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A2C5–SEASONAL AND LATITUDINAL ADAPTATIONS TO TEMPERATURE: MECHANISMS, CONSTRAINTS AND FISHERY IMPLICATIONS

**Organised by N.M. Whitely and H.O. Pörtner for the Animal Respiration and Thermal Biology
Groups**

A2/C5.1–Climate oscillations and recruitment in fish: results of modelling

A. Colosimo, P. Sirabella, University 'La Sapienza', Rome; A. Giuliani, 1st. Sup. Sanità, Rome, J. Dippner, Inst. Fur Ostseeforschung, Warnemunde

The general problem we address is how much of the observed variability in marine ecosystems can be attributed to climate variability. Our approach relies on the combined use of principal component analysis (PCA) and canonical correlation analysis (CCA), a couple of relatively simple statistical tools successfully used to dissect out significant correlations among variables in complex systems of any kind [1,2].

We used yearly recorded data of Atlantic cod recruitment collected from the area surrounding the Kola peninsula (Barents Sea) and from the North Sea, in conjunction with sea surface temperatures in the same regions and NAO values.

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 recruitment [3]. In the North Sea, the situation is simpler: the data are compatible with only one major interaction mechanism. Moreover, in the two regions, the effect of temperature on cod recruitment has a reversed direction: direct correlation for the Barents Sea, inverse correlation for the North Sea. This is probably due to an optimal temperature regime for cod recruitment lying in between the 'cold' Barents Sea and the 'warm' North Sea.

[1] Rencher A. (1972). Interpretation of canonical discriminant functions, *Am. Stat.*, 46, 217–225.

[2] Presendorfer R.W. (1988). PCA in meteorology and oceanography, Elsevier, Amsterdam.

[3] Sirabella et al. (2001). Breaking down the climate effects on cod recruitment, *Mar. Ecol. Prog. Ser.*, 216, 213–222.

A2/C5.2–Temperature selection at high latitudes: autecologic studies with Arctic fish and Kamchatka crab

J.S. Christiansen, Norwegian College of Fishery Science, University of Tromsø, Norway

Fish may respond to temperature differences of less than 0.1 °C. This makes temperature a key environmental component that limits the distribution and affects the migratory behaviour of marine fish and other aquatic ectotherms. This talk intends to: (1) describe the heterogeneous nature of sub-arctic and arctic waters, i.e. temperature, salinity and density fields; (2) give an overview of the underlying determinants of temperature selection ('preference') in fish; and (3) present some preliminary results from case studies on temperature selection in the polar cod (*Gadidae: Boreogadus saida*) and the Kamchatka crab (*Crustacea: Paralithodes camtschatica*). The polar cod is a true arctic fish species that lives at sub-zero temperatures for most of its life. In a free choice laboratory situation, however, polar cod often select temperatures that are well above those found in its natural habitat. Thus, there is an apparent discrepancy between the fundamental (laboratory) and the realized (nature) thermal niches in polar cod, which may be caused by non-thermal stimuli. The Kamchatka crab was transferred from the Okhotsk Sea to the Murman coast by Soviet authorities during the 1960s. In recent years, the number of crabs has increased exponentially and the species has spread westwards into warmer waters along the Finnmark coast to the North Cape. Therefore, it is of considerable interest to examine the thermal behaviour of this introduced species in order to estimate how far west and south it might disperse.

Christiansen, J.S., Schurmann, H., Karamushko, L.I. (1997). *Cybium*, 21 (4), 353–362.

A2/C5.3–Effect of temperature change and hypoxia on the distribution of Atlantic cod (*Gadus morhua*)

M.F. Petersen, J.F. Steffensen, Marine Biological Laboratory, University of Copenhagen, Denmark

Different haemoglobin components are common within a single fish species, and since 1961, the existence of the polymorph haemoglobin of Atlantic cod (*Gadus morhua*) has been known. With agar gel electrophoresis, the haemoglobin components can be separated into two homozygous types, HbI-1 and HbI-2, and one heterozygous type HbI-1/2. The distribution of Atlantic cod with different haemoglobin genotypes is heterogeneous, with a high frequency of the HbI-2 allele in the waters off Greenland, Iceland, the Faeroe Islands and northern Norway, while the HbI-1 allele is dominant in warmer areas.

To investigate if temperature is the selective parameter in the heterogeneous distribution of Atlantic cod, the preferred temperature of HbI-1 and HbI-2 cod was measured. Significant differences were observed, as HbI-2 cod preferred 8.2 ± 1.5 °C, while HbI-1 cod preferred 15.4 ± 1.1 °C. The effect of hypoxia (35% oxygen saturation) on the preferred temperature was also measured. Previous studies show that the preferred temperature of fish decreases during hypoxia, this was also the case for HbI-1 cod, which preferred 9.8 ± 1.8 °C during hypoxia, while this effect was absent for HbI-2 cod.

The results from this study indicate that a distributional change of the different haemoglobin types of Atlantic cod will occur due to environmental temperature changes. Global warming will result in an advantage for the HbI-1 cod. However, since HbI-1 cod prefer a low temperature under hypoxic conditions, an unfavourable situation for Atlantic cod stocks can be predicted if a combination of increased water temperature and hypoxia occurs.

