierten Pejus-Temperaturen lagen bei 1,6°C (T_p I) und bei 7,3°C (T_p II). Erreichte die Temperatur höhere Werte als 16,0 ± 1,2°C wurde eine starke Abnahme im venösen PO_2 beobachtet (siehe hierzu Einzelergebnisse in Publikation IV, Fig. 5B; aufgrund der individuellen Temperaturabhängigkeit vom P_{VO2} der einzelnen Tiere ist der stärkere Abfall im venösen PO_2 oberhalb von 16°C bei den gemittelten Werten nicht sichtbar), was auf das Erreichen der oberen kritischen Temperatur (T_c) von *G. morhua* hindeutet.

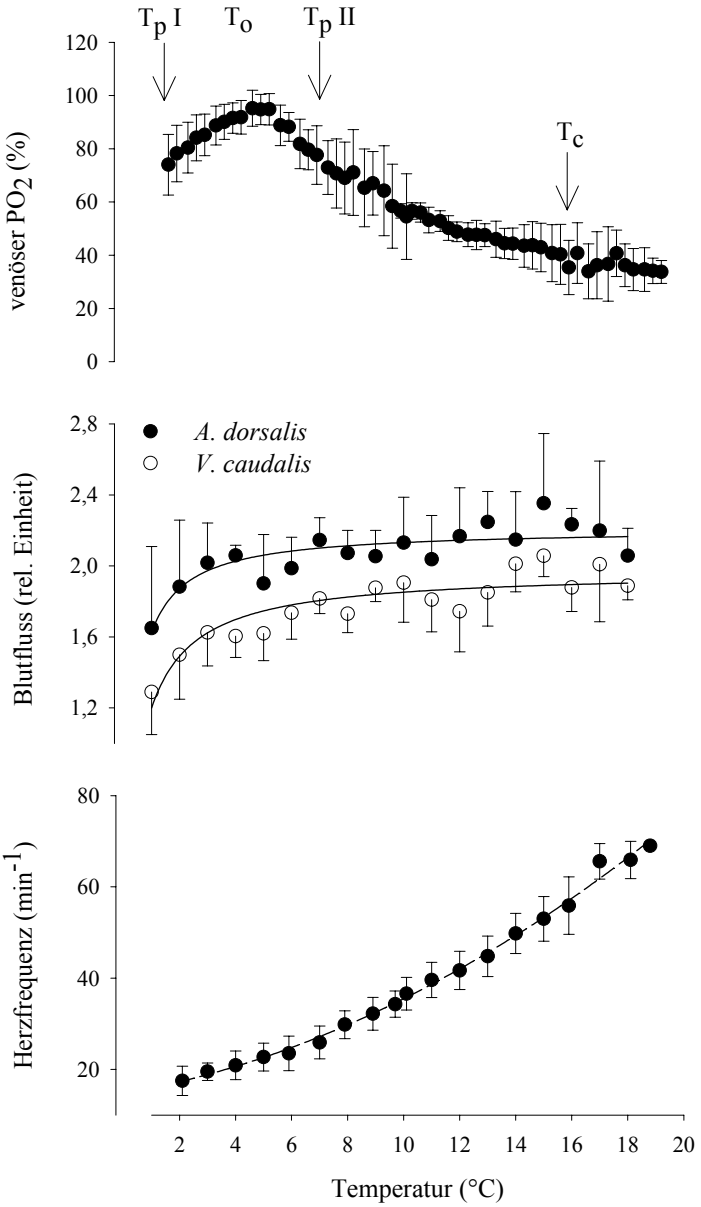


Abb. 14 Venöser PO_2, arterieller und venöser Blutfluss und Herzfrequenz bei *G. morhua* bei progressiver Temperaturänderung (1°C h^{-1}) ausgehend von 10°C. Die beiden Pejus-Temperaturen, T_p I und T_p II begrenzen den Bereich mit maximalem PO_2 (T_o), in dem eine maximale aerobe Aktivität möglich ist. Ab diesen Temperaturen ergaben sich signifikant niedrigere PO_2-Werte, und eine Limitierung in der aeroben Kapazität tritt ein. Ab der oberen kritischen Temperatur (T_c) fiel der venöse PO_2 stärker (Der starke Abfall oberhalb von T_c ist deutlicher in den Einzeldaten zu sehen, aufgrund individueller Temperaturabhängigkeit des venösen PO_2 der Tiere (Publikation IV, Fig. 5B)), und Rhythmusstörungen in der Herzfrequenz traten auf. (Abbildung verändert nach Publikation IV).

Ab 16°C war ebenfalls eine Tendenz zu erkennen, dass Kabeljau seine Herzrate nicht weiter steigern konnte, und es kam zu Unregelmäßigkeiten in der Herzfrequenz (Publikation IV, Fig. 2 B&3). Der Abfall im venösen PO_2 und die beobachteten Herz-Rhythmus-Störungen bei Temperaturen > 16°C bewirken möglicherweise auch den beobachteten Zusammenbruch des Energiestoffwechsels im weißen Muskelgewebe oberhalb von 16°C (Publikation II, Fig. 6). Sobald ein Einbruch im Energiestoffwechsel zu erkennen war, führte ein schnelles Abkühlen der Wassertemperatur zu keiner Erholung, und das Tier starb innerhalb einer

Stunde. Im Gegensatz dazu erholten sich die Tiere, wenn die Temperatur beim Auftreten von Herz-Rhythmus-Störungen erniedrigt wurde. Die Ergebnisse deuten darauf hin, dass die beobachteten Herz-Rhythmus-Störungen bei hohen Temperaturen in *G. morhua* ein früher *in vivo*-Indikator für das Erreichen kritischer Temperaturen bei Fischen ist, und dass die Grenzen der Temperaturtoleranz von *G. morhua* eher durch das Herz-Kreislauf-System als durch die Ventilationsleistung gesetzt werden.

