Review article

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*)

H.O. Pörtner[a,*], B. Berdal[b], R. Blust[c], O. Brix[b], A. Colosimo[d], B. De Wachter[c], A. Giuliani[d], T. Johansen[e], T. Fischer[a], R. Knust[a], G. Lannig[a], G. Naevdal[e], A. Nedenes[b], G. Nyhammer[e], F.J. Sartoris[a], I. Serendero[a], P. Sirabella[d], S. Thorkildsen[b], M. Zakhartsev[c]

[a] *Departments of Ecosystems Research and of Ecophysiology, Alfred-Wegener-Institute for Polar and Marine Research, D-27515, Bremerhaven, Germany*
[b] *Departments of Zoology and University of Bergen, Norway*
[c] *Department of Biology, RUCA, Antwerp, Belgium*
[d] *Department of Biochemistry, University of Rome "La Sapienza", Italy*
[e] *Fisheries and Marine Biology, University of Bergen, Norway*

Abstract

Effects of global warming on animal distribution and performance become visible in many marine ecosystems. The present study was designed to develop a concept for a cause and effect understanding with respect to temperature changes and to explain ecological findings based on physiological processes. The concept is based on a wide comparison of invertebrate and fish species with a special focus on recent data obtained in two model species of fish. These fish species are both characterized by northern and southern distribution limits in the North Atlantic: eelpout (*Zoarces viviparus*), as a typical non-migrating inhabitant of the coastal zone and the cod (*Gadus morhua*), as a typical inhabitant of the continental shelf with a high importance for fisheries.

Mathematical modelling demonstrates a clear significant correlation between climate induced temperature fluctuations and the recruitment of cod stocks. Growth performance in cod is optimal at temperatures close to 10°C, regardless of the population investigated in a latitudinal cline. However, temperature specific growth rates decrease at higher latitudes. Also, fecundity is less in White Sea than in

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*Corresponding author. Alfred-Wegener-Institut, Marine Biology/Ecological Physiology, Postfach 12 01 61/ Columbusst., D-27568 Bremerhaven, Germany. Tel.: +49-471-4831-1307; fax: +49-471-4831-1149.

E-mail address: hpoertner@awi-bremerhaven.de (H.O. Pörtner).

North and Baltic Sea cod or eelpout populations. These findings suggest that a cold-induced shift in energy budget occurs which is unfavorable for growth performance and fecundity. Thermal tolerance limits shift depending on latitude and are characterized by oxygen limitation at both low or high temperatures. Oxygen supply to tissues is optimized at low temperature by a shift in hemoglobin isoforms and oxygen binding properties to lower affinities and higher unloading potential. Protective stimulation of heat shock protein synthesis was not observed.

According to a recent model of thermal tolerance the downward shift of tolerance limits during cold adaptation is associated with rising mitochondrial densities and, thus, aerobic capacity and performance in the cold, especially in eurythermal species. At the same time the costs of mitochondrial maintenance reflected by mitochondrial proton leakage should rise leaving a lower energy fraction for growth and reproduction. The preliminary conclusion can be drawn that warming will cause a northern shift of distribution limits for both species with a rise in growth performance and fecundity larger than expected from the Q_{10} effect in the north and lower growth or even extinction of the species in the south. Such a shift may heavily affect fishing activities in the North Sea. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

The impact of decadal-scale climate variations on marine communities and populations has been well documented (Cushing, 1982; Beamish, 1995; Bakun, 1996). Therefore, warming of the atmosphere as currently reported will affect, directly or indirectly, marine populations during all life stages. However, a mechanistic cause and effect relationship has not yet been established. Recently, a wider comparison of the mechanisms characterizing thermal intolerance between and within species has led to the development of a unifying physiological concept, with the general conclusion that oxygen limitation characterizes the first line of thermal intolerance in water breathing and possibly, air breathing animals (Pörtner et al., 2000; Pörtner, 2001). The present study attempts to detail the physiological and ecological aspects of this concept for fish, focusing on the principle mechanisms and applying them to two eurythermal species characterized by northern and southern distribution limits in the North Atlantic: the eelpout (*Zoarces viviparus*), as a typical non-migrating inhabitant of the coastal zone, with a k-selective reproduction strategy (viviparous) and the cod (*Gadus morhua*), as a typical inhabitant of the continental shelf with a high importance for fisheries. These species both have a boreal distribution. The southernmost distribution limits are, for eelpout, in the Wadden Sea of the southern North Sea and, for cod, in the northern part of the Bay of Biscay (Whitehead et al., 1984). Through a combination of data collected on ecological and physiological processes in a latitudinal cline and their respective modelling the present study not only allows to test the validity of this concept in the field but also leads to a treatment of the energetic consequences with respect to temperature effects on growth and reproduction. The principles are illustrated by comparison of the eurythermal species with the stenothermal Antarctic eelpout, *Pachycara brachycephalum*.

A comparison between summer water temperature and abundance of *Z. viviparus* in the German Wadden Sea over the last 40 years, shows a distinct relationship between hot summer events and low abundance in the following year (plotted from data by Tiews, 1984).

For cod, the effect of temperature changes differs between populations (Daan, 1994). Temperature is held responsible for most of the observed differences in growth (Brander, 1994). Populations dwelling in colder waters (Labrador, Greenland, Iceland and Barents Sea) seem to augment stock size, recruitment and growth rates when temperature is increasing. In the Arcto-Norwegian cod stock, an increased abundance of 0-group cod (August/September after spawning) associated with increased body length was recorded repeatedly during the period 1965–1992 and associated with elevated water temperatures close to 5°C (Nakken, 1994). Conversely, cold temperatures in the Barents Sea appear to limit growth performance and recruitment. Similarly, a recent collapse of cod populations occurred on Georges Bank and close to Newfoundland during a period of cooling. At the other end of the temperature spectrum, populations in warmer regions like the North Sea appear to suffer from elevated temperatures. Currently, a severe drop in the recruitment of North Sea cod has become visible which is associated with a rise in sea surface temperature above 8°C, averaged for the period February–June for each year (O'Brien et al., 2000). In the southern North Sea, cod is no longer found at high summer temperatures.

As a corollary the effect of global warming on these fish species would be large enough to be relevant for short-term catch forecasts. In addition, overfishing has led to significant drops in fish populations below the 50% level of virgin biomass and extremely low rates of recovery have been observed, for example in cod (Hutchinson, 2000). The observation of low recovery becomes explainable if normally acceptable fishing activities turn into overfishing owing to climate-induced decreases in recruitment.

For a mechanistic understanding of the effects of climate change the present study brings together retrospective studies, field data analysis, ecological and physiological experiments, genetical investigations and modelling. Since modelling activities are hampered by the complex interactions between climate and fish populations (Claireaux et al., 1995; Brander, 1996; Lindahl et al., 1998), we chose a semi-empirical and essentially data-driven approach for the modelling of ecological findings. Ecological analyses for such modelling efforts focus on recruitment, growth and fecundity of cod (*G. morhua*) and eelpout (*Z. viviparus*) from different climatic regions in field campaigns and laboratory experiments including historical information.

Since oxygen limitation characterizes the onset of thermal intolerance, temperature dependent adjustments in aerobic capacity are interpreted to shift tolerance limits associated with temperature dependent tradeoffs in energy budgets (Pörtner et al., 1998, 2000; Pörtner, 2001). These shifts might lead to changes in growth, fecundity and recruitment, ecological key processes which determine population structure and dynamics. Adjustments of oxygen transport via hemoglobin are essential in this context. Study of hemoglobin isoforms also provides information on biochemical genetic variations (Sick, 1961; Smith et al., 1990). The HbI-polymorphism of Atlantic cod hemoglobin with the genotypes HbI_{11}, HbI_{12} and HbI_{22} was described more than 30 years ago (Frydenberg et al., 1965; Sick, 1965a, b). The frequency of the two alleles HbI(1) and HbI(2) shows a north-south cline along the Norwegian coast with a frequency of the HbI(1) allele of about 10% in the Barents Sea (Arctic Norwegian cod), 20–50% along the coast of northern and western Norway, and 70% in the Kattegat (Frydenberg et al., 1965). A similar cline, although less clear, can be seen along the North American East Coast (Sick, 1965b). According to these studies, genetic differences exist linked to the respective differences in the functional properties of HbI_{11}, HbI_{12} and HbI_{22} components (Karpov and Novikov, 1980). The present study extends on the

functional analyses and includes analyses for cod from the Baltic Sea for comparison with the respective data from the other areas.