A2/C5.4—Cold temperature as a shaping force in the structure and physiology of fish tissues

B.D. Sidell, Marine Sciences, University of Maine, USA

Because the majority of fishes are ideal ectotherms, exposure to cold water temperatures immediately translates to life at very cold cellular temperatures. Decreases in temperature lead to increases in aqueous gaseous solubilities, decreases in catalytic rates, increased viscosity of both aqueous and lipid phases of the cell, and consequently, decreases in rates of molecular diffusion. In combination, these factors can have profound effects on the physiology and biochemistry of fish tissues. Oxidative muscle tissues of cold-bodied fishes from both high temperate and polar latitudes display structural and biochemical characteristics, which contribute to overcoming constraints of cold temperature upon normal cellular function. These include very dense mitochondrial populations, high cellular content of lipid compounds (both as neutral lipid deposits and membranous structures), and enhanced activities of enzymes from aerobic metabolic pathways (via differences in either amount of

enzyme or in catalytic properties of the enzyme). Exposure to chronically cold temperatures over evolutionary time can also apparently lead to the retention of otherwise physiologically deleterious traits, when the appropriate ecological conditions prevail. An excellent example is found in Antarctic Channichthyid icefishes, a group that uniformly lacks hemoglobin in their circulation and encompasses several species that do not express the intracellular oxygen-binding protein, myoglobin. Lack of these proteins coupled with slow gaseous diffusion exacerbates the challenge of oxygen delivery.

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A2/C5.5—Effects of temperature on intracellular diffusion of phosphorous metabolites in muscle.

T.S. Moerland, O.H. Vanderlinde, Biological Science, Florida State University, USA

The thermal sensitivity of molecular diffusion may be a determinant of acclimatory and evolutionary responses to temperature in fish muscle. Pulsed-field gradient NMR (PFG-NMR) permits non-invasive measurements of intracellular diffusion in living cells, and this technique has been used to examine creatine phosphate (PCr) and ATP diffusion in fish red and white skeletal muscle. At brief diffusion times (19 ms), intracellular diffusion coefficients for PCr and ATP (D_{PCr} and D_{ATP}) in both muscle types are $\sim 70\%$ of their values in bulk solution. The Q_{10} of diffusion is 1.28 (5–25 °C). At longer diffusion times, both D_{PCr} and D_{ATP} become highly anisotropic: diffusion measured along the long axis of muscle cells is approximately independent of diffusion time, whereas D_{PCr} and D_{ATP} measured radially decrease with increasing time until a new steady-state value ($\sim 50\%$ of the value in bulk solution) is reached at ~ 100 ms. The time dependence of apparent diffusion coefficients is indicative of interaction between the diffusing solute and the structure of the medium through which diffusion occurs. Mathematical simulation shows that neither the thick/thin filament lattice of muscle nor the sarcolemma cause diffusive anisotropy. Rather, intracellular membranous structures, including mitochondria and the sarcoplasmic reticulum (SR), are the principal elements that inhibit mobility in an orientation-dependent manner. The ultrastructural responses of fish muscle to low environmental temperatures may compensate for the thermal sensitivity of diffusion and the inherent structural restrictions to radial diffusion of phosphorous metabolites.

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A2/C5.6—Mechanisms underlying seasonal and latitudinal changes in muscle metabolic capacities in fish and molluscs

H.E. Guderley, Département de Biologie, Université Laval, Québec, P.Q. G1K 7P4

In the earth's geophysical environment, temperature and photoperiod are the major abiotic factors to change on a seasonal and latitudinal basis. Thermal fluctuations are of particular importance for aquatic ectotherms, since their body temperature is in equilibrium with their environment. In the laboratory, many ectotherms respond to cold acclimation with increases in muscle aerobic capacity, partly due to increases in mitochondrial protein and partly due to changes in the phospholipid composition of mitochondrial membranes. In nature, seasonal fluctuations in aquatic productivity can modulate such acclimatory responses, particularly given the important energy storage role played by muscle. Reproduction in aquatic ectotherms typically leads to considerable mobilisation of energy from muscle, potentially depleting stored fuels and protein levels. Given that glycogen is a binding site for glycolytic enzymes, glycogen mobilisation may indirectly reduce enzyme activities. Reproductive cycles can also modify the properties of muscle mitochondria. As reproduction is typically seasonal, its influence must be considered when interpreting seasonal changes in muscle physiology. When an organism faces the challenge of cold temperatures, it can either remain active or enter into metabolic depression, adopting the strategy that best suits its time-averaged energy budget. Thus, the thermal biology of skeletal muscle mitochondria from cold active species differs considerably from that of species that spend their winters in a metabolically depressed state. The challenge to thermal biologists is to tease out the direct and indirect responses to temperature presented by living systems.

A2/C5.7—Glacial retreats, warm visits and other hearty destinations: a fish's perspective

A.P. Farrell, H.A. Shiels, S. Clutterham, C.G. Lee, Biological Sciences, Simon Fraser University

Over the last 10 000 years, salmonids in British Columbia have faced profound changes in their thermal environment, including a glacial retreat and also warming to perhaps 4 °C higher than present day. Using rainbow trout and various salmon stocks, we are investigating how the salmonid cardiac and exercise capabilities evolved to function at these extremes of temperature. At the level of cardiac myocytes, evidence is accumulating for good regulation of calcium delivery over a wide temperature range through three interactive mechanisms. Foremost, temperature modulates the shape of the car-

diac action potential through alterations in both K⁺-channels activities and the kinetics of L-type Ca²⁺-channel flux, which together maintain trans-sarcolemmal calcium delivery, despite the direct effects of temperature on each protein in isolation. Secondly, at high temperatures, adrenergic stimulation of Ca²⁺-channels is decreased. Thirdly, at low temperatures and low heart rates, the sarcoplasmic reticulum plays an increased role in calcium delivery, responses that are augmented by temperature acclimation. In intact fish, venous oxygen tension is protected during exercise, so that the heart is not starved of an oxygen supply via the cardiac circulation (i.e. venous return to the heart), and venous oxygen tensions during swimming are elevated with acclimation temperature. At the population level, maximum oxygen uptake differs among salmonids within the same genus and species, but the mechanisms underlying these differences remain a mystery. Funded by NSERC Canada.