Es stellt sich die Frage, inwieweit diese Befunde mit den Verbreitungsgebieten von Kabeljau in seiner natürlichen Umgebung übereinstimmen. Wie in der Einleitung erwähnt, ist Kabeljau über den gesamten Nord-Atlantik verbreitet, wobei die nördlichste Verbreitungsgrenze in der Barentssee und das südlichstes Verbreitungsgebiet in der nördlichen Biskaya liegt. Adulter Kabeljau ist bei Temperaturen zwischen –0,5 und 12-14°C zu finden, wobei er sich jedoch hauptsächlich zwischen 1 bis 5°C aufhält (Jones 1968). Righton et al. (2001) vermuteten einen Zusammenhang zwischen Beutevorkommen (nicht untersucht) und der beobachteten geringen Vertikalbewegung von *G. morhua* aus der Nordsee, da sich die Tiere im Sommer hauptsächlich in Bodennähe aufhielten. Berücksichtigt man jedoch, dass die Oberflächentemperatur der Nordsee im Jahresverlauf zwischen 7 und 16°C schwankt (Laevastu 1993), könnte das Verhalten von *G. morhua* auf die hohen Sommertemperaturen zurückzuführen sein. Zudem kann sich während der Sommerzeit eine starke Temperatursprungschicht in der Nordsee bilden, welche zu Grundtemperaturen zwischen 6 und 8°C führt (Brander 1994, Dippner CLICOFI-Bericht). In der Ostsee wird die Verbreitung von Kabeljau vorwiegend durch die Salinität und den Sauerstoffgehalt limitiert, da die ausgeprägte Schichtung in der Ostsee eine vertikale Durchmischung zwischen O_2-reichem Oberflächenwasser und O_2-armen Tiefenwasser limitiert (Bagge et al. 1994; Tomkiewicz et al. 1998; Neuenfeldt 2002). Beobachtungen von R. Knust (pers. Mitteilung), dass Kabeljau in warmen Sommern aus der südlichen Nordsee trotz eines ausreichenden Nahrungsangebot in kühlere, nördliche Gewässer abwandert, bestätigen die Annahme, dass das Migrationsverhalten von Kabeljau eher auf die hohen Temperaturen als auf ein verringertes Beutevorkommen zurückzuführen ist. Gegen eine beuteabhängige Verbreitung von Kabeljau sprechen auch die Arbeiten von Chouinard (1994) und von Schwalme und Chouinard (1999). Aus einer Magenanalyse von Kabeljau aus dem südlichen Sankt Lorenz Golf ging hervor, dass Kabeljau im Winter die Temperatursprungschicht für eine Beutejagd nicht überschreitet, sondern sich in den tieferen und wärmeren Gewässern bei 5 bis 6°C aufhält. Die Verbreitungsgebiete von *G. morhua* werden somit wahrscheinlich stärker von der Temperatur als von dem verfügbaren Beutevorkommen limitiert.

Die in der Natur vorkommende temperaturabhängige Abundanz von *G. morhua* spiegelt den gefundenen optimalen Temperaturbereich für Kabeljau aus der Nordsee zwischen 2°C und 7°C wider. Zwischen 7°C und ungefähr 16°C liegt für *G. morhua* der obere Pejus-Bereich. In diesem Bereich ist Überleben im physiologischen Sinne möglich, die Fähigkeit, aus der Ruhesituation heraus aerob aktiv zu werden, jedoch verringert (siehe Übersichtsartikel Pörtner 2002a, Farrell 2002). Die sich ergebende kritische Temperatur bei 16,0 ± 1,2°C ist in Übereinstimmung mit einer 88%igen Mortalitätsrate von Kabeljau der Islandküste, der bei 15.6°C gehältert wurde (Björnsson et al. 2001). Im Wachstumsexperiment mit *G. morhua* der Nordsee bei den Temperaturen 4°C, 8°C, 12°C und 15°C ergab sich ebenfalls bei den 15°C-akklimatisierten Tieren die höchste Mortalität, wobei die geringste in der bei 4°C gehälterten Versuchsgruppe gefunden wurde (siehe Fischer 2002).

Obiger Befund eines optimalen Energiehaushalts bei 5°C, aufgrund eines Maximums im venösen PO_2, ist in Übereinstimmung mit der in der Natur gefundenen Verbreitung von *G. morhua*, steht jedoch auf den ersten Blick im Widerspruch zu den gefundenen höheren Wachstumsraten bei den 12°C gegenüber den 4°C gehälterten *G. morhua* (Pörtner et al. 2001; Fischer 2002). Zum einen besteht jedoch ein negativer Zusammenhang zwischen Tiergröße und optimaler Temperatur für Wachstum und Futterverwertung (Björnsson et al. 2001). Zum anderen erfolgten die Wachstumsuntersuchungen bei unlimitierter Fütterung. Brett et al. (1969) und Jobling (1994) fanden erniedrigte Optimumtemperaturen für das Wachstum bei Reduzierung des Futterangebots (siehe Publikation IV).

Die eingeschränkte aerobe Leistungskapazität von Kabeljau der südlichen Nordsee oberhalb von 7°C und die in der Natur bevorzugten kühleren Wasserschichten deuten möglicherweise darauf hin, dass der Energieaufwand für eine Beutejagd bei höheren Temperaturen nicht mehr über das Herz-Kreislauf-System gedeckt werden könnte. Aus diesen Gründen, liegt die optimale Temperatur für *G. morhua* in seiner natürlichen Umgebung mit großer Wahrscheinlichkeit bei geringeren Temperaturen, als unter Laborbedingungen mit unlimitiertem Futterangebot und vor allem mit verringertem Energieaufwand für Beutejagd, gefunden wurde.

5. Zusammenfassende Betrachtung und Ausblick

Das Ziel dieser Arbeit war es, ein mechanistisches Verständnis auf physiologischer Ebene zu gewinnen, inwieweit sich die temperaturbedingten Anpassungsmechanismen der aeroben Kapazität sowohl zwischen kaltakklimatisierten borealen und kaltadaptierten polaren als auch zwischen kaltadaptierten eurythermen (Subarktis und Arktis) und kaltadaptierten stenothermen (Antarktis) Fischen unterscheidet und inwieweit diese das Wachstum der Tiere beeinflussen könnten. Die Wärmeakklimatisation der antarktischen Aalmutter *P. brachycephalum* sollte einen Hinweis geben, ob wie bei eurythermen Organismen nachgewiesen wurde, kaltstenotherme Tiere in der Lage sind, ihre aerobe Kapazität an erhöhte Temperaturen anzupassen. Zur Klärung und Identifizierung der thermalen Toleranzgrenzen und möglicher Konsequenzen der globalen Erwärmung auf die Verbreitungsgebiete von Kabeljau der südlichen Nordsee sollten die Messungen des Herz-Kreislauf-Systems bei *G. morhua* beitragen.