Poikilotherms also rely on cellular defence mechanisms such as increased levels of heat shock proteins (HSP) that may protect the organism against temperature induced damages. The HSP family plays a central role in protein homeostasis and protection of proteins against temperature induced protein aggregation and denaturation. As a consequence HSPs may increase temperature tolerance and play a role during acclimatisation and adaptation (Sanders, 1993, Iwama et al., 1998).

2. Material and methods

2.1. Modelling of historical data

In the present study the pattern of temporal correlations between cod recruitment, sea surface temperatures and atmospheric pressure variability as traced by the North Atlantic oscillation (NAO) index, was investigated by means of a combined use of principal component analysis (PCA) and canonical correlation analysis (CCA) in three specific areas of the North Atlantic, namely the Barents Sea (Kola region), the Baltic Sea and the North Sea. First, the principal components (also known as empirical orthogonal functions) of each time series were computed, so that most of the available information concentrated in a smaller number of new mutually independent dimensions (leading eigenmodes). Secondly, for each region a CCA was carried out over the leading eigenmodes of any possible couple of time series, in order to highlight the possible correlation channels linking the corresponding data fields.

The time series for the Kola region derived from sea surface temperatures recorded on a yearly basis at the Russian Kola Hydrographic Section between 1943 and 1995 as well as on data for Arcto-Norwegian cod recruitment (Sundby, 1998). This treatment assumes that survival of eggs and larvae hatched in the Norwegian current and of recruits drifting Northward into the Barents Sea are similarly affected by temperature. In the Baltic Sea and in the North Sea, the time series for cod recruitment (combined for western and eastern Baltic stocks, courtesy of Dr. Joachim Dippner, Warnemünde) spanned a shorter time interval: 1966–1997 and 1963–1997, respectively.) For these regions the sea-surface temperatures were obtained from the GISST (Global sea ice coverage and sea surface temperature) data-bank and the three-year running means of the NAO winter index were adopted from Sundby (1998).

The sea-surface temperature, cod recruitment and NAO time series, for each of the three above mentioned regions, were submitted to PCA and checked for the presence in the first three principal components of the largest ($\geqslant 60\%$) fraction of the total information. The subsequent canonical correlation study over the principal components' triplets allowed a straightforward comparison of global correlation patterns between temperature and cod recruitment in the three regions.

The PCA method, being not constrained by stationarity and/or harmonic constraints (Vautard et al., 1992), is particularly suited for the study of relatively short and "noisy" time series and allows dissecting of the independent contributions (the extracted modes are orthogonal by construction) to the time series under study. In order to produce the multivariate matrix required by PCA, each time series was subjected to a time delay embedding (Broomhead and King, 1986),

namely to a shift of a constant lag (equal to 1 year in our case) for n times ($6 \leqslant n \leqslant 8$). Thus, a collection of n surrogate series corresponding to delayed copies of the original series was generated and, from the resultant $n + 1$ rank matrix (having as columns the original series plus the n surrogates) the principal modes organizing the temporal variability of the series were extracted (Ghil and Vautard, 1991).

CCA (Rencher, 1992) is a technique for analysing relationships between two sets of variables, in our case the recruitment–temperature, recruitment–NAO and NAO–temperature couples of principal components triplets. CCA finds, from each of the two sets, a linear combination of variables, called a canonical variable, such that the correlation between the two canonical variables is maximized. This correlation is the first canonical correlation, and CCA continues by finding a second pair of canonical variables, independent of the first one, that produces the second highest correlation coefficient. The process goes on until the number of pairs of canonical variables equals the number of variables in the smaller set, and permits the identification of canonical correlations as independent "correlation channels" between the two sets, corresponding to distinct mechanisms through which the linkage between sets takes place (Rencher, 1992).

2.2. Ecological studies

Growth performance and fecundity of cod and eelpout were calculated from literature data and from field data collected in 1998 and 1999 in various regions (Norwegian coast, Arctic, White Sea and southern North Sea). Growth rates in the field were evaluated in this study from reading growth rings on otoliths. The number of eggs in the gonads of cod (Bleil and Oeberst, 1993) and the number of larvae of eelpout (this study) were estimated as fecundity parameters. Growth experiments at different temperatures were carried out with Norwegian coastal cod (CC), North East Arctic cod (NEAC) and with cod from the southern German Bight. Offspring of broodstocks from CC and NEAC (spring 1997) were raised under laboratory conditions from spring 1998 to March 1999. The fish were individually tagged (PIT tags), kept at three different water temperatures: 8°C, 12°C and 15°C, and fed ad libitum with commercial dry food (Nor/Aqua). Total body weights and total lengths of the cod were measured once every 6 weeks and covered the size range between 120 and 462 g for North Sea cod or 130–669 g for CC and NEAC at the onset of the experiments. The experimental design for German Bight cod differed slightly: the fish were raised from April to June 1999 and tagged with floy tags, an additional experiment was carried out at 4°C and body weights and lengths were measured once every 3 weeks.

2.3. Physiological studies

Eelpout from the German Bight and the Baltic (Kiel bight) were acclimated at 11°C. Upper lethal temperatures were determined in Baltic and North Sea eelpout as the temperature values at which loss of equilibrium or the onset of spasms were observed during an acute temperature increase (1°C/min). This experiment was repeated for 5 consecutive days to test for heat hardening effects. Oxygen consumption (M_{O_2}) and ammonia excretion (AE) of Baltic eelpout were measured in a closed respirometer during a slow temperature increase (1 °C/2 days). The ammonia quotient ($AQ = AE/M_{O_2}$) was calculated to indicate a potential imbalance in nitrogen metabolism.

Oxygen consumption in Antarctic eelpout (*Pachycara brachycephalum*) and North Sea common eelpout (*Z. viviparus*) were measured by intermittent flow or flow through respirometry (Clark type electrodes, Eschweiler, Kiel, Germany) keeping water oxygen levels above 80% air saturation at all times. In parallel experiments fish were exposed to increasing temperatures starting from 0°C in Antarctic eelpout and from 3°C (acclimation temperature) in North Sea eelpout. Temperature increments per day were by 1°C in Antarctic and by 3°C in North Sea eelpout. At each temperature tissue samples were collected from fish killed after anaesthesia using a solution of MS 222 in sea water at 0.25 g/l. Samples were freeze-clamped immediately and stored in liquid nitrogen. Succinate as an indicator of oxygen deficiency at the mitochondrial level was analysed using standard enzymatic procedures (Bergmeyer, 1985).

For an analysis of the temperature dependence of oxygen transport to tissues blood P_{O_2} was monitored online with implanted fiber optic oxygen sensors (optodes, Pre Sens, Neuburg a. d. Donau, Germany). Oxygen microoptodes were implanted in the gill arches of cod, following the principles of cannulation outlined by Larsen et al. (1997). The tip of the optode was coated with teflon to shield the sensor from the oxygen independent fluorescent signals caused by the degradation products of hemoglobin. The long-term stability of these optodes enabled online measurements of P_{O_2} for more than one week at a measurement frequency of 1/min. The results were tested against a model of the physiological mechanisms underlying thermal tolerance, which has been developed based on analyses of temperature dependent changes in haemolymph P_{O_2} in crustaceans (Frederich and Pörtner, 2000; Pörtner, 2001).

Cod used for hemoglobin studies were collected in the southern North Sea (February 1999), and in the Baltic Sea (Kiel bight, Bornholm, and South of Gotland and North of Gdansk) in April 1999. Blood was sampled from anaesthetised fish (Metacainum, 0.7–0.9 g/10 l sea water, Norsk Medisinal Depot) by cardiac puncture with ice-cold heparinized syringes. About 0.2 ml of blood from each fish was used for genotyping by gel electrophoresis (Sick, 1965a, b; Jørstad, 1984) and isoelectric Multiphor II-system (Pharmacia). In total 803 fish were genotyped.