A2/C5.8—Latitudinal effects on vascular control in fishes

S. Egginton, J.V. Hill, Physiology, University of Birmingham, UK

The branchial arteries of Antarctic and temperate nototheniids constrict more strongly in the presence of serotonin and carbachol than with adrenaline or noradrenaline (Egginton et al., 2001; Forster, et al., 1998). This pattern may be due to phylogeny or adaptation to a low environmental temperature, which, for the temperate species, has not been lost upon migration to higher temperatures. To elucidate the pattern of vascular control with latitude, and to clarify the phylogenetic effects, phylogenetically distant species from the Northern hemisphere were compared with the southern nototheniids. The species examined were the northern polar cod (*Boreogadus saida*), the northern temperate short-horned sculpin (*Myoxocephalus scorpius*), the southern temperate black cod (*Paranotothenia angustata*), two southern polar nototheniids (*Notothenia coriiceps*, *Trematomus bernacchii*) and rainbow trout (*Oncorhynchus mykiss*). Vasomotor control of efferent branchial arteries via noradrenaline, serotonin and carbachol was measured using in vitro myography at two temperatures, 10 °C apart. In addition, the total nerve density of the branchial arteries was measured. Vessels generally behaved similarly amongst species, with some exceptions, and the Q_{10} effects were surprisingly limited. Branchial artery nerve densities can differ between species, vessel types and along the vessels.

Egginton, S., Forster, M.E., Davison, W. (2001). The control of vascular tone in notothenioid fishes is determined by phylogeny, not environmental temperature. *Am. J. Physiol.* 205, R1197–R1205.

Forster, M.E., Forster, A.H., Davison, W. (1998). Effects of serotonin, adrenaline and other vasoactive drugs on

the branchial blood vessels of the Antarctic fish *Pagothenia borchgrevinki*. Fish Physiol. Biochem. 19, 103–109.

A2/C5.9—Haemoglobin isoforms and oxygen binding characteristics in fish populations in a latitudinal cline

O. Brix, Department of Zoology, Bergen, Norway

The haemoglobin-polymorphism of Atlantic cod with the phenotypes *HbI*1/1*, *HbI*1/2* and *HbI*2/2* was described more than 30 years ago (Frydenberg et al., 1965). 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 approximately 10% in the Barents Sea, 20–50% along the coast of northern and western Norway, and 70% in the Kattegat (Frydenberg et al., 1965). Brix et al. (2002) and Pörtner et al. (2001) clearly demonstrated that *HbI*2/2* is better fitted to cold temperatures than *HbI*1/1*, being able to transport more oxygen from the environment to the tissues. This could result in a higher growth rate, supported by length and weight data. However, it could also, not excluding the other possibility, be a result of a higher oxygen demand in response to cold adaptation, compensating for some of the energy loss caused by proton leakage in the mitochondria (Pörtner et al., 2001).

The results suggest selection to be important for the evolution and distribution of cod populations. The studies further support the general hypothesis of cold water species having preferentially more anodal haemoglobins—often few components being more sensitive to ligands, than warm water species—less anodic components being less sensitive to ligands (Brix et al., 1999).

Brix et al. (1999). J.Comp.Physiol. B. 169, 323–334.

Brix et al. (2002). (submitted).

Frydenberg et al. (1965). Hereditas 53, 257–271.

Pörtner et al. (2001). Continental Shelf Res., 21, 1975–1997.

A2/C5.10—Oxygen limited thermal tolerance: implications for physiological and ecological performance at various latitudes

H.O. Pörtner, R. Knust, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

For an understanding of the effects of global warming on marine ecosystems, we attempt to explain ecological findings based on physiological processes, focusing on eelpout (*Zoarces viviparus*), cod (*Gadus morhua*) and comparative studies in other species. These studies indicate that thermal tolerance limits are set at the highest complexity levels in animals, by ventilatory and circulatory capacities for oxygen uptake. These capacities are

set to levels just sufficient to meet maximum oxygen demand between average highs and lows of environmental temperatures. The adjustment of aerobic scope, reflected by mitochondrial densities and capacities, is identified as crucial in thermal adaptation on top of parallel adjustments at molecular or membrane levels. The costs of mitochondrial maintenance are also adjusted, reflected by changes in transmembrane proton leakage. Growth performance in cod is optimal close to 10 °C, in all populations investigated in a latitudinal cline. However, temperature-specific growth decreases at higher latitudes. Also, fecundity is less in the White Sea than in the North and Baltic Sea cod or eelpout populations. Accordingly, a shift in energy budget unfavorable for growth performance and fecundity occurs, which does, however, not extend to limit muscular performance. We suggest that the rise in proton leakage in cold adapted eurytherms may leave less energy for growth and reproduction in the cold, but also limits heat tolerance to species specific levels. As a corollary, tradeoffs in the adjustment of physiological mechanisms are suitable to understand temperature-dependent biogeography and performance changes elicited by global warming.