Eine Kälte- bzw. Wärmeakklimatisation von borealer *Z. viviparus* und *G. morhua* (Nordsee) und polarer *P. brachycephalum* (Antarktis) und *G. morhua* (Barentssee) führte zu einer Steigerung bzw. Erniedrigung der aeroben Kapazität in der Leber der Tiere. Die vorliegende Arbeit zeigte damit, dass die Fähigkeit die aerobe Kapazität an unterschiedliche Temperaturen anzupassen auch auf die kaltstenotherme antarktische Aalmutter *P. brachycephalum* zutrifft. Nach einer erstmalig durchgeführten Langzeit-Wärmeakklimatisation hatten die 5°C gehälterten Tiere im Vergleich zu den Kontrolltieren (0°C) eine erniedrigte aerobe Kapazität in der Leber. In einer früheren Arbeit wurden antarktischen Fische der endemischen Familie Nototheniidae, *Trematomus bernachii* und *T. newnesi*, für 2 bis 5 Wochen bei 4°C gehältert. Diese Temperatur war die höchste, bei der die Tiere für wenige Wochen akklimatisiert werden konnten (Weinstein und Somero 1998). *P. brachycephalum* wurde für mindestens 10 Monate bei 5°C gehalten, und bei den Tieren wurde keine Stresssituation aufgrund der höheren Umgebungstemperatur festgestellt (siehe Publikation III). Weiterhin zeigte diese Art keine Reduzierung des Sauerstoffverbrauchs über Protonen-Leckströme, wie von Pörtner et al. (1998) für kaltstenotherme Tiere der Antarktis postuliert wurde. Dies spricht dafür, dass es sich bei *P. brachycephalum* vermutlich nicht um eine rein kaltstenotherme Art handelt. Im Gegensatz zu kaltadaptierten eurythermen *G. morhua* der Barentssee wurde bei kaltadaptierter *P. brachycephalum* jedoch eine erniedrigte oxidative ATP-Synthese-Kapazität, jedoch ebenfalls erhöhte Enzymaktivitäten im Vergleich zu kaltakklimatisierten borealen Tieren gefunden.

Aus diesen Ergebnissen kann geschlussfolgert werden, dass *P. brachycephalum*

vermutlich aufgrund ihrer benthischen und trägen Lebensweise (Hypometabolismus) in der Lage ist, sowohl in der Antarktis wie auch bei höheren Temperaturen zu leben. Höhere Temperaturen scheinen für *P. brachycephalum* bis zu einem gewissen Grad tolerierbar zu sein und durch eine globale Erwärmung scheint diese antarktische Fischart nicht direkt bedroht zu sein, über den Einfluss höherer Temperaturen auf Nahrungsangebot und Reproduktion kann jedoch keine Aussage getroffen werden.

Polare Tiere scheinen im Vergleich zu borealen Eurythermen nur in eingeschränktem Maße zu einer Regulierung ihrer aeroben Kapazität in der Lage zu sein. Sowohl die antarktische Aalmutter als auch der arktische Kabeljau hatten im Vergleich zu borealen Tieren, die bei gleicher Temperatur gehältert wurden, erhöhte Enzymaktivitäten. Im Gegensatz zu *P. brachycephalum* hatte jedoch *G. morhua* der Barentssee ebenfalls eine höhere mitochondriale Respiration (oxidative Phosphorylierung und *proton leakage*) und einen höheren Standardstoffwechsel als boreale Nordseetiere bei gleicher Akklimatisationstemperatur (siehe Abb. 11; van Dijk et al. 1999; Fischer 2002). Adaptive Kältekompensationsmechanismen scheinen somit die Anpassungsfähigkeit an wärme Temperaturen bei den nördlichen kaltadaptierten eurythermen Populationen zu minimieren. Dieser Unterschied spiegelt sich in der hohen Mortalitätsrate von Kabeljau aus der Barentssee wider, die bei 12°C und 15°C fast 50% betrug. Im Gegensatz dazu lag die Mortalität von Kabeljau aus der Nordsee bei 12°C nur bei 20% und stieg erst in der bei 15°C gehälterten Versuchsgruppe auf über 40% an (siehe Fischer 2002).

Weiterhin wurde für alle Kabeljaupopulationen ein Optimum in der Wachstumskurve zwischen 10°C und 11°C gefunden, wobei das Wachstum der Tiere von der Nordsee zur Barentssee abnahm. Eine Kälteakklimatisation vor allem jedoch eine Kälteadaptation scheint aufgrund einer gesteigerten aeroben Kapazität mit einem höheren Energieaufwand gekoppelt zu sein und damit zu einem geringeren Wachstum in der Kälte beizutragen. Diese Annahme wird ebenfalls durch den geringeren Lipidgehalt in der Leber von kalt- versus warmakklimatisierten Kabeljau der Nordsee impliziert. Einen weiteren Hinweis auf erhöhte Kosten aufgrund einer Kälteakklimatisation ergibt eine Arbeit von Farrell und Clutterham (2003), in der niedrigere venöse PO_2-Werte in kalt- versus warmakklimatisierten Regenbogenforellen, *O. mykiss* beschrieben wurden, die sich mit zunehmender Schwimmleistung bei der jeweiligen Hälterungstemperatur signifikant unterschieden. Zudem trat bei den kaltakklimatisierten Tieren schon bei geringerer Schwimmleistung ein signifikant erniedrigter venöser PO_2-Wert im Vergleich zum Kontrollwert (keine Aktivität) ein. Somit führt die Kälteanpassung nicht nur zu erhöhten Kosten während des Ruhestoffwechsels,

sondern vor allem zu einem erhöhten energetischen Aufwand bei Aktivität (siehe dazu auch Pörtner 2002b). Welchen Einfluss die erhöhten Kosten aufgrund eines gesteigerten Sauerstoffverbrauchs durch Protonen-Leckströme haben, könnten die momentan durchgeführten Wachstumsexperimente an Aalmutter der Nordsee und der Antarktis (E. Brodte) zeigen.