Washing and stripping of the erythrocytes (RBC) were carried out as described by Brix et al. (1998). Stripped hemoglobin was equilibrated with Hepes buffer (100 mmol/l) containing NaCl (100 mmol/l) at pH 7.5 and 8.0. pH was measured with a micro pH-meter system (BMS2 Mk2, Radiometer). Hemoglobin concentrations were 0.200–0.675 mg/ml (DADE) and thus in the range recommended by Giardina and Amiconi (1981) for the tonometric method (100–0.1 mg/ml). The chloride concentration was about 90 mmol/l (CMT10 Chloride titrator, Radiometer) and ATP was added to a level of 5 mmol/l. Immediately before analysis the sample was then adjusted to the appropriate pH (7.5 or 8.0). An aliquot of 0.5 ml was transferred to a modified glass tonometre thermostatted to 4°C and 8°C and equilibrated with various gas mixtures. Oxygen binding was analysed at 541, 560 and 576 nm a thermostatted scanning photometer (Beckman model 25) according to Brix et al. (1998).

Gene frequencies were calculated on hemoglobin allele basis, the homozygote having two allele's each (1–1 and 2–2 respectively) and the heterozygote having one of each (1–2). The saturation, Y, was calculated as described by Brix et al. (1998). The oxygen tension for half saturation, P_{O_2}, was estimated from Hill plots by linear regression analysis.

Heat shock proteins (HSP70) were measured in the livers of cod (from the Arctic, Norwegian coast or German Bight) grown at 8°C, 12°C and 15°C and in North Sea cod exposed to an acute temperature increase of 1°C/day from 11°C to 16.5°C. HSP70 levels in the liver were measured

with an indirect non-competitive ELISA. After homogenisation and centrifugation (16 000 g) sample supernatants were diluted with coating buffer (15 mmol/l Na_2CO_3, 39 mmol/l $NaHCO_3$, 0.02% NaN_3, pH 9.6) to a total protein concentration of 35 μg/ml; boiled for 5 min and coated on microtiterplates (COSTAR, Cambridge MA, USA) overnight at 4°C. After washing (10 mmol/l Tris, 150 mmol/l NaCl, 0.1% NaN_3, 0.05% Tween 20, pH 7.2) wells were blocked (6 h, 20°C) with 3% BSA, washed again and incubated with monoclonal anti-HSP70 (BRM22 Sigma, 1/500 dilution) at 4°C overnight. Subsequently, plates were washed and incubated with anti IgG alkaline phosphatase conjugate (Sigma, 1/2000 dilution) for 2 h at room temperature, washed again and incubated with freshly prepared substrate buffer. Absorbance was measured at 405 nm (BioRad 3550-UV microplate reader) and concentrations calculated.

2.4. Calculations

Differences were tested for significance using Student's *t*-test, analysis of variance (ANOVA, SuperAnova, Abacus concepts 1991) or analysis of covariance (ANCOVA, SuperAnova, Abacus concepts 1991) and the Student–Newman–Keuls posthoc test at the $p < 0.05$ level. Data are given as means $\pm$ standard deviation.

3. Results

Fig. 1 shows the first three components of the cod recruitment and sea-surface temperatures concerning the Kola region, together with the reconstruction of the original time series as linear combinations of those components. The results obtained for the other regions are qualitatively identical. It is evident from the figure that the first three components are both trend-like and quasi-harmonic (actually they appear in the form of sine–cosine associated pairs) and allow an almost complete reconstruction of the original series. Table 1 shows the result of the CCA carried out over the three main principal components of the time series for each of the studied regions. Highly significant correlations between cod recruitment and temperature fluctuations over time emerge for all three regions. In the Kola region this correlation takes place along two distinctly independent correlation channels which correspond to the two significant canonical correlations between cod recruitment and temperature. In North Sea and Baltic regions the data highlight the presence of only one statistically significant mechanism of correlation between cod recruitment and temperature. The NAO and temperature spaces are fully interconnected (canonical correlations near unity between NAO and temperature spaces) in all the three areas, thus confirming, from an empirical data-driven point of view, the importance of this global-scale climate mode on local temperature dynamics. Despite the strong link existing between NAO and temperature spaces, cod recruitment and NAO spaces did not show any significant canonical correlation. This result shows that temperature is the climate "variable" most directly acting on cod populations.

Fecundity and growth performance in both eelpout and cod decrease with increasing latitude (Figs. 2 and 3). The drop in the number of eggs in cod does not appear to be compensated for by an increase in egg size since a clear effect of ambient temperature on egg size is not apparent (Brander, 1994). Similarly, preliminary data available on the larval body lengths of viviparous
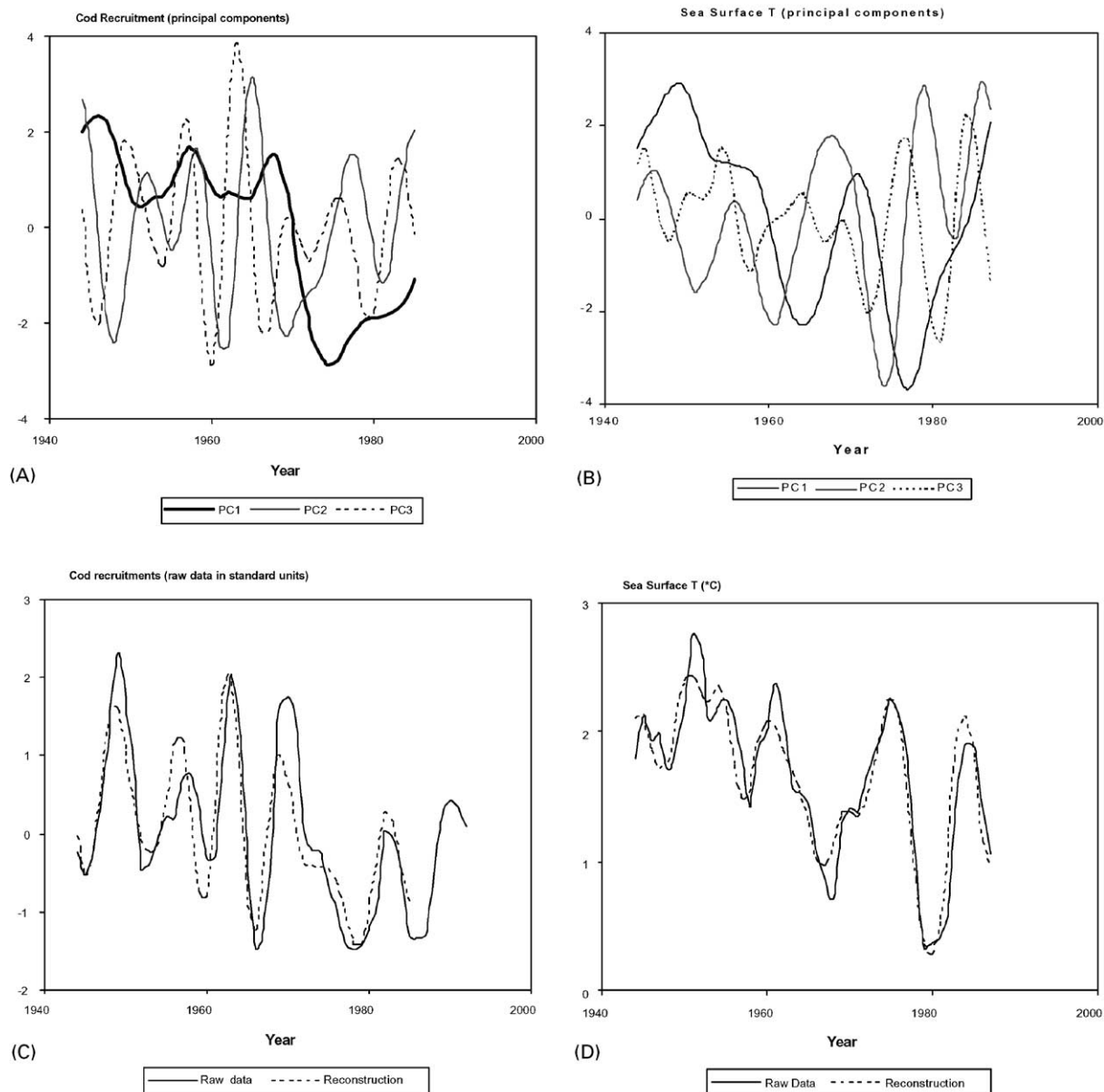
Fig. 1. Temperature and cod recruitment time series in the Kola region: raw data and principal components (see Table 1). The two left panels refer to cod recruitment: (A) shows the first three principal components of the temperature time series, and (C) contains the original time series as well as its reconstruction solely by means of the three components in (A). The two left panels refer to temperatures, and have been drawn according to identical criteria. As for cod recruitment, the fractions of the total variability explained by PC1, PC2 and PC3, were, 33.6%, 28.8% and 27.0%, respectively. The corresponding values for the temperature principal components were 42.8%, 33.6% and 16.0%.