A2/C5.11—Abstract withdrawn

A2/C5.12—Protein metabolism at low temperatures

K.P.P. Fraser, L.S. Peck, A. Clarke, British Antarctic Survey, Natural Environment Research Council, Cambridge, UK

Growth in most polar ectotherms is very slow, and the rates of most cellular processes is considerably reduced in comparison to temperate and tropical species. Organisms essentially grow by the process of protein growth, the net balance of protein synthesis and degradation. Growth in polar ectotherms is constrained because of low temperatures and a highly seasonal food supply. The current work examined whether low polar growth rates were the result of reduced rates of protein synthesis or elevated rates of protein degradation. In vivo whole body fractional protein synthesis rates were measured in the Antarctic limpet *Nacella concinna* at four points throughout the year by injecting a radiolabelled amino acid. Protein degradation was estimated from the difference between protein synthesis and protein growth. RNA to protein ratios and RNA translational efficiencies were also measured. Fractional protein synthesis rates varied seasonally, ranging between 0.27 and 0.56% day⁻¹, and were only slightly lower than temperate species, RNA to protein ratios were very high ranging between 17 and 54 µg RNA mg⁻¹ protein and RNA translational efficiencies were very low (mean 0.14 mg protein mg⁻¹ RNA day⁻¹). Overall growth rates were

low because only 21% of synthesized proteins were retained as protein growth, the remaining proteins were degraded. *N. concinna* achieves respectable protein synthesis rates by maintaining high tissue RNA concentrations to counteract the low translational efficiency of RNA at polar water temperatures. However, the cost of maintaining elevated tissue RNA concentrations and the low retention efficiency of synthesized proteins suggest the costs of growth may be considerably higher in polar than temperate ectotherms.

A2/C5.13—The energetic costs of in vitro protein synthesis in stenothermal and eurythermal pectinids: a direct approach

D. Storch, H.O. Pörtner, Ecophysiology and Ecotoxicology, Alfred-Wegener-Institute, Bremerhaven

Translationally active cell-free systems from gills of the Antarctic scallop *Adamussium colbecki* Smith (1902) and the European scallop *Aquiptecten opercularis* (Linnaeus 1758) were developed, characterized and optimised for an analysis of translational capacity. The aim was to determine the energetic cost of protein synthesis in the in vitro cell free system by directly measuring the required energy equivalents in the lysates. Protein synthesis rate in assays conducted with lysates of *A. colbecki* ($1.029 \pm 0.061 \mu\text{mol Phe}/\text{min}$ at 15°C) were higher compared to lysates of *A. opercularis* ($0.087 \pm 0.013 \mu\text{mol Phe}/\text{min}$ at 15°C and $0.156 \pm 0.023 \mu\text{mol Phe}/\text{min}$ at 25°C). This can in part be attributed to the naturally occurring higher RNA content in lysates of *A. colbecki* ($0.883 \pm 0.037 \text{ mg RNA}/\text{ml lysate}$) compared to *A. opercularis* ($0.468 \pm 0.013 \text{ mg RNA}/\text{ml lysate}$).

There was no significant difference in the energetic costs of protein synthesis in cell free systems of gill lysates of the cold stenothermal *A. colbecki* with 4.3 ± 0.7 energy equivalents/peptide bond formed and the eurythermal *A. opercularis* with 5.6 ± 0.6 energy equivalents, indicating that there are no differences in the efficiency of the translation machinery. The energetic costs specified for protein synthesis in gill lysates of *A. colbecki* almost correspond with the generally accepted theoretical value of four energy equivalents per peptide bond formed, whereas the value for gill lysates of *A. opercularis* was slightly higher. We suggest that cold adaptation occurs at the level of increased protein stability leading to a decrease in overall turnover rates at no increase in the costs of protein synthesis, however, at significant cold compensation of the capacity of translation.

A2/C5.14—Fish performance and temperature

G. Claireaux, J.-M. Guarini, CREMA; L'Houmeau, CNRS-IFREMER; P. Lazure, DEL AO, IFREMER, France

Temperature has been implicated as an important factor in regulating the seasonal migrations of numerous fish species, although the direct causal relationship has rarely been proven. Evaluating the biological significance of temperature in regulating fish distribution is important if we are to predict how the seasonal movements and distribution of fish may change in response to changes in seawater temperature. This information is a crucial element in understanding the relationship between adult biomass (reproductive potential) and ensuing recruitment (breeding success) from which biological reference points for sustainable management of fisheries are derived. The control of metabolic activity by thermal conditions is generally considered the central issue in attempts by fish for an optimised use of an ecosystem. Our investigation aimed at elucidating the ways in which the environment sets the limits (scope) within which physiological processes, particularly energy allocation, take place. For that purpose, we examined the influence of water temperature on the distribution of the European sea bass in the English Channel and around the British Isles. We argue that winter offshore migration alleviates the environmental constraints on bass bioenergetics by preserving the aerobic metabolic power output. Existing models of the relationships between aerobic metabolic scope or scope for heart rate and temperature were used to estimate population movements in dynamic seawater temperature fields calculated by a hydrographic model. Taking into account bass swimming performance, we investigated the physiological outcome, and potential benefit, of previously report seasonal movements and distribution patterns.

A2/C5.15—Effects of seasonal temperature changes on the swimming performance of trout

N. Day, P.J. Butler, School of Biosciences, The University of Birmingham, UK

Adult brown trout (*Salmo trutta*) were acclimated to 'seasonal' temperatures (5°C in winter; 15°C in summer) and 'reversed seasonal' temperatures (15°C in winter; 5°C in summer) while exposed to the natural (seasonal) photoperiod. In both winter and summer, the mean critical swimming speed (U_{crit}) for fish acclimated to the reversed seasonal temperature was more than 30% lower than that obtained for those acclimated to the correct seasonal temperature. This lowering of U_{crit} in fish acclimated to the reversed seasonal temperature appeared to be mainly due to the inability of white muscle to function optimally. This was supported by the

observed mean levels of both lactate and ammonia in the white muscle of these individuals after exercise, which were both significantly lower than those observed in the fish swum at the correct seasonal temperatures. These observations, together with other data obtained from the biochemical and morphometrical analysis of the muscle tissue, suggest that swimming ability is influenced, at least in part, by seasonal factors other than changes in environmental temperature.