Die *in vivo*-Untersuchungen an Kabeljau der Nordsee ergaben eine deutliche Temperaturabhängigkeit des Herz-Kreislauf-Systems. Anhand von Herzfrequenz, Blutfluss und venösem PO_2 konnten erstmalig Pejus-Temperaturen (Übergang von optimaler zu verminderter aerober Leistung) bei Fischen eindeutig definiert werden. Bei 10°C gehälterten Tieren lag das Maximum der aeroben Leistung (höchster venöser PO_2) bei 5,0 ± 0,2°C und der gefundene energetische Optimumbereich zwischen 2°C und 7°C, korreliert mit dem in der Natur gefundenen Verbreitungsgebiet und Reproduktionszeitraum (zwischen Februar und März bei einer Durchschnittstemperatur von 4,5°C, siehe Fischer 2002) von Kabeljau.

Neben der Temperatur ist auch das Nahrungsangebot für die Verbreitungsgrenzen verantwortlich, jedoch scheint die Temperatur der Hauptfaktor zu sein, da *G. morhua* trotz ausreichender Beute die südliche Nordsee im Sommer meidet. Möglicherweise ist die verminderte aerobe Leistungskapazität oberhalb von 7°C der Grund, dass *G. morhua* bei höheren Umgebungstemperaturen in kühlere Gewässer Richtung Norden abwandert.

Aus einer Arbeit von Farrell (2002) geht hervor, dass bei Rotlachs *O. nerka* die maximale Schwimmgeschwindigkeit, ihre aerobe Leistungskapazität und die Herz-Leistungskapazität bei Akklimatisationstemperaturen unter- und oberhalb ihrer Präferenztemperatur abnahm. Die von Farrell (2002) getroffene Vermutung, dass die Kapazität des Herz-Kreislauf-Systems eine ausreichende Sauerstoffversorgung der Gewebe aufrechtzuerhalten mit Erreichen der oberen Pejus-Temperatur abnimmt, konnte in dieser Arbeit bestätigt werden. Die verminderte Leistungsfähigkeit von jenseits der Präferenztemperatur gehälterten Tieren und der in dieser Arbeit gefundene Optimumbereich (2°C – 7°C) für den bei 10°C akklimatisierten Kabeljau lassen vermuten, dass es sich bei der Herz-Kreislauf-Kapazität um einen adaptiven Mechanismus handelt, der sich nicht bei einer Temperatur-Akklimatisation ändert. Jedoch müssten zur Bestätigung der Annahme, dass sich das Optimum für eine aerobe Leistungskapazität zwischen 2°C und 7°C nicht mit der Akklimatisationstemperatur verschiebt, weiter Versuche mit 5°C gehältertem *G. morhua* der Nordsee folgen, da die bei Farrell (2002) beschriebenen Parameter bei der jeweiligen Akklimatisationstemperatur gemessen wurden.

Im Hinblick auf eine globale Erwärmung würde es somit auch zu einer Verschiebung der südlichen Verbreitungsgrenze bei Kabeljau kommen, so dass im Extremfall diese Art in der Nordsee ganzjährig nicht mehr vertreten wäre. Physiologische Parameter könnten somit zur Klärung von ökologischen Beobachtungen in der Natur beitragen.

Im Gegensatz zu den Ergebnissen bei Crustaceen (Frederich und Pörtner 2000) zeigten die Untersuchungen an *G. morhua*, dass mit einer Temperaturerhöhung keine Erniedrigung im arteriellen PO_2 einhergeht und erst mit Erreichen der kritischen Temperatur ein Abfall im arteriellen PO_2 zu erkennen war. Dagegen wurde im venösen PO_2 der Fische ein ähnlicher temperaturabhängiger Verlauf wie für den arteriellen PO_2 von Crustaceen gefunden, der eine Einteilung in Optimum, Pejus- und Pessimum-Bereiche erlaubte. Abbildung 15 stellt ein nach Frederich und Pörtner (2000) modifiziertes Temperatur-Toleranzmodell vor. Während im oberen Pejus-Bereich der arterielle PO_2 konstant bleibt, fällt der venöse PO_2 ab. Diese allmähliche Verschiebung zwischen Sauerstoffversorgung und –verbrauch ist auf eine verminderte Herz-Kreislauf-Leistung zurückzuführen. Diese Verschiebung scheint für gehälterte Tiere tolerierbar zu sein (zur Erinnerung: größtes Wachstum bei 12°C Akklimatisation). Dies gilt vor allem, wenn man berücksichtigt, dass unter Laborbedingungen kaum Energie für die Beutejagd aufgewendet werden muss und die Tiere nicht in eine Situation kommen, in der ihre aerobe Leistungskapazität nicht mehr über das Kreislauf-System gedeckt werden kann.

Dieses modifizierte Temperatur-Toleranzmodell für eine aerobe Leistungskapazität trifft möglicherweise allgemein für Fische zu, wie das durchgeführte Wärmeakklimatisations-experiment mit der antarktischen Aalmutter vermuten lässt. Unter Berücksichtigung der Arbeiten von van Dijk et al. (1999) und Mark et al. (2002) an *P. brachycephalum* kann eine obere Pejus-Temperatur um 5°C angenommen werden, was eine Langzeit-Hälterung laut Modell erlaubte. Es bleibt zu überprüfen, ob eine langfristige Hälterung dieser Art auch bei höheren Temperaturen, jedoch unterhalb ihres kritischen Temperaturbereichs (9°C - 13°C, van Dijk et al. 1999; Mark et al. 2002) möglich wäre und vor allem ob sich der thermale Optimumbereich mit der Akklimatisationstemperatur verschiebt.