Table 1
Canonical correlations analysis of the first three principal components of cod recruitment and environmental time series[a]

Kola region

	Temp./Recr. (0.0001)	NAO/Recr. (0.1919)	NAO/Temp. (0.0001)
Can. correlation 1	0.671 ($<$0.0001)	0.419 (0.192)	0.906 (0.0001)
Can. correlation 2	0.535 (0.003)	0.330 (0.271)	0.854 (0.0001)
Can. correlation 3	0.311 (0.051)	0.148 (0.363)	0.462 (0.002)

North Sea region

	Temp./Recr. (0.0048)	NAO/Recr. (0.1756)	NAO/Temp. (0.0001)
Can. correlation 1	0.770 (0.005)	0.603 (0.176)	0.800 (0.0001)
Can. correlation 2	0.326 (0.062)	0.288 (0.714)	0.718 (0.0001)
Can. correlation 3	0.010 (0.962)	0.061 (0.766)	0.523 (0.0007)

Baltic region

	Temp./Recr. (0.0001)	NAO/Recr. (0.2144)	NAO/Temp. (0.0001)
Can. correlation 1	0.878 ($<$0.0001)	0.538 (0.214)	0.859 (0.0001)
Can. correlation 2	0.466 (0.213)	0.377 (0.287)	0.809 (0.0001)
Can. correlation 3	0.199 (0.363)	0.295 (0.172)	0.445 (0.033)

[a] The table reports the correlation coefficients, together with their significance values, relative to the pairs of canonical variables indicated in the column labels. The statistically significant correlations ($p \leqslant 0.05$) are underlined. The significance values attached to column labels indicate the global significance of the comparison provided by the most conservative among a panel of tests, namely Wix-lamda, Pillai's trace, Otelling-Lawley and Royce max. root, suggested by Rao (1973) for the multivariate linear regression. As for the significance of single regressors, the estimate is based on Rao's modified approximate F statistic (Rao, 1973).

eelpout from the Norwegian and German Wadden Seas indicate that there is no difference (E. Brodte, pers. comm.). However, the number of offspring per female eelpout was significantly lower in the White Sea than in eelpout of the same age in the German Wadden Sea. A two year old female eelpout from the German Wadden Sea gave birth to 60–100 juveniles, whereas eelpout from the colder White Sea only reached this level of fecundity at an age of 5–6 years. Cod and eelpout populations living in colder waters displayed a significantly lower growth performance than cod from the southern North Sea (Fig. 3). The growth experiments confirmed this climate dependent growth performance in cod. In all three populations (Norwegian coastal cod, North East Arctic cod and cod from the German Bight) the highest growth rates were measured between a water temperature of 10–11°C. At this optimal temperature the cod population of the German Bight displayed a significantly higher growth performance than the populations from the Norwegian coast and from Arctic waters (Fig. 4). On the basis of the mean water temperatures in Norwegian coastal waters and in the southern North Sea and on the basis of the data obtained in growth experiments, the growth performance of the two populations in the field were estimated. The growth curves estimated for German Bight and Norwegian coastal cod fit those calculated
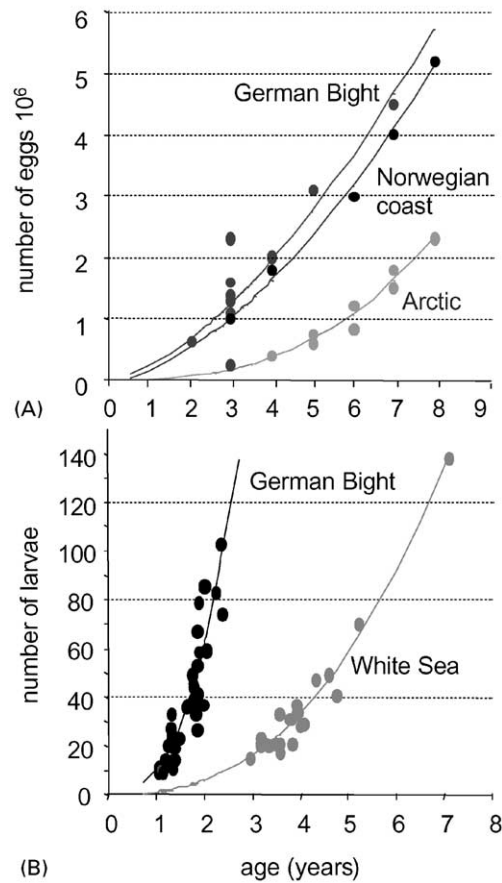
Fig. 2. (A) Relationship between fecundity (number of eggs) and age (years) in cod (*Gadus morhua*) in different climatic regions. German Bight: data by Schopka (1971) and T. Fischer and R. Knust; Norwegian coast: Botros (1962); Arctic: Kjesbu et al. (1998); (B) Relationship between fecundity (number of larvae) and age (years) in common eelpout (*Zoarces viviparus*) in the German Bight and the White Sea (data by J. Ulleweit and R. Knust).

from field data (Fig. 3B). Such an analysis proved impossible for North East Arctic cod, due to the incomplete data basis on mean water temperatures for that area.

Temperatures for the onset of spasms differed significantly between Baltic ($28.0 \pm 1.0°C$, $n = 7$) and North Sea eelpout ($29.1 \pm 0.2°C$, $n = 5$, t-test, $p < 0.01$). However, this difference was only present during the first day. From day two on no difference between both populations could be established (data by M. Zakhartsev, B. De Wachter and R. Blust).

According to a previously developed model (Fig. 5) the first line of thermal intolerance is most likely defined by limited oxygen availability to tissues (see Section 4). The availability of oxygen in the tissues depends upon oxygen uptake by ventilation and oxygen distribution via the circulatory system. Capacity limitations of ventilation and circulation are responsible for the development of progressive hypoxia during warming when heart rate and especially ventilation operate at maximum capacity until beyond the high T_c anaerobic metabolism set in (Frederich and Pörtner,
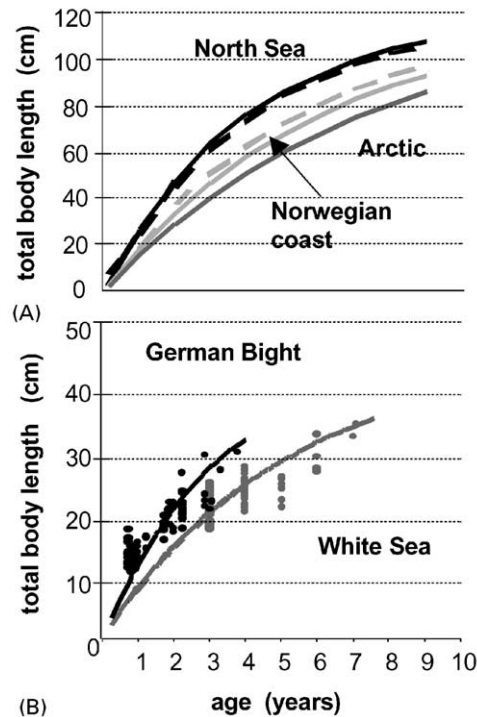
Fig. 3. A: Relationship between age (years) and body length (cm) in cod, *Gadus morhua*. Comparison of field data (solid line) and growth rates predicted from experimental growth studies (dotted line) in fish from various regions. Field data for the North Sea by Daan (1974), for the Norwegian coast by Botros (1962) and for the Arctic by Michaelsen et al. (1998). Experimental data for the North Sea by T. Fischer and R. Knust, for the Norwegian coast and the Arctic by T. Johansen and G. Naevdal. B: Relationship between age (years) and body length (cm) in common eelpout, *Zoarces viviparus* in the German Bight and the White Sea (data by J. Ulleweit and R. Knust).