A2/C5.16—Seasonal changes in the stress response of rainbow trout: a 2-year study

P. Koldkjær, A.R. Cossins, Biological Sciences, University of Liverpool; T.G. Pottinger, CEH, Windermere, UK

Previous work suggested profound seasonal rhythms in the β -adrenosensitivity of red blood cells (RBCs) in salmonid fish. We have thus conducted an extensive monthly sampling regime in mature rainbow trout over 2 years with simultaneous examination of β -adrenergic receptors, β -adrenergically stimulated RBC Na influx, liver cortisol receptors as well as plasma hormones, ions, lactate and glucose. Water temperature fluctuated between 4 and 20 °C, and the fish were exposed to natural light in outdoor tanks supplied with lake Windermere water. The RBCs possessed membrane-bound β -adrenoceptors on all sampling occasions. Surprisingly, the number or affinity of β -adrenoceptors did not govern the magnitude of the adrenergically stimulated RBC Na influx. However, the Na influx, determined in vitro at 15 °C by use of $^{22}\text{[Na]}$, was reduced by 55 and 30% in December 1999 and February 2001, respectively, compared to the average Na influx. When reduced, there was no apparent compensation by a reduced desensitization of the Na/H exchanger. The Na influx did not consistently co-occur with the spawning period. The adreno-responsiveness of young RBCs was significantly higher than that of older cells, which is likely to contribute to seasonality of the response. Seasonal variations in plasma cortisol levels were not sufficient to modify the density of membrane-bound β -adrenoceptors. In contrast, there was a loss of specific liver cortisol binding in 50–88% of the fish during December 1999–June 2000 and February–June 2001. We hope to have completed an ongoing study of the consequences of the apparent lack of liver cortisol receptors on metabolic enzymes.

A2/C5.17—Adaptation of red blood cell anion exchange to temperature

F.B. Jensen, Biology, SDU Odense; T. Wang, Aarhus; J. Brahm, Copenhagen, Denmark

Presence of an anion exchange protein (known as AE1 or band 3) endows the vertebrate red blood cell (RBC) membrane with a high chloride and bicarbonate permeability. Anion exchange across the RBC membrane is

a rate-limiting step in CO₂ transport, and it is strongly temperature sensitive in endothermic animals such as man and chicken. In ectotherms that experience large variations in body temperature, similar temperature sensitivity would create problems for CO₂ transport upon cooling. We have analysed acute and chronic effects of temperature on RBC Cl⁻ self-exchange in different animals. We show that Q_{10} for anion exchange in ectothermic animals (trout and turtle) is smaller than in endotherms. Furthermore, the Q_{10} for anion exchange is close to Q_{10} for CO₂ excretion, revealing a proper match between the acute temperature sensitivities of RBC anion exchange and metabolic CO₂ production. Thus, in the ectotherms, the degree of rate limitation offered by anion exchange in CO₂ transport does not change appreciable as temperature changes. When compared at constant temperature, the RBC Cl⁻ permeability shows large differences among species (trout, carp, eel, cod, turtle, alligator, chicken, man). Cl⁻ permeabilities are, however, remarkable similar when compared at the preferred body temperatures, reflecting an appropriate evolutionary adaptation of RBC anion exchange function to the different thermal niches encountered by animals.

Jensen, F.B., Wang, T., Brahm, J. (2001). *J. Exp. Biol.* 204, 39–45.

A2/C5.18—Feast and famine: seasonal impact on feeding and starvation in fish

Iris Hardewig, F. Hölker, C. Wolter, S. Volkmann, P. van Dijk, Institute of Freshwater Ecology, Berlin, Germany

Many fish species experience regular periods of starvation during their life. Starvation is often induced by reduced food availability during winter, when plankton densities are low. During this period, many fish species have to rely on body reserves to meet their energetic needs, which results in a loss of body weight. In order to minimize weight loss, winter starving fish decrease their metabolic rate, which is favoured by low water temperatures during this season.

The fresh water gadoid, *Lota lota*, however, reduces its food uptake during summer, when water temperatures reach 20 °C and above. We calculated seasonal variations of energy influx from the stomach contents of wild catches of burbot. While energy influx decreases dramatically during summer, metabolic costs are likely to be high due to high water temperatures. Model calculations show that surplus energy for growth is minimal during this period. Our field observations, however, revealed that juvenile *Lota lota* show a gain in weight and length throughout the year. Physiological investigations explain this apparent contradiction and show how *Lota lota* is able to grow despite reduced energy influx at high water temperatures during summer.

A2/C5.19—Metabolic aspects of temperature adaptations in an eurythermal marine gastropod *Littorina saxatilis*: latitudinal vs. microscale comparisons

I.M. Sokolova, Zoology Department, University of Guelph, Canada; H.O. Pörtner, Alfred-Wegener Institute, Bremerhaven, Germany

In order to analyse the temperature-related changes in metabolic regulation and their role in adaptation to different thermal environments in a eurythermal marine gastropod *Littorina saxatilis*, we compared the rates of aerobic and anaerobic metabolism in air and water and activities of key metabolic enzymes in snails from different latitudes (the North and the White Seas) and shore levels. Aerobic metabolism was the main energy-supplying pathway in *L. saxatilis* in both aquatic and aerial environments, whereas anaerobic metabolism contributed 0–3% to the total energy production under all the conditions tested. Increasing environmental temperatures led to a switch from fully aerobic to partly anaerobic pathways of energy production in *L. saxatilis*. Analysis of the upper critical temperatures (T_c), characterized by the onset of anaerobiosis and by the adverse changes in cellular energy status, revealed a complex pattern with latitudinal cold adaptation resulting in an upward shift in T_c , in contrast to cold acclimation leading to a downward shift of T_c . This suggests wider temperature windows at northern latitudes of the White Sea. Adaptation to the colder environments involved increased activity of key metabolic enzymes both on a latitudinal scale and between shore levels suggesting metabolic cold compensation of enzyme activities in this eurythermal species. In general, our data suggest that the ability to efficiently regulate metabolism and to match energy supply with energy demand in the face of temperature change may be crucial for temperature adaptation of *L. saxatilis*.

