The Journal of Experimental Biology 202, 2233–2238 (1999)
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JEB2022

SEASONAL AND TEMPERATURE EFFECTS ON THE ADRENERGIC RESPONSES OF ARCTIC CHARR (*SALVELINUS ALPINUS*) ERYTHROCYTES

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Accepted 21 May; published on WWW 19 July 1999

Summary

In the present study, we have examined the adrenergic responses of Arctic charr (*Salvelinus alpinus*) erythrocytes acclimated to different temperatures (2, 8 and 14 °C) during different seasons. We measured the changes in cellular water and ion contents after noradrenaline stimulation using different noradrenaline concentrations and external pH values. Furthermore, the effects of acute temperature changes on the magnitude of the adrenergic response were studied. The adrenergic response of Arctic charr erythrocytes showed pronounced seasonal variation. The $[Na^+]/[Cl^-]$ accumulation ratio after adrenergic stimulation was greatest in May, indicating an enhanced activity of the Na^+/H^+ exchanger. The noradrenaline-induced change in $[Na^+]_i$ was greatest in spring. In addition to a seasonal effect, the exchanger seemed to be most active in erythrocytes from charr acclimated to low temperature (2 °C) early in May: the EC_{50} value was lower and the calculated maximal increase in $[Na^+]_i$ was greater in the 2 °C-acclimated group than in the other acclimation groups. In contrast, acclimation to different temperatures did not affect these responses (measured at a constant temperature) in February. An acute temperature change has a smaller effect on the adrenergic response of Arctic charr erythrocytes than on rainbow trout (*Oncorhynchus mykiss*) erythrocytes.

Key words: Na^+/H^+ exchange, noradrenaline, pH-dependency, EC_{50} value, acute temperature change, $[Na^+]/[Cl^-]$ accumulation ratio, Arctic charr, *Salvelinus alpinus*.

Introduction

The adrenergic responses of teleost erythrocytes are pronounced during acute changes in oxygen availability and oxygen demand (for reviews, see Motais et al., 1992; Nikinmaa, 1992; Thomas and Perry, 1992; Nikinmaa and Salama, 1998). Changes in temperature are associated with marked changes in the activity of teleost fishes, the resting rate of oxygen consumption increasing more than twofold with a 10 °C increase in temperature (Brett and Glass, 1973). Furthermore, an increase in temperature above the optimum usually leads to a reduction in the aerobic scope of activity (Brett and Glass, 1973). Thus, any responses that are related to the oxygen homeostasis of the animal may be greatly affected by temperature changes. In view of this, it is surprising that the effects of temperature on the adrenergically activated Na^+/H^+ exchange of teleost erythrocytes has been very little studied. In rainbow trout (*Oncorhynchus mykiss*) erythrocytes, an acute increase in temperature, over the temperature range 2–20 °C, causes a marked increase in the activity of the Na^+/H^+ exchanger, the Q_{10} value being 5.4 (Cossins and Kilbey, 1990). No information is available about the effect of long-term temperature acclimation on the adrenergic response. Studies on the seasonality of the adrenergic response of teleost erythrocytes have also been carried out almost exclusively using rainbow trout. Some of these studies have demonstrated that there is either a decline in the adrenergic Na^+/H^+ exchange activity (Cossins and Kilbey, 1989) or a total lack of adrenergic response (Nikinmaa and Jensen, 1986) in winter. Other investigations (Tetens et al., 1988; Milligan et al., 1989), however, found no effect of season on the adrenergic responses of trout erythrocytes. At present, the reasons for these differences remain unexplained. They may be a consequence of ‘genetic’ adaptation of different strains to prevailing environmental conditions, different acclimation procedures used in the studies or variations in the environmental or hormonal cues preceding the studies.

There is marked variation in the adrenergic responsiveness of teleost erythrocytes (e.g. Salama and Nikinmaa, 1989; Cossins and Kilbey, 1991; Val et al., 1998) measured at a constant temperature. The temperature optima of the species studied are markedly different, as are the temperature ranges they tolerate. However, to date, the data do not allow comparisons of the temperature-dependency of the adrenergic responses in erythrocytes from species with different thermal

preferences, since closely related species with different thermal optima have not been investigated systematically.

In view of the extensive data about the behaviour of rainbow trout erythrocytes, we have investigated the effects of season and temperature on the catecholamine responses of another salmonid species, the Arctic charr (*Salvelinus alpinus*), which is a relatively stenothermal cold-water species. The optimum temperature range for Arctic charr (5–16 °C) is lower than that for rainbow trout (10–22 °C; Elliott, 1981). Thus, comparisons of the temperature responses of this species with those of rainbow trout provide important insights about the thermal behaviour of the catecholamine-stimulated transport pathways of erythrocytes. In the present study, we have investigated the effects of season, temperature acclimation and acute temperature changes on the adrenergic responses of Arctic charr erythrocytes.

Materials and methods

Experiments were carried out at the Finnish Game and Fisheries Research Institute, Saimaa Fisheries Research and Aquaculture, at Enonkoski, Finland, in February, early May (1–10), late May (25–31) and August 1996 and in February 1997. The ambient water temperatures at the experimental periods were 1.6, 4.6, 8.8 and 1.4 °C, respectively. Sexually mature 4-year-old Arctic charr [S*alvelinus alpinus* (L.) 550–1360 g] and rainbow trout [*Oncorhynchus mykiss* (Walbaum) 700–1200 g] of both sexes were held in 1500 l tanks supplied with a constant flow of lake water. The actual flow rate was not measured but was adequate to maintain greater than 90 % air saturation in the outlet. Water temperature was adjusted to 2, 8 and 14 °C by mixing cold and warm (18 °C) water. The warm water was heated using automatic, external electrical heating elements. Temperature was measured daily and readjusted when necessary by adjusting the flows of cold and heated water. The animals were subjected to a 12 h:12 h light:dark photoperiod and fed regularly to satiation with commercial pellet (Tess Vital 5, Rehuraisio, Finland). The fish were allowed to acclimate to the three temperatures for at least 3 weeks before their erythrocytes were used in experiments.

Blood samples were taken from anaesthetized (MS 222; 0.1 g l^{-1} water) fish by caudal puncture into heparinized syringes. Plasma and erythrocytes were separated by centrifugation (10 000 ***g***, 2 min), and the erythrocytes were washed twice with the saline used in the experiments. The composition of the saline (in mmol l^{-1}) was as follows: 128 NaCl, 3 KCl, 1.5 $MgCl_2$, 1.5 $CaCl_2$, 5 glucose, 3 pyruvate and 10 Hepes, pH 7.6 at 20 °C. The washed red blood cells were left overnight at 4 °C to equilibrate. This procedure ensures that cells from the different acclimation groups have exactly the same acute temperature history. Thus, the differences measured reflect long-term acclimation of the erythrocyte membrane. Before the experiments began, the erythrocytes were resuspended to a haematocrit of 20 %.

Experimental protocol

The dose–response relationship of erythrocytes for noradrenaline was first determined at pH 7.1 and at 20 °C. This low pH and