2000). In cod the preliminary data suggest that temperature does not exert a strong influence on arterial P_{O_2} however venous P_{O_2} showed a linear drop during progressive warming (Fig. 6). Oxygen deficiency at the mitochondrial level characterizes the T_c and became visible in liver (Fig. 7B), an organ with a high mitochondrial density and a large, usually aerobic energy (oxygen) demand. This T_c was found between 21°C and 24°C in North Sea eelpout and at about 9°C in Antarctic eelpout, at temperatures below the onset of spasms.

Oxygen consumption analyses in North Sea and Antarctic eelpout revealed an exponential rise with increasing temperature. When North Sea eelpout were acclimated to cold temperatures their temperature specific rate of oxygen consumption rose to similar levels as in Antarctic eelpout. Owing to lower levels of Arrhenius activation energies in the metabolic rate of the North Sea species, a drastic rise in metabolic rate only occurred beyond 22°C whereas the Antarctic eelpout displayed such a drastic increase already when temperature approached 9°C (Fig. 7A).

Only a small temperature effect on the ammonia quotient (ratio of ammonia excretion over oxygen consumption rates) was found (ANOVA, $p < 0.05$). The ammonia quotient in Baltic eelpout decreased significantly from 0.13 ± 0.02 at 11.6°C to 0.07 ± 0.02 at 13.2°C and increased
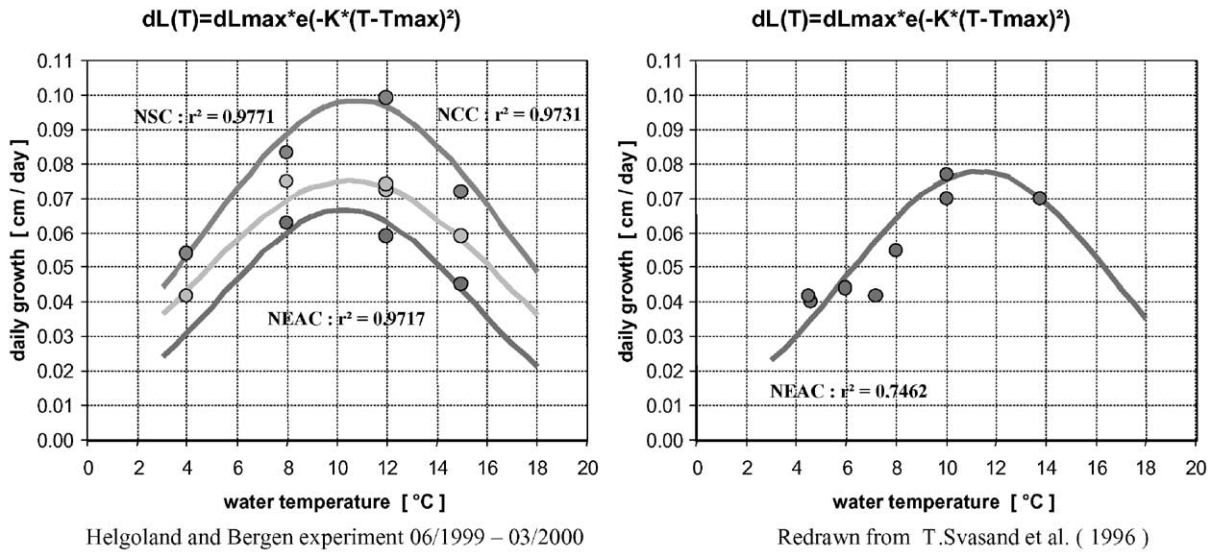
$$dL(T)=dLmax*e(-K*(T-Tmax)^2)$$

Fig. 4. Relationship between growth parameters and temperature (°C) for cod (*Gadus morhua*) from different climatic regions as derived from experimental growth studies. German Bight data by T. Fischer and R. Knust. Norway coastal and Norway arctic waters data by T. Johansen and G. Naevdal. The relationship between water temperature and daily growth rates were recalculated from daily growth data and water temperatures given in Svasand et al. (1996).
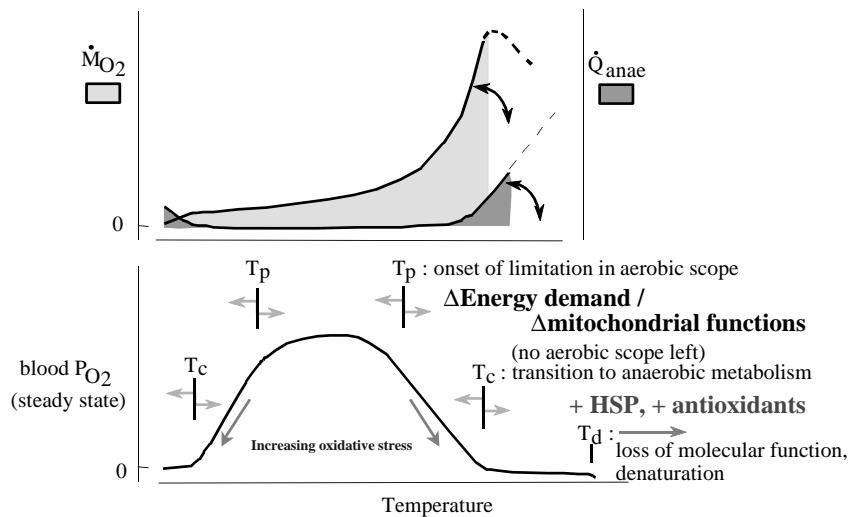

Fig. 5. Model of oxygen limitation of thermal tolerance adopting the law of tolerance (modified after Frederich and Pörtner, 2000; Pörtner, 2001). Pejus temperature (T_p) and critical temperatures (T_c) are set by the capability of oxygen supply to meet tissue oxygen demand and, therefore, depend on ventilatory and circulatory performance. Heat shock proteins and antioxidative defense may extend tolerance to high (and low) thermal extremes by shifting denaturation temperatures (T_d), but are likely not to influence pejus temperatures. The model is supported by studies on fish and on marine invertebrates of different phyla (see text).
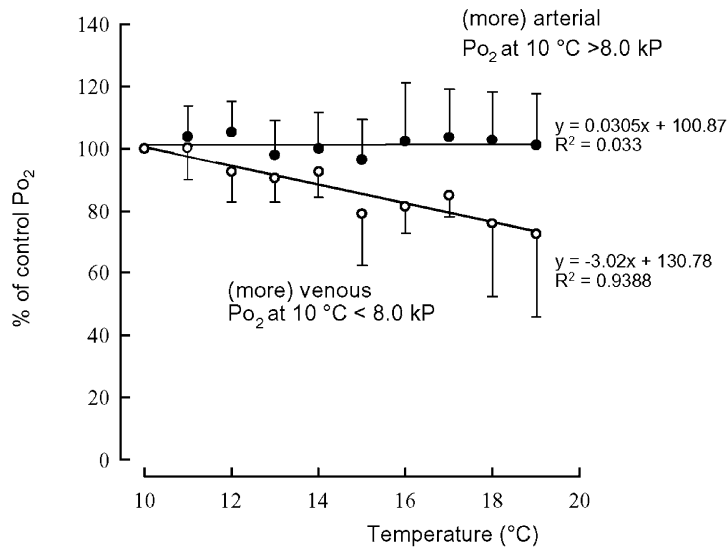
Fig. 6. P_{O_2} decrease in cod gill blood (quantified as % of initial P_{O_2}) during warming by $1°C\,h^{-1}$. When according to low P_{O_2} readings a large fraction of venous blood influenced P_{O_2} measurements a stronger, significantly different temperature dependence became visible (means $\pm$ SD, data by I. Serendero, G. Lannig, F. J. Sartoris and H. O. Pörtner).

again to 0.15 ± 0.02 at $17°C$ (ANOVA, $p < 0.05$, df: 27, $n = 4-6$) with no clear correlation with the onset of mortality.