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A2/C5.20—Relationship between temperature and population structure of North Sea plaice: evidence from electronic tags

E. Hunter, J.D. Metcalfe, CEFAS, Lowestoft; J.D. Reynolds, UEA, Norwich, UK

Between December 1993 and September 1999, 752 mature female plaice were tagged with electronic data storage tags (DST) and released at eight North Sea locations. Tidal data recorded by returned DST were used to reconstruct the movements of 145 free-ranging plaice for periods of up to 512 days, with a total behavioural rec-

ord of 20 403 days of data. The results revealed a seasonal subdivision of the population sampled into three geographically distinct sub-units. Between May and October, these sub-units were centred on Amrum Ground to the east, Ekofisk Field to the north, and between the north coast of East Anglia and Dogger Bank to the west. During the spawning period (December–April), individuals from both the eastern and northern sub-units were found simultaneously on the German Bight spawning grounds, while individuals from all three sub-units visited spawning grounds in Southern Bight. The only fish in the current study to leave the North Sea were a sub-set of the western sub-unit that visited spawning grounds in the eastern English Channel. The western and eastern sub-groups recorded a seasonal rise and fall in ambient temperature, whereas the northern sub-group spent the summer in deep, cold thermally-stratified water. Here we consider the role of thermal stratification, the tidal-streams, and other possible factors involved in the maintenance of the observed distribution.

A2/C5.21—Seasonal vs. latitudinal cold adaptation of mitochondrial functions in *Arenicola marina*

A.M. Sommer, H.-O. Pörtner, Alfred Wegener Institute, Bremerhaven, Germany

Latitudinal cold adaptation proved to be energetically costly in the eurythermal lugworm *Arenicola marina* (Sommer and Pörtner, 2002, submitted). To obtain more information about the trade-offs and therewith the limits of thermal adaptation, mitochondrial functions were characterised in latitudinally cold adapted White Sea animals and compared with seasonally cold acclimatised North Sea lugworms.

Mitochondria from subpolar animals displayed higher succinate oxidation rates and reduced Arrhenius activation energies (E_a) at low temperatures in state 3 respiration and higher H^+ -leakage rates when compared with boreal animals. These differences, an increased activity of cytochrome *c*-oxidase and elevated numbers of mitochondria indicate increased aerobic capacity in subpolar worms necessary to maintain motor activity at low but variable environmental temperatures, however, at the expense of a higher metabolic rate. The reduced activity of citrate synthase (CS) may compensate for this increased O_2 -demand. In contrast, elevated CS activities and mitochondrial respiration rates accompanied acclimation of boreal lugworms to winter conditions. With falling acclimation temperatures the significant Arrhenius break temperature in state 3 respiration (11 °C) became insignificant (5 °C) or even disappeared (0 °C), a phenomenon known from hibernating vertebrates [Geiser and McMurchie (1985). *J. Comp. Physiol.-B* 155, 711–715]. The efficiency of aerobic energy pro-

duction in winter animals rose, as H^+ -leakage in relation to state 3 decreased with cold acclimation, indicated by higher RCR values and increased ADP/O ratios, but at the expense of regulatory flexibility. Metabolic depression might be involved, a mechanism possible only for a time-limited situation, and therefore, unsuitable for latitudinal cold adaptation in eurythermal animals.

A2/C5.22—Does temperature cause different metabolic adaptations during starvation in fish?

M. Binner, P. van Dijk, I. Hardewig, Institute of Freshwater Ecology, Berlin, Germany

The influence of temperature on metabolic adaptations during long-term starvation was investigated in two freshwater species, roach (*Rutilus rutilus*) and burbot (*Lota lota*).

Roach shows a rather typical feeding and fasting cycle during the year, which is correlated to the seasonal availability of food. In wintertime, when the water temperature is low and little food is available, the roach starts fasting, accompanied by reduced locomotive activity. Thus, roach is a winter fasting species. The feeding behaviour of the burbot, however, seems to be independent from the seasonal availability of food. *Lota lota*, the only freshwater gadoid, seems to reduce its locomotive activity and food consumption during summer, when it is exposed to high temperatures. It dissipates its energy stores during the warmest period of year and appears to be a summer fasting fish.

To examine the effects of temperature on the fasting metabolism in fish, summer and winter conditions were simulated in a comparative study on *Rutilus rutilus* and *Lota lota*. During 4 weeks of food deprivation at 20 and 4 °C, the combined effect of starvation and temperature on oxygen consumption and on the mobilisation of energy reserves was investigated. Long-term respiratory measurements were conducted to determine whether starvation leads to a depression of the metabolic rate of the investigated species. To examine the effect of temperature on substrate preferences of the oxidative metabolism during starvation, the sequential mobilisation of different substrates (glycogen, protein, fat) in muscle and liver tissue was analysed.

A2/C5.23—Growth, production and respiration in scallops

O. Heilmayer, H.O. Pörtner, T. Brey, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

Temperature is a major factor controlling metabolism, and hence, growth rates. To obtain a better understanding of eco-physiological effects of temperature on polar invertebrates, we analysed the influence of temperature

on the metabolism and growth of three pectinid species (Mollusca, Bivalvia) along a latitudinal cline.