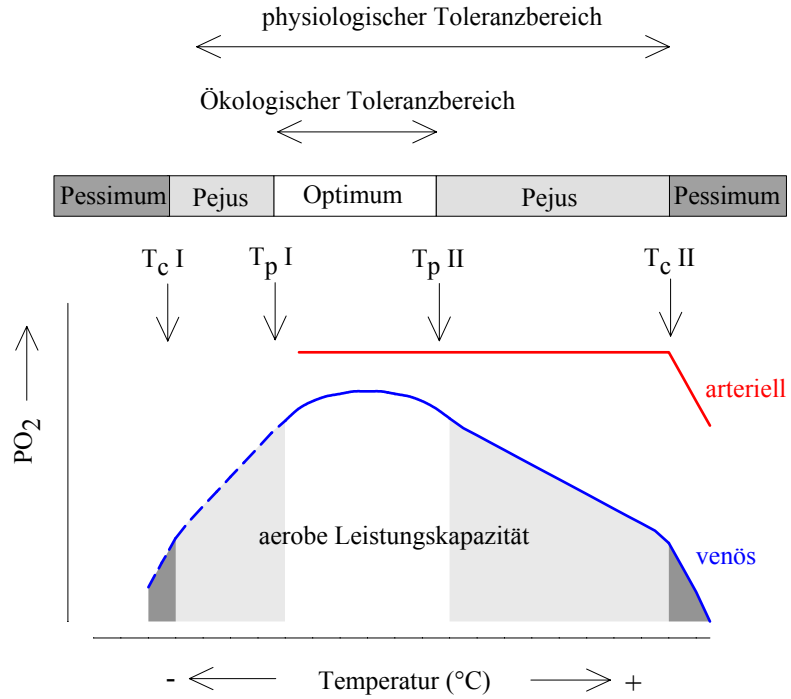


Abb. 15 Temperatur-Toleranzmodell für Fische (modifiziert nach Frederich und Pörtner 2000). Die aerobe Leistungskapazität (Fläche unterhalb des venösen PO_2) ist im thermalen Optimumbereich maximal. Im unteren bzw. oberen Pejus-Bereich ist die aerobe Leistungskapazität zunehmend limitiert, vom Tier jedoch noch tolerierbar, bis der kritische Temperaturbereich erreicht wird. Weitere Erklärung siehe Text.

6. Literaturverzeichnis

Alp RP, Newshole EA, Zammit VA (1976) Activities of citrate synthetase and NAD^+ -linked and $NADP^+$ -linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. Biochem J 154: 689-700

Anderson ME (1990) Zoarcidae. In: Gon O, Heemstra PC (Hrsg.) Fishes of the Southern Ocean. Smith Institute for Ichthyology, S. 256ff

Archer SD, Johnston IA (1991) Density of cristae and distribution of mitochondria in the slow muscle fibres of Antarctic fish. Physiol Zool 64: 242-258

Arntz WE (1973) Periodicity of diel food intake of cod *Gadus morhua* in the Kiel Bay. Oikos 15: 138-145

Arntz WE, Tarazona J (1990) Effects of El Niño 1982 - 83 on benthos, fish and fisheries off the South American Pacific coast. In: Glynn PW (Hrsg.) Global ecological consequences of the 1982 – 83 El Niño – Southern Oscillation. Elsevier Oceanogr. Series, S. 323ff

Arntz WE, Brey T, Gallardo VA (1994) Antarctic Zoobenthos. Oceanography and Marine Biology: an Annual Review 32: 241-304

Bagge O, Thurow F, Steffensen E, Bay J (1994) The Baltic cod. Dana 10: 1-28

Bakun A (1996) Patterns in the Ocean-Ocean Processes and Marine Population Dynamics. California Sea Grant College System, La Jolla, S. 323ff

Beamish RJ (1995) Climate Change and Northern Fish Populations. Canadian Special Publication of Fisheries and Aquatic Science 121: 739ff

Beukema JJ, de Vlas J (1979) Population parameters of the lugworm, *Arenicola marina*, living on tidal flats in the dutch wadden sea. Neth J Sea Res 13: 331-353

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

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917

Bouchard P, Guderley H (2003) Time course of the response of mitochondria from oxidative muscle during thermal acclimation of rainbow trout, *Oncorhynchus mykiss*. J Exp Biol 206: 3455-3465

Brand MD (1990) The contribution of the leak of protons across the mitochondrial inner membrane to standard metabolic rate. J Theor Biol 145: 267-286

Brander K (1994) Spawning and life history information for North Atlantic cod stocks. ICES Cooperative Research Report Nr. 205, S. 150ff

Brander KM (1996) Effects of climate change on cod (*Gadus morhua*) stocks. In: Wood C, Mc Donald DG (Hrsg.) Implications For Freshwater and Marine Fish. Soc Exp Biol Sem Ser, Cambridge University Press, 61: 255-278

Brett JR, Shelbourn JE, Shoop CT (1969) Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J Fish Res Board Can 26: 2363-2394

Brookes PS, Buckingham JA, Tenreiro AM, Hulbert AJ, Brand MD (1998) The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty acid composition. Comp Biochem Physiol B 119: 325-334

Campbell C, Davies P (1978) Temperature acclimation in the teleost, *Blennius pholis*: changes in enzyme activity and cell structure. Comp Biochem Physiol B 61: 165-167

Chouinard GA (1994) Distribution of groundfish and herring during the 1994 Cabot Strait survey. DFO Atlantic Fish Res Doc 94(68) S: 24

Clarke A (1983) Life in cold water: The physiological ecology of polar marine ectotherms. Oceanogr Mar Biol Ann Rev 21: 341-353

Clarke (1991) What Is Cold Adaptation and How Should We Measure It? J Am Zool 31: 81-92

Clarke A, North AW (1991) Is the growth of polar fish limited by temperature? In: di Priso G, Maresca B, Tota B (Hrsg.) Biology of the Antarctic fish. Springer Verlag, Berlin, Heidelberg, 54pp

Clarke A, Peck LS (1991) The physiology of polar marine zooplankton. Polar Res 10: 355-369

Cohen DM, Inada T, Iwamoto T, Scialabba N (1990) Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. FAO Fish Synop 125(10): 442ff

Cordiner S, Eggington S (1997) Effect of seasonal temperature acclimatization on muscle metabolism in rainbow trout, *Oncorhynchus mykiss.* Fish Physiol Biochem 16: 333-343

Cossins AR, Sinensky M (1986) Adaptations of membranes to temperature, pressure and exogenous lipids. In: Shinitzsky M (Hrsg.) Physiology of Membrane Fluidity. CRC Press, S. 1ff

Crockett EL, Sidell BD (1990) Some pathways of energy metabolism are cold adapted in Antarctic fishes. Physiol Zool 63: 472-488

Cushing DH (1982) Climate and Fisheries. Academic Press, London, S. 373ff

Cushing DH (1984) The gadoid outburst in the North Sea. J Cons Ciem 4(2): 159-166

Das AB (1967) Biochemical changes in tissues of goldfish acclimated to high and low temperatures. II. Synthesis of protein and RNA of subcellular fractions and tissue composition. Comp Biochem Physiol 21: 469-485

Dippner JW (1997) Recruitment success of different fish stocks in the North Sea in relation to climate variability. Deutsche Hydrogr Zeitschr 49: 277-293

Dippner JW (CLICOFI-Bericht) Retrospective studies on long term data series of climate conditions and populations dynamics of cod and eelpout – Data Report, Climatology and Climate Variability.