The results of the hemoglobin studies in cod are shown in Fig. 8. The HbI(1) allele frequency distribution showed a marked gradient from west to east from 66% at the White Bank to 1% at Gotland and Gdansk and confirmed previous gene frequency analysis, in that it shows an increased fraction of HbI_{22} in the Baltic Sea from east to west with falling temperature. Animal weights and lengths in the samples obtained from the North Sea population were significantly larger than the respective data for the Baltic Sea population. Furthermore, heterozygotic animals were larger than the homozygotes, and HbI_{22} animals had the lowest weights and smallest sizes. In the Baltic Sea, however, HbI_{22} fish were larger and heavier than HbI_{11} fish, and there was an increase in weight and length from west to east in the Baltic Sea.

Oxygen affinities were determined for the three genotypes at $4°C$ and $8°C$ at pH 7.5 and 8.0. In the figure the change in P_{O_2} values between pH 7.5 and 8.0 (ΔP_{50}) is shown for the two homozygotes for a comparison of the O_2 unloading potential at $4°C$ or $8°C$, respectively. Typical for population comparisons we found relatively high standard deviations for P_{50} (5.47 on average). However, our results show a clearly higher unloading potential of HbI_{22} compared to HbI_{11} at $4°C$, while there were no differences at $8°C$. These conclusions are valid with similar arteriovenous differences in pH and Po_2 in all populations. The pH dependence expressed as the Bohr factor ($\Delta \log P_{50}/\Delta pH$) was very high for all genotypes (means $\pm$ SD $= -0.91 \pm 0.06$, -0.90 ± 0.12, and -0.72 ± 0.06, for HbI_{11}, HbI_{12} and HbI_{22}, respectively), but there were no differences according to temperature or habitat.

No effect of an acute temperature increase on liver HSP70 could be observed in cod (ANOVA, $p > 0.05$, $n = 24$). However, a 10 months acclimation to $8°C$, $12°C$ and $15°C$ revealed that sex and
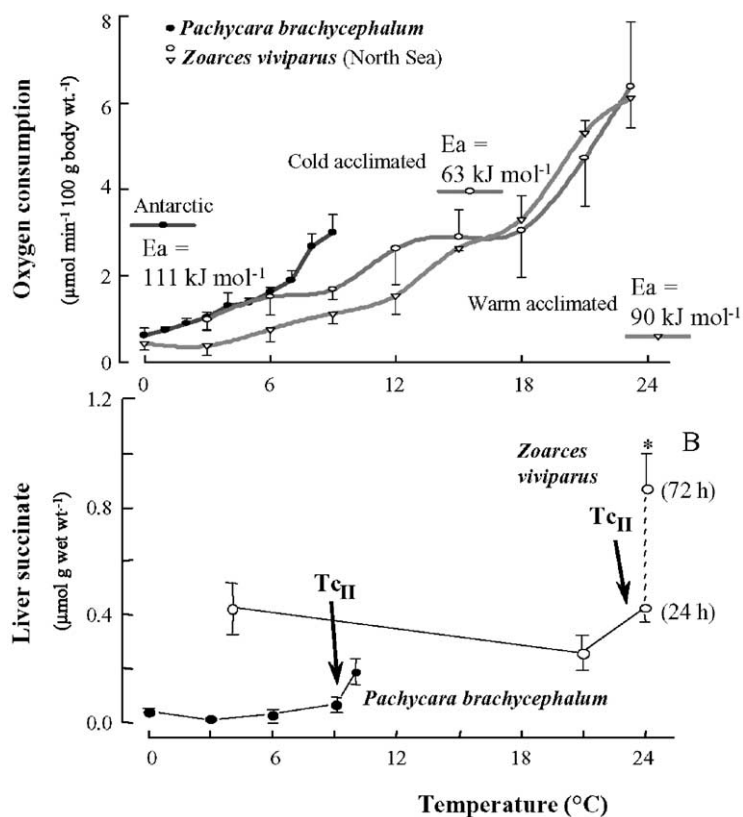
Fig. 7. Changes in (A) the rates of oxygen consumption (standard metabolic rate) of Antarctic and common eelpout compared to (B) succinate levels in the liver (modified after van Dijk et al., 1999). Note the drastic rise in oxygen consumption during the period when succinate accumulation indicates the onset of mitochondrial oxygen deficiency.

temperature both had a significant influence on HSP70 level (ANOVA, $p < 0.001$ for sex and $p < 0.01$ for temperature). Females had higher levels (29.8 ± 8.9 ng equivalents/μg protein, $n = 93$) than males (23.9 ± 8.9, $n = 85$) and animals grown at 12°C had 25% higher levels than at 8°C or 15°C. A significant correlation was found between HSP70 levels and hepato-somatic index ($r = -0.42$, $p < 0.001$). No differences existed between cod populations (Arctic, Norwegian coast or German bight) with respect to HSP70 levels.

4. Discussion

4.1. Temperature and population dynamics

PCA (Ghil and Vautard, 1991) and CCA revealed a significant influence of temperature on cod population dynamics. It is worth noting the differences between the correlation structure highlighted by our approach and the information accessible by the simple correlation coefficients
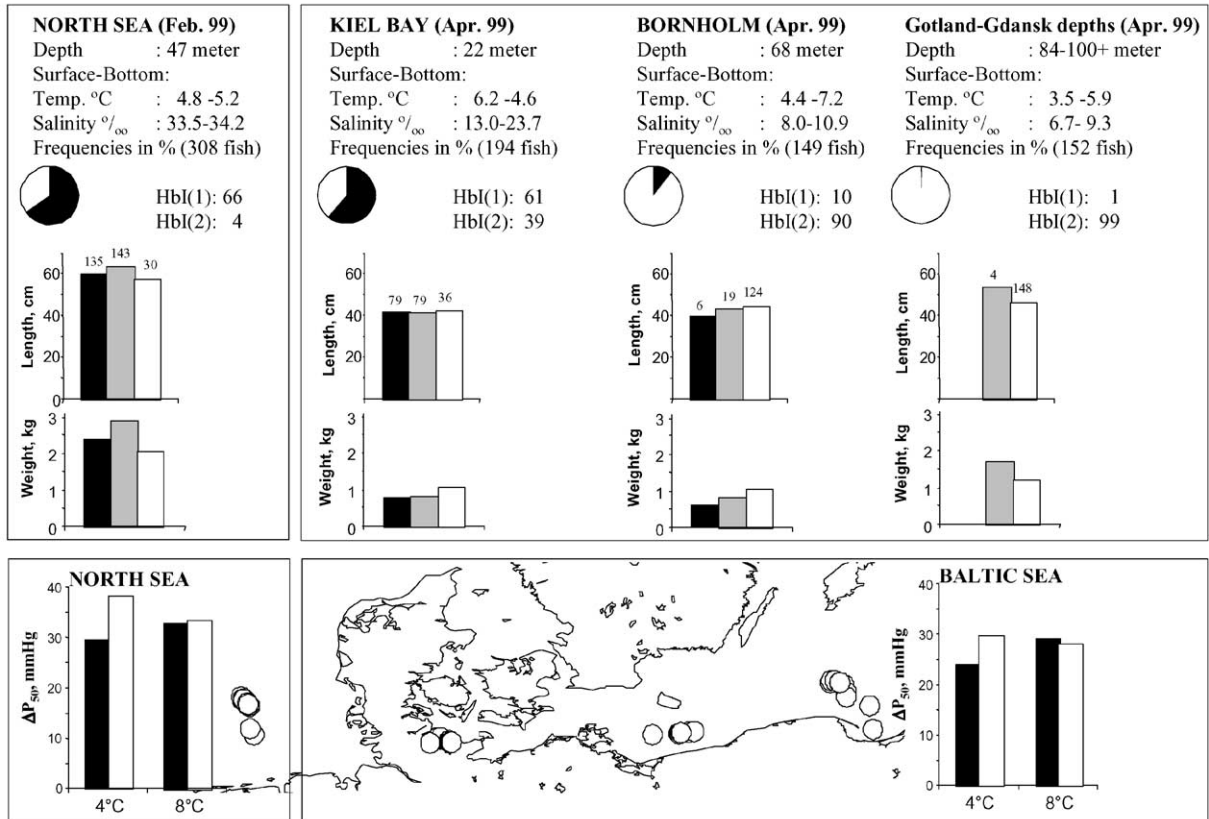
Fig. 8. HbI(1) and HbI(2) frequencies, body lengths, and growth in Atlantic cod from the North Sea and the Baltic Sea. The actual catch areas are indicated by circles. The oxygen unloading potential is calculated as the significant difference (Δ) in P_{50} values found at pH 8.0 (post branchial blood) and pH 7.5 (venous blood) at 4°C and 8°C. The different genotypes analysed from the Baltic Sea for oxygen affinity came from different locations. Five specimens were used in each case for each genotype (data by O. Brix, B. Berdel, A. Nedenes and S. Thorskildsen).