Growth rate, growth performance (ϕ) and productivity of the cold stenothermal, endemic Antarctic scallop (*Adamussium colbecki*), the stenothermal Patagonian scallop (*Zygochlamys patagonica*) and the eurythermal Queen scallop (*Aequipecten opercularis*) were compared with literature data obtained from other scallop populations world-wide. To evaluate the metabolic effect of long-term adaptation to cold, we compared physiological processes at two different levels. Besides basal metabolism (measured as oxygen consumption rate), we investigated the characteristics of citrate synthase (CS), isolated from the adductor muscle of the three species. Our study showed that average annual growth rate and overall growth performance were inversely correlated to latitude. Respiration measurements of whole animals (56 populations/nine species) at ambient temperatures (0–25 °C) revealed a significant negative impact of temperature on metabolic rate with no evidence for metabolic cold adaptation. In contrast, a comparison of maximum CS activity revealed higher activities for *A. colbecki* over the whole temperature range and therefore indicates a metabolic cold compensation of CS. A difference in the thermal properties of the enzyme is indicated by a significantly lower Arrhenius-break-temperature in CS of *A. colbecki*. Further investigations are necessary to explain the contradictory results of low basal metabolism and elevated enzyme capacity in the cold stenothermal Antarctic scallop.

A2/C5.24—Adrenergic stress responses in high latitude fishes: Antarctic vs. Arctic

N.M. Whiteley, Biological Sciences, University of Wales Bangor, UK; J.S. Christiansen, Norwegian College of Fishery Science, University of Tromsø, Norway; S. Egginton, Physiology, University of Birmingham, UK

Adrenergic control of the cardiovascular system in Antarctic nototheniids appears to be reduced relative to temperate-zone teleosts, relying more on cholinergic or serotonergic mechanisms (Egginton and Davison, 1997; Egginton and Hill, this meeting). In particular, there is minimal lactacidosis and a strikingly weak adrenergic stress response, which may, in part, be explained by an apparently inadequate capacity for catecholamine synthesis (Whiteley and Egginton, 1999). We, therefore, studied the stress response of Polar Cod (*Boreogadus saida*), a resident of the Arctic Ocean, to determine whether a similar physiology accompanies cold exposure at both poles. Animals of 30–50 g mass were held at 0 °C and subjected to an exercise stress regime. Haematocrit changed little (0.24 ± 0.01 vs. 0.26 ± 0.01), as did other haematological parameters, while blood lactate

showed a small but significant rise (0.67 ± 0.06 vs. 1.03 ± 0.14 mmol l^{-1} , $P < 0.05$). Acute or chronic exposure to 5 °C only accentuated the response slightly. Similarly, plasma catecholamine levels were not markedly raised, and head kidney tyrosine hydroxylase activity, as a measure of the capacity to synthesise catecholamines, was low compared with other temperate-zone species. These data suggest that a similar depressed stress response is present in phylogenetically distant species from opposite poles. It is unclear whether this is a consequence of low environmental temperature or locomotory activity.

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Whiteley, N.M., Egginton, S. (1999). J. Exp. Biol. 202, 3623–3629.

A2/C5.25—Do seasonal changes in protein metabolism influence thermal tolerances in an intertidal crustacean?

L.S. Faulkner, N.M. Whiteley, Biological Sciences, University of Wales, Bangor, UK

Temperature tolerances in the intertidal semi-terrestrial isopod, *Ligia oceanica*, vary seasonally with CT_{max} falling from 37.4 °C in the summer to 34.9 °C in the winter. Below these values, animals survive temperatures of 25–31 °C by synthesising heat shock proteins (hsp). Examination of hsp synthesis patterns in seasonally-acclimatised *L. oceanica*, showed a different response in summer vs. winter. In the summer, animals synthesised a protein with a molecular weight of 60 kDa (hsp 60) at two induction temperatures of 27 °C and 31 °C. Animals collected in the winter, however, synthesised hsp 60 at the lower induction temperature of 25 °C, and continued to express hsp 60 at 27 and 29 °C. Unlike summer animals, winter animals did not survive heat shock at 31 °C, suggesting that survival of *L. oceanica* at elevated temperatures is linked to the ability to synthesise hsps.

Metabolic costs of protein synthesis also vary between summer and winter-acclimatised *L. oceanica*, which may explain seasonal differences in hsp synthesis patterns. Isopods collected from the shore in the winter were characterised by high costs of whole animal protein synthesis ($40.9 \mu\text{mol O}_2 \text{ mg protein}^{-1}$), which accounted for 70% of mean oxygen uptake rates. In contrast, metabolic costs were 4.6 times lower in animals collected in the summer ($3.9 \mu\text{mol O}_2 \text{ mg protein}^{-1}$), accounting for 17% of mean oxygen uptake. Consequently, hsp synthesis, and therefore, survival of intertidal isopods at elevated temperatures in the winter may be restricted by the high costs of protein synthesis at a time when energy stores and food resources are limited.

A2/C5.26—Mechanisms of enzyme adaptation to seasonal temperature change in cod (*Gadus morhua*)

M.V. Zakhartsev, R. Blust, Biology, University of Antwerp, Belgium

The aim of this study was to determine the effects of seasonal temperature change on properties of lactate dehydrogenase (LDH) from muscle and liver of Norwegian coastal cod *Gadus morhua*. Two groups of fishes were acclimated to 4 °C and 12 °C for 1 year. Acclimation temperature (AT) has a clear effect on the growth rate, but not on the physiological condition of the cod. However, differences between the groups in allele frequencies of the four tested allozymes were not found. In addition, AT has no effect on the LDH's iso-electric profile.

Neither kinetic (K_M , K_{si} , V_{max}) nor thermodynamic (E_a , ΔG) differences among LDH-phenotypes were found. However, there were clear kinetic differences among the tissues and the relative catalytic efficiency is higher in liver. Despite these kinetic differences, the thermodynamic requirement to fulfil the pyruvate reduction in crude homogenate is the same for both tissues ($E_a = 47 \text{ kJ mol}^{-1}$) at AT = 12 °C. At AT = 4 °C, the E_a becomes higher ($E_a = 53\text{--}59 \text{ kJ mol}^{-1}$). Acclimation does not have a significant effect on the ΔG of the reaction ($56\text{--}58 \text{ kJ mol}^{-1}$).