Egginton S, Sidell B (1989) Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. Am J Physiol 256: R1-R10

Eshky AA, Taylor AC, Atkinson RJA (1996) The effect of temperature on aspects of respiratory physiology of the semi-terrestrial crabs, *Uca inversa* (Hoffmann) and *Metopograpsus messor* (Forskal) from the Red Sea. Comp Biochem Physiol A 114: 297-304

Farrell AP (1996) Effects of temperature on cardiovascular performance. In: Wood CM, McDonald DG (Hrsg.) Global Warming: Implications for fresh water and marine fish. Cambridge University Press, S. 135ff

Farrell AP (2002) Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. Comp Biochem Physiol A 132: 797-810

Farrell AP, Clutterham SM (2003) On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. J Exp Biol 206: 487-496

Fischer T (2002) The effects of climate induced temperature changes on cod (*Gadus morhua* L.): Linking ecological and physiological investigations. Dissertation an der Universität Bremen, Fachbereich 2

Fonds M, Jaworski A, Iedema A, Puyl PVD (1989) Metabolism, food consumption, growth and food conversion of shorthorn sculpin (*Myoxocephalus scorpius*) and eelpout (*Zoarces viviparus*). ICES Demersal fish committee, CM G:31

Foster AR, Hall SJ, Houlihan DF (1993) The effect of temperature acclimation on organ/tissue mass and cytochrome c oxidase activity in juvenile cod (*Gadus morhua*). J Fish Biol 42: 947-957

Frank KT, Perry RI, Drinkwater KF (1990) Predicted response of Northwest Atlantic invertebrates and fish stock to CO_2-induced climate change. Trans Am Fish Soc 119: 353-365

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

Gaebel K, Roots BI (1989) Stereological analysis of mitochondria from brains of temperature acclimated goldfish, *Carrasius auratus* L (5 and 30°C). J therm Biol 14: 187-190

Götting KJ (1976) Fortpflanzung und Oocyten – Entwicklung bei der Aalmutter (*Zoarces viviparus*) (Pisces, Osteichthyes). Helgoländer wissenschaftliche Meeresuntersuchungen Nr. 28: 71-89

Gornall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of biuret reaction. J Biol Chem 117: 751-66

Guderley H (1990) Functional significance of metabolic responses to thermal acclimation in fish muscle. Am J Physiol 259: R245-R252

Guderley H (1998) Temperature and growth rates as modulators of the metabolic capacities of fish muscle. In: Pörtner HO, Playle RC (Hrsg.) Cold ocean physiology. Cambridge University Press, S. 58ff

Guderley H, Leroy P (2001) Family origin and the response of threespine stickleback, *Gasterosteus aculeatus*, to thermal acclimation. J Comp Physiol B 171: 91-101

Günther CP (1992) Dispersal of intertidal invertebrates: a strategy to react to disturbances of different scales? Neth J Sea Res 30: 45-56

Haase A (1990) Snapshot FLASH MRI. Applications to T1, T2 and chemical-shift imaging. Magn Reson Med 13(1): 77-89

Hardewig I, van Dijk PLM, Moyes CD, Pörtner HO (1999a) Temperature-Dependent Expression of Cytochrome-C Oxidase in Antarctic and Temperate Fish. Am J Physiol 277: R508-R516

Hardewig I, Peck LS, Pörtner HO (1999b) Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid *Lepidonotothen nudifrons*. J Comp Physiol B 169: 597-604

Heath AG, Hughes GM (1973) Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). J Exp Biol 59: 323-338

Hochachka PW, Somero GN (1984) Biochemical adaptation. Princeton University Press, Princeton, New Jersey

Holton GF (1973) Respiration of Arctic charr (*Salvelinus alpinus*) from a high Arctic lake. J Fish Res Bd Can 6: 717-723

Holton GF (1974) Metabolic cold adaptation of polar fish: fact or artefact? Physiol Zool 47: 137-152

Hummel H, Sommer A, Bogaards RH, Pörtner HO (1997) Variation in genetic traits of the lugworm *Arenicola marina*: temperature related expression of mitochondrial allozymes? Mar Ecol Prog Ser 159: 189-195

Hutchings JA, Myers RA (1994) What can be learned from the collapse of renewable resource? Atlantic cod, Gadus morhua, of Newfoundland and Labrador. Can J Fish Aquat Sci 51(9): 2126-2146

ICES (1991) Report of the ICES Advisory Committee on Fishery Management. ICES Cooperative Research Report Nr. 179

ICES (1997) Report of the Working Group on the Assesment of Demersal Stocks in the North Sea and Skagerrak. ICES CM Nr. 6

Jankowsky HD, Hotopp H, Seibert H (1984) Influence of thermal acclimation on glucose production and ketogenesis in isolated eel hepatocytes. Am J Physiol 246: R471-R476

Jobling M (1994) Fish bioenergetics. Chapman and Hall, London. S. 309ff

Johnston IA (1990) Cold adaptation in marine organisms. Phil trans R Soc Lond B 326: 655-667

Johnston IA, Guderley H, Franklin CE, Crockford T, Kamunde C (1994) Are mitochondria subject to evolutionary temperature adaptations? J Exp Biol 195: 293-306

Johnston IA, Calvo J, Guderley H, Fernandez D, Palmer L (1998) Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. J Exp Biol 201: 1-12

Jones FRH (1968) Fish Migration. Spottiswood, Ballantyne & Co. Ltd., London and Colchester. S. 144f

Kawall HG, Torres JJ, Sidell BD, Somero GN (2002) Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain. Mar Biol 140: 279-286

Kent J, Koban M, Prosser C (1988) Cold-acclimation-induced protein hypertrophy in channel catfish and green sunfish. J Comp Physiol B 158: 185-198

Knijn RJ, Boon TW, Heessen JL, Hislop JRG (1993) Atlas of the North Sea fishes. ICES Cooperative research report Nr. 194 S. 268ff

Krogh A (1914) The quantitative relation between temperature and standard metabolism in animals. Int Z Phys Chem Biol 1: 491-508

Laevastu T (1993) Marine climate, weather and fisheries. The Effects of Weather and Climate Changes On Fisheries and Ocean Resources. Blackwell Scientific Publications Ltd., Oxford. S. 67

Lambert Y, Dutil J (1997) Can simple indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? Can J Fish Aquat Sci 54: 104-112

Londraville R, Sidell B (1990) Ultrastructure of aerobic muscle in Antarctic fishes may contribute to maintenance of diffusion fluxes. J Exp Biol 150: 205-220

Love R (1970) The Chemical Biology of Fishes. New York, Academic Press

Lucassen M, Schmidt A, Eckerle LG, Pörtner HO (2003) Mitochondrial proliferation in the permanent versus temporary cold: enzyme activities and mRNA levels in Antarctic and temporate zoarcid fish. Am J Physiol Regul Integr Comp Physiol, online, DOI: 10.1152/ajpregu.00111.2003

Lythgoe J, Lythgoe G (1974) Meeresfische. BLV Verlagsgesellschaft mbH, München

Madon SP, Schneider DW, Stoeckel JA (1998) In situ estimation of zebra mussel metabolic rates using the electron transport system (ETS) assay. J Shellfish Res 17: 195-203

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

McKeown BA (1984) Fish Migration. Croom Helm Ltd, Beckenham, Kent, S. 37ff

Miranda EJ, Hazel JR (1996) Temperature-induced changes in the transbilayer distribution of phosphatidylethanolamine in mitoblasts of rainbow trout (*Oncorhynchus mykiss*) liver. J Exp Biol 274: 23-32

Moyes CD, Matthieu-Costello OA, Tsuchiya N, Filburn C, Hansford RG (1997) Mitochondrial biogenesis during cellular differentiation. Am J Physiol 272: 1345-1354

Müller H (1983) Fische Europas. Neumann Verlag, Leipzig, Radebeul

Myers RA, Hutchings JA, Barrowman NJ (1996) Hypotheses for the decline of cod in the North Atlantic. Mar Ecol Prog Ser 138: 293-308

Nakken O (1994) Causes of trends and fluctuations in the Arcto-Norwegian cod stock. ICES Mar Sci Symp 198: 212-228

Neuenfeldt S (2002) The influence of oxygen saturation on the distributional overlap of predator (cod, Gadus morhua) and prey (herring, Clupea harengus) in the Bornholm Basin of the Baltic Sea. Fish Oceanogr 11(1): 11-17

Netzel J, Kuczynski J (1995) The abundance of flounder (*P. flesus* L.) and eelpout (*Z. viviparus* L.) in the Gulf of Gdansk area are estimated by swept area method. ICES, Baltic fish Committee CM, J: 30

O`Brien CM, Fox CJ, Planque B, Casey J (2000) Climate variability and North Sea cod. Nature 404: 142

Palsson OK (1994) A review of the trophic interactions of cod stocks in the North Atlantic. ICES Mar Sci Symp 198: 553-575

Parsons LS, Lear WH (2001) Climate variability and marine ecosystems impacts : a North Atlantic perspective. Prog Oceanogr. 49(1-4): 167-188

Peck LS (1989) Temperature and basal metabolism of two Antarctic marine herbivores. J Exp Mar Biol Ecol 125: 1-12

Pogson GH (1988) Constraints on the genetic process of biochemical adaptation. Can J Zool 66: 1139-1145

Pörtner HO, Hardewig I, Sartoris FJ, van Dijk PLM (1998) Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? in: Pörtner HO, Playle R (Hrsg.) Cold Ocean Physiology. Cambridge University Press, Cambridge, S.88ff

Pörtner HO, Hardewig I, Peck LS (1999) Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternulls elliptica*. Comp Biochem Physiol A 124: 179-189

Pörtner HO, van Dijk PLM, Hardewig I, Sommer A (2000) Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: Davison W, Williams CH (Hrsg.) Antarctic Ecosystems: Models for Wider Ecological Understanding. Christchurch, New Zealand, Caxton Press, S. 109ff

Pörtner HO, 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 FJ, 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*). Cont Shelf Res 21: 1975-1997

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88: 137-146

Pörtner HO (2002a) Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarcy of thermal tolerance in animals. Comp Biochem Physiol A 132: 739-761

Pörtner H (2002b) Physiological basis of temperature dependent biogeography: tradeoffs in muscle design and performance in polar ectotherms. J Exp Biol 205: 2517-2530

Pörtner HO, Lucassen M, Storch D (2003 eingereicht) Metabolic biochemistry and the capacities of physiological and ecological function: an integrative view.

Prosser CL (1991) Environmental and metabolic animal physiology. Wiley-Liss, New York, S. 109ff

Rafael J, Braunbeck T (1988) Interacting effects of diet and environmental temperature on biochemical parameters in the liver of *Leuciscus idus melanotus* (Cyprinidae: Teleostei). Fish Physiol Biochem 5: 9-19

Righton D, Metcalfe J, Connolly P (2001) Different behaviour of North and Irish Sea cod. Nature 411: 156

Rose GA (1993) Cod spawning on a migration highway in the north-west Atlantic. Nature 366: 458-461

Scholander PF, Flagg W, Walters V, Irving L (1953) Climatic adaptation in arctic and tropical poikilotherms. Physiol Zool 26: 67-92

Schwalme K, Chouinard GA (1999) Seasonal dynamics in feeding, organ weights, and reproductive maturation of Altantic cod (*Gadus morhua*) in the southern Gulf of St Lawrence. ICES J Mar Sci 56: 303-319

Scott WB, Scott MG (1988) Atlantic fishes of Canada. Can Bull Fish Aquat Sci Nr. 219 University of Toronto Press, Toronto

Seddon W, Prosser C (1997) Seasonal variations in the temperature acclimation response of the channel catfish, *Ictalurus punctatus*. Physiol Zool 70: 33-44

Serendero-Krause IC (2000) Bedeutung der Umgebungstemperaturen für den aeroben Stoffwechsel von Kabeljau (Gadus morhua) aus Nordsee und Nord-Norwegischer See. Diplomarbeit, Fachbereich 2, Universität Bremen

Shelford VE (1913) Animal communities in temperate America. Chicago

Shelford VE (1931) Some concepts of bioecology. Ecology 12(3): 455-467

Sidell W, Driedzic WR, Stowe DB, Johnston IA (1987) Biomedical correlations of power development and metabolic fuel preferenda in fish hearts. Physiol Zool 60: 221-232

Sokolova IM, Pörtner HO (2001) Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. obtusata* from different latitudes and shore levels. Mar Biol 139: 113-126

Somero GN, Fields PA, Hofmann GE, Weinstein RB, Kawall H (1998) Cold Adaptation and Stenothermy in Antarctic Notothenioid Fishes: What Has Been Gained and What Has Been Lost? In: di Prisco G, Pisano E, Clarke A (Hrsg.) Fishes of Antarctica. Springer Verlag Italia, Milano, S.97ff

Sommer AM, Klein B, Pörtner HO (1997) Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina* (L.). J Comp Physiol B 176: 25-35

Sommer AM, Pörtner HO (2002) Metabolic cold adaptation in the lugworm *Arenicola marina* (L.): comparison of White Sea and North Sea population. Mar Ecol Prog Ser 240: 171-182

Southward AJ, Hawking SJ, Burrows MT (1995) Seventy years` observation of changes in distribution and abundance of zooplancton and intertidal organisms in the western englich channel in relation to rising sea temperature. J therm Biol 20: 127-155

Storelli C, Acierno R, Maffia A (1998) Membran lipid and protein adaptations in Antarctic fish. In: Pörtner HO, Playle RC (Hrsg.) Cold Ocean Physiology. Cambridge University Press, S: 166ff

Tomkiewicz J, Lehmann KM, St.John, MA (1998) Oceanographic influences on the distribution of Baltic cod, *Gadus morhua*, during spawning in the Bornholm Basin of the Baltic Sea. Fish Oceanogr 7(1): 48-62

Torres J, Somero G (1988) Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. Mar Biol 98: 169-180

Tylor S, Sidell BD (1984) Changes in mitochondrial distribution and diffusion distances in muscle of goldfish upon acclimation to warm and cold temperatures. J exp Zool 232: 1-9

van den Thillart G, Modderkolk J (1978) The effect of acclimation temperature on the activation energies of state III respiration and on the unsaturation of membrane lipids of goldfish mitochondria. Biochim Biophys Acta 510: 38-51

van Dijk PLM, Hardewig I, Tesch C, Pörtner HO (1999) Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic, and eurythermal temperate eelpouts (Zoarcidae). J Exp Biol 202: 3611-3621

Weinstein RB, Somero GN (1998) Effects of temperature on mitochondrial function in the Antarctic fish *Trematomus bernachii*. J Comp Physiol 168: 190-196

Wohlschlag DE (1960) Metabolism of an Antarctic fish and the phenomenon of cold adaptation. Ecol 41: 287-292

Yeager DP, Ultsch GR (1989) Physiological regulation and conformation: a Basic program fort he determination of critical points. Physiol Zool 62(4): 888-907

Zielinski S, Pörtner HO (1996) Energy metabolism and ATP free-energy change of the intertidal worm, *Sipunculus nudus*, below a critical temperature. J Comp Physiol B 166: 492-500

Danksagung

Mein Dank gilt Herrn Prof. Dr. H.O. Pörtner für die gute wissenschaftliche Betreuung und vor allem für seine stete Diskussionsbereitschaft. So manches Mal gingen die Diskussionen über wissenschaftliche Belange hinaus, bei denen mein fränkisches Temperament zum Glück „nit bös“ aufgefasst wurde.

Herrn Prof. Dr. W.E. Arntz und Herrn Prof. Dr. G.O. Kirst möchte ich für die Übernahme der Begutachtung und die Zusage für die Prüfungskommission dieser Arbeit danken.

Herrn Dr. F.J. Sartoris danke ich für die gute Betreuung. Zudem konnte er mit aufmunternden Lebensweisheiten wie „Alles wird gut“ meine Stimmung bessern, und vor allem habe ich es ihm zu verdanken, dass ich es doch noch auf die Polarstern geschafft habe. Schade fand ich nur, dass er meine kreativen Wort-Neuschöpfungen nicht so ganz nachvollziehen konnte ☺.

Ein besonderes Dankeschön geht an Herrn Dr. Christian Hermann Bock (so viel Zeit muss sein!), der mir nicht nur in wissenschaftlichen Belangen zur Seite stand und steht.

Weiterhin gilt mein Dank

Torsten F.: für die unterhaltsamen Labornachmittage mit „summer jam“ und neuen Geschichten aus Fishtown.

Daniela N. S.: Wir hatten eine schöne Zeit, sei es im Labor, zum „Stress-Abrocken“ im Yesterday oder beim Kuchen- und Plätzchenbacken; vor allem in der Endphase unserer Labortätigkeit, in der wir sprichwörtlich Hand-in-Hand arbeiteten.

Timo H.: ohne viel Worte: Du bist die gute Seele der Arbeitsgruppe! …, und danke für den „Frodo-Zug“. ☺

Rolf W.: für alle Rechnerfragen und Rettungen in letzter Not.

Boris K.: für diverse Norwegen-Trips und für das illustrationsreiche Handbuch zum Aquarium-Container; trotz so kleiner Reibereien, kann man „Yoda“ nicht lange böse sein.

Magnus L.: für die biochemische Hilfestellung und für die Organisation des Stressabbaus durch Freizeitsport.

Olaf H.: für die stete Hilfsbereitschaft und Aushilfe, sei es mit Keksen oder Optoden.

Eva B.: für so manch` beantwortete ökologische Frage und natürlich für die „ereignisreiche“ Kutterfahrt, die ja trotz widriger Wetterlage doch noch geklappt hat.

Frank M.: für seine ruhige Hand als „OP-Schwester“ und für den Schokoladenvorrat, obwohl wir uns über die Geschmacksrichtung nicht ganz einig werden konnten.

Felix M.: für seine Lektortätigkeit und seiner Kreativität in der Namensgebung; von Liesl zu Gislbert.

Nils K.: dass Du Hugo in Deine Obhut übernommen hast.

Carsten B.: für die Twin Peaks Collection

Barbara N.: für konstruktive Raucher-Eck-Gespräche

Und natürlich allen anderen aus der Arbeitsgruppe und Kaffeerunde für die nette Atmosphäre!