between temperature and cod recruitment series. In no case such correlation reaches statistical significance, which impairs discovering the ecological role exerted by temperature. The combined use of PCA and CCA (Table 1), however, allows both filtering the original series from their noise components and exploring the existence of complex links between biological and environmental variables. Two independent effects of temperature variability on cod recruitment emerged for the Kola region, pointing to the existence of at least two different mechanisms of comparable importance by which temperature may affect cod populations. Any reasonable hypothesis on the nature of these two mechanisms must take into account a number of physiological, metabolic, and genetic factors (see below). In the Baltic Sea and in the North Sea the situation is somewhat simpler, and the data are compatible with only one major interaction mechanism.

Although growth and fecundity fell significantly at higher latitudes the optimum temperature for growth was the same in all cod populations studied. However, despite long-term maintenance

at different temperatures, temperature specific growth rates were different between southern North Sea, Norwegian coast and North East Arctic cod. This strongly suggests a genetical difference between populations that cannot be overcome during an acclimatization process. It is well established that genetical exchange between cod populations is limited (Pogson et al., 1995; for review see Boutilier, 1998). This may well have led to the establishment of physiological characters that are no longer and can no longer be brought to complete agreement between populations. Matching growth curves for experimental and field conditions suggest that the influence of temperature on growth becomes predictable for the different populations (Fig. 3B). Similar growth rates in the lab and in the field may also indicate that growth in the field was not food limited.

Overall, the findings obtained in the ecological and statistical analyses confirm that cod and eelpout productivity (indicated by growth rates and fecundity) decreases in the range of low temperatures reached in the Barents Sea or White Sea, respectively. In these environments cod and eelpout will come closer to their lower thermal limits. Maximum growth performance found in the southern North Sea for cod and in the Baltic for eelpout might suggest that these environments are most suitable to maintain highly productive cod and eelpout populations, however, only if spring and summer temperatures remain below the upper thermal limits. Recent summer temperatures in the southern North Sea may have been too high and have forced adult cod to migrate further North. Also, spring temperatures above $8°C$ appear fatal for recruitment (O'Brien et al., 2000). For eelpout, limited survival of larger specimens in the shallow areas of its habitat, the Wadden Sea, has also been reported for warm summers (see Section 1).

4.2. Mechanisms of thermal limitation and geographical distribution

Our data do suggest that HSP70 is a poor indicator of temperature stress in cod, as differences between males and females and between the different acclimation temperatures were small ($<20\%$), and no effect of acute temperature exposure could be found. The changes observed agree with earlier findings, in which changes of HSP70 levels due to other stressors were small in fish (Koban et al., 1991; Yu et al., 1994).

Instead, oxygen limitation appears as a unifying principle suitable to explain the first limits of thermal tolerance in water breathing animals (Pörtner et al., 1998, 2000; Pörtner, 2001). Recent investigations in marine invertebrates of various phyla and in fish indicate that the progressive reduction of oxygen levels in the body fluids and, finally, the transition to an anaerobic mode of mitochondrial metabolism characterize the oxygen limitation of thermal tolerance at both low and high temperature extremes. Since oxygen supply to tissues becomes limited, the performance of ventilation and circulation likely plays an important role in defining thermal tolerance (cf. Zielinski and Pörtner, 1996; Sommer et al., 1997; van Dijk et al., 1999; Frederich and Pörtner, 2000; Figs. 6 and 7, for review see Pörtner et al., 1998, 2000; Pörtner, 2001). The transition to anaerobiosis at critical temperatures indicates strict limits of tolerance. Critical temperatures differ between species and populations depending on latitude or seasonal temperature acclimatisation and are related to geographical distribution. For example, a within species comparison of *Arenicola marina* populations in a latitudinal cline revealed that both low and high T_c's were lower in cold adapted, sub-Arctic animals from the Russian White Sea than in boreal, North Sea specimens (Sommer et al., 1997). Among fish high T_c values, indicated by the onset of

succinate formation in the liver, were found lower in Antarctic than in North Sea eelpout (van Dijk et al., 1999, Fig. 7). Also, the thermal tolerance window (between critical temperatures) is much narrower in the stenothermal Antartic than in North Sea eelpout, however, in both cases it is characterized by an exponential rise in oxygen demand and oxygen deficiency at the mitochondrial level.

Further work by Frederich and Pörtner (2000), carried out in the spider crab *Maja squinado*, reports the progressive development of oxygen limitation from a reduction towards an elimination of aerobic scope at both low and high temperature extremes. According to this model an optimum range of maximum P_{O_2} and, accordingly, oxygen availability to tissues characterizes the temperature window of optimum aerobic performance and scope for aerobic activity (Fig. 5). The model presented in Fig. 5 was developed adopting the characteristics of the "law of tolerance" (Shelford 1913, 1931) and largely, the temperature dependence of body fluid P_{O_2} as found in marine invertebrates (Zielinski and Pörtner, 1996; Frederich and Pörtner, 2000; Pörtner, H. O., T. Hirse and L. S. Peck, unpublished). Thermal tolerance becomes progressively time limited once aerobic scope starts to fall within pejus ranges (Schwerdtfeger, 1977; pejus = getting worse), prior to when anaerobic metabolism sets in. The model suggests that short term thermal tolerance is improved by antioxidative defence and heat shock proteins, especially when survival becomes a question of time due to insufficient oxygen availability, enhanced formation of oxygen radicals owing to progressive hypoxia and, at high temperature, elevated O_2 demand in the pejus range and, finally, transition to anaerobic metabolism and protein denaturation (see Pörtner (2001) for a review). Transitions from optimum to pejus range at the so-called pejus temperatures, T_p limit the temperature range of maximum aerobic activity (Fig. 5) and very likely characterize long term environmental temperature limits or the ecological tolerance range, as discussed for a crustacean (*Maja squinado*, Frederich and Pörtner 2000, based on ecological data by Dauvin et al., 1991; Sournia and Birrien, 1995). In accordance with this model, pejus temperatures well below critical temperatures could recently be defined for Antarctic and temperate eelpout as being characterized by the maximum capacity of blood circulation (F. Mark, C. Bock, F. J. Sartoris, H. O. Pörtner, unpublished). The data indicate that aerobic scope becomes limiting already at $15°C$ in North Sea eelpout acclimated to $12°C$, emphasizing our conclusion that, owing to physiological limits, this species lives at a temperature determined Southern distribution boundary in the Wadden Sea.

From a comparative point of view ventilation became limiting earlier than circulation in a crustacean in the warm (Frederich and Pörtner, 2000). The heart (being provided with oxygenated haemolymph from the gills) can only pump oxygenated haemolymph to the extent to which arterial P_{O_2} is maintained at the gills. The data obtained in cod (Fig. 6) suggest a different situation in fish in that arterial P_{O_2} remains high and independent of temperature. In fish the capacity for oxygen uptake at the gills may be maximized such that arterial oxygen uptake does not become limiting. In contrast to crustaceans, the heart in fish receives the venous return from the tissues and is not exposed to arterial blood. However, the drop in venous P_{O_2} indicates that excessive oxygen uptake from the blood is not fully compensated for by circulatory performance. In accordance with the above characterization of pejus temperature in eelpout circulatory rather than ventilatory performance likely sets the limits of thermal tolerance. These relationships need to be investigated in more detail in order to develop the full picture of thermal limitations in fish.

4.3. Tradeoffs in cold adaptation

Cold adaptation requires an adaptational shift of both, T_p and T_c values towards lower temperatures to maintain sufficient aerobic activity. T_ps, T_cs and the width of the tolerance window are likely to be set by the adjustment of density and kinetic properties of mitochondria, e.g. maximum respiratory as well as proton leakage rates and their temperature dependence (Pörtner et al., 2000). Elevated mitochondrial densities and aerobic capacities have been found in many cold-adapted ectotherms (e.g. Johnston et al., 1998) and could also be confirmed for cold adapted cod including populations at high latitudes (I. Serendero, F. J. Sartoris, T. Fischer, H. O. Pörtner, unpublished). At the low T_p and finally T_c the progressively insufficient capacity of mitochondria will contribute to functional failure, among others of ventilation and circulation, thereby explaining the breakdown of oxygen transport and aerobic metabolism (e.g. Zielinski and Pörtner, 1996; Frederich and Pörtner, 2000). Mitochondrial proliferation will provide a rise in aerobic capacity as required to meet energy demands and maintain function in the cold. However, the increase in mitochondrial density will ultimately cause a rise in oxygen demand which becomes detrimental during warming when it can no longer be covered by oxygen uptake through ventilation and circulation. Baseline mitochondrial oxygen demand without concomitant ATP production is caused by the passive influx of protons through the inner mitochondrial membrane (proton leakage) which is driven by the proton gradient set up during respiration (Brand, 1990, cf. Pörtner et al., 1998). More than 50% of cellular M_{O_2} at rest may be attributed to mitochondrial proton leakage (Brand, 1990). As a tradeoff mitochondrial proliferation in the cold may reduce energy availability to growth and reproduction owing to elevated costs of mitochondrial maintenance (see below).

Adaptive flexibility of eurythermal fish to adjust to seasonal temperature changes is not only suggested by the observation of metabolic cold compensation during winter (van Dijk et al., 1999) but also by the comparison of acute temperature tolerance between Baltic and North Sea eelpout which initially indicates a difference in thermal sensitivity. However, heat hardening increased heat tolerance in Baltic eelpout and resulted in the disappearance of the difference in temperature sensitivity between both populations. The mechanisms behind need to be investigated. However, the respective temperatures are found much above the pejus and critical values and most likely far above those ever experienced by the animals in the natural environment (cf. Pörtner, 2001). Considering the short term heating protocol, mechanisms are likely involved which support short term but not long term thermal tolerance (cf. Fig. 5).

The limiting role of circulatory performance with a non-limiting capacity for gill oxygen uptake in fish emphasizes the role of the hemoglobin as a means to optimize oxygen transport, also reflected in the expression of various isoforms depending on ambient temperature. Karpov and Novikov (1980) suggested that the HbI_{22} molecule is the most efficient O_2 carrier at low temperatures with the highest O_2 affinity, while the HbI_{11} molecule has the highest affinity at about 20°C. However, their results were based on single measurements and are in contradiction to the general view, that anodic components like HbI_{22} are expected to display a lower affinity, which could safeguard oxygen unloading over a larger P_{O_2} range. Recent unpublished data (O. Brix) confirm this general view and clearly emphasize that HbI_{22} is more suitable to function at cold temperatures than HbI_{11} by being able to transport more oxygen from the environment to the tissues. This might support a higher aerobic capacity and, in this way, an extension of the

optimum range to lower temperatures. Moreover, it could also support a higher oxygen demand in response to cold adaptation, elicited by elevated mitochondrial densities and proton leakage. In both cases the HbI_{22} genotype reflects adaptation to life in cold environments. All genotypes display a very high pH sensitivity of oxygen binding. Any temperature change would thus greatly affect oxygen affinities owing to temperature dependent pH or pK changes. Since Bohr factors close to unity were found in HbI_{11}, this genotype may not benefit from an arteriovenous pH difference, while the Bohr factor identified for HbI_{22} (-0.72) is optimal to facilitate O_2 unloading (Brix et al., 1981).

4.4. Effect of cold adaptation on energy budgets

Decreasing growth rates (at the same temperature!) with rising latitude indicate a change in energy budget unfavourable for growth. This conclusion is corroborated by the finding of a decreasing fecundity in Northern populations of cod and eelpout. Energy allocations to growth and reproduction are only possible after the requirements for physiological and biochemical homeostasis (maintenance) have been met (Wieser, 1994) including the cost of swimming and food consumption (Boutilier, 1998). The question arises which energy consuming processes are elevated in the cold and lead to decreased energy availability to growth and reproduction.

In this context the degree of metabolic cold adaptation (MCA) may play an important role. MCA is frequently observed as a rise in temperature specific metabolism in temperate zone aquatic ectotherms during winter (e.g. in eelpout, *Z. viviparus*, van Dijk et al., 1999) but is minor or absent in polar stenotherms (Clarke and Johnston, 1999). A comparison of eurythermal species in a latitudinal cline (from marine intertidal zones and shallow waters of the Northern hemisphere) with stenothermal animals (from stable polar environments of the Antarctic) suggested that the level of metabolic cold adaptation depends upon the extent of diurnal and seasonal temperature fluctuations leading to higher costs of mitochondrial maintenance in eurythermal than in stenothermal animals (Pörtner et al., 2000). Also, at constant levels of eurythermy, cold adapted populations of the same species would exhibit elevated costs of mitochondrial maintenance.

The following hypothesis arises: As a tradeoff, increased fractions of oxygen and energy are allocated to the maintenance of elevated mitochondrial densities in the cold, especially in eurythermal animals. This might lead to a reduction of growth performance and fecundity which is larger than expected from the principle rate dependence of temperature alone, as evidenced for cod and eelpout. The minimization of this tradeoff may explain why in a latitudinal cline MCA is no longer evident in polar stenotherms. A more detailed elaboration of the cellular energy budget is therefore required to come to a clear picture of the tradeoffs involved in cold adaptation. Accordingly, a fish bioenergetics model needs to be developed in order to account for the effects of temperature on the physiological conditions and growth performance of cod and eelpout. The fluxes of energy in and out of the organisms are modelled in terms of metabolism, assimilation, excretion, growth and reproduction. Energy is converted into biomass using the caloric density of predator and prey. This "macroscopic" part of the model is coupled to a "microscopic" part describing the effects of temperature on cellular processes

like mitochondrial energy turnover and demand and its thermal sensitivity as well as protein synthesis.

5. Conclusions

As a consequence of the tradeoffs associated with the adjustment of upper and lower thermal tolerance limits the following predictions arise for Atlantic cod and common eelpout: Cold acclimation and adaptation at the population level leads to higher mitochondrial densities and capacities and thereby, elevated mitochondrial maintenance metabolism at constant level of eurythermy. These differences appear to be genetically fixed between populations of the same species. They may explain a species specific or even population specific sensitivity to heat and cold. They may also explain the overproportional reduction in growth performance and fecundity observed in Northern populations in both field and experimental studies.

As a preliminary picture for Atlantic cod and common eelpout, global warming will lead to a northward shift of populations. These species will experience increased growth performance and fecundity at more northern latitudes as water temperatures rise. The expected shift in energy budgets suggests that this stimulating effect of warming on growth in Northern populations may be larger than expected from the typical rate effect of temperature on biochemical reactions (the Q_{10} effect) alone. However, if polar water temperatures remain the same as of today the limitation of growth performance and fecundity by cold temperatures in the North together with a more restricted range of geographical distribution will lead to a reduced overall population size of the species.

Further research will need to follow this line of thought. The specific tradeoffs involved in cod and eelpout performance in a latitudinal cline require further study at the mitochondrial and whole animal level. Changes in the energy budget and the mechanisms behind need to be quantified for a complete understanding of the tradeoffs involved in temperature adaptation and the limitation of adaptation processes.

Acknowledgements

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