Differences in K_M values among the acclimation groups were not observed for both tissues. Two-phase linear regression analysis revealed that at temperatures above 16 ± 1 °C, K_M becomes temperature dependent, which has important consequences for the anaerobic capacity. Thus, the main strategy of LDH adjustment to seasonal temperature variations involves changes in LDH concentration, adjustment of thermodynamic (E_a) and kinetic (K_{si}) properties of the enzymes being present, but not the expression of alternative forms. The ecological consequences of global temperature rise could be that the cod will probably follow colder waters.

A2/C5.27—Abstract withdrawn

A2/C5.28—Temperature adaptation in cod (*Gadus morhua*): comparison of mitochondrial enzyme capacities and growth in boreal and arctic populations

G. Lannig, T. Fischer, I. Serendero, L. Eckerle, F.J. Sartoris, H.O. Pörtner, Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany

Comparative studies indicate that the processes and limits of thermal tolerance of marine ectotherms are linked

with the adjustment of aerobic scope and capacity of the whole animal accompanied by adjustments at the cellular and molecular level. The present study investigated temperature effects on growth rates, liver composition and aerobic enzyme activities in white muscle and liver of three cod populations in a latitudinal cline between North Sea, Norwegian Coast and Barents Sea. Our results show a cold-induced rise in enzyme activities in the white muscle of all populations accompanied by enhanced Arrhenius activation energies (E_a) for citrate synthase of North Sea and Arctic cod. Higher enzyme activities provided clear evidence for permanent cold adaptation in Northern compared to Southern populations. Growth rates were highest at temperatures between 10 and 11 °C in all populations.

In conclusion, cold compensation for enhancing aerobic capacity likely includes mitochondrial and enzymatic proliferation (St. Pierre et al., 1998; Wilson, 1973), whereas E_a may be adjusted high to minimize metabolic flux (Pörtner et al., 2000). These adjustments may also reflect a change in energy budget, contributing to lower liver lipid contents and decreasing growth at low temperatures.

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Pörtner, H.O., van Dijk, P.L.M., Hardewig, I., Sommer, A. (2000). In: Davison, W., Williams, C.H. (Eds.) *Antarctic Ecosystems: Models for Wider Ecological Understanding*. Caxton Press, Christchurch.

A2/C5.29—Thermal tolerance of a variety of temperate crab species

M. Cuculescu, Biology, University of Northumbria at Newcastle; R.S. Hopkin, S. Qari, K. Bowler, D. Hyde, Biology, University of Durham, UK

It is possible that the critical thermal maximum (CT_{max}) of a given species is adaptive and that it is related to the thermal properties of the environment that a species inhabits. In drawing conclusions regarding the adaptive nature of the CT_{max} of a particular species, it is important to demonstrate the extent to which phylogenetic relationships influence the magnitude of CT_{max} . To this end, we conducted a preliminary study of CT_{max} on nine species of winter acclimatized malacostracan crustacea. Species sampled included the anomurans *Pagurus bernhardus* and *Munida rugosa*, and the brachyurans *Carcinus maenas*, *Cancer pagurus*, and *Inachus dorsettensis*. CT_{max} values ranged from 19.8 ± 2.2 °C for *C. pagurus* to 31.9 ± 1.8 °C for *C. maenas*. Animals

were subsequently exposed to a 1-h heat shock at a sublethal temperature (1–2 °C below the CT_{max}) and allowed a further 1-h recovery period at 10 ± 1 °C before determination of CT_{max} . This heat shock treatment induced significant increases in the CT_{max} of the majority of species studied. For *C. maenas*, the elevation of CT_{max} with heat shock was correlated with characteristics of neuromuscular transmission measured electrophysiologically.

A2/C5.30—Regulation of the cardiovascular system of common carp (*Cyprinus carpio*) during severe hypoxia at three acclimation temperatures

J.A.W. Stecyk, A.P. Farrell, Department of Biological Sciences, Simon Fraser University, Burnaby, Canada

Unlike the large amount of information that exists for short-term moderate hypoxia, the cardiovascular responses of anoxia-tolerant teleosts, such as the common carp, to a prolonged exposure of severe hypoxia are limited. The first direct measurements of cardiac output during prolonged severe hypoxia in common carp (*Cyprinus carpio* L.) described an appreciable cardiac depression with severe hypoxia at acclimation temperatures of 6, 10 and 15 °C (1). Our ongoing studies now confirm the earlier prediction that that common carp utilize a Pasteur effect to maintain cardiac function during severe hypoxic exposure at these temperatures. Cardiovascular controls were also elucidated through injections of alpha-adrenergic, cholinergic, purinergic, and beta-adrenergic antagonistic drugs under normoxic and severely hypoxic conditions. These experiments revealed that cardiac depression during severe hypoxia was mainly mediated by increased vagal tone. Injection of phentolamine, which decreased systemic resistance and increased heart rate, illustrated that a strong alpha-adrenergic tone and a barostatic reflex were retained during severe hypoxia. Injection of the purinergic antagonist in hypoxic fish had no cardiovascular effect, but interestingly increased ventilation rate. Injection of the beta-adrenergic antagonist propranolol significantly reduced cardiac output, heart rate and stroke volume indicating the presence of an adrenergic tone during severe hypoxic exposure, a finding in agreement with the earlier observation of the presence of elevated catecholamine levels during hypoxia in the common carp (2).

(1) Stecyk and Farrell, JEB, in press.

(2) van Raaij et al. (1995). *Am. J. Physiol.* 268, R1163–R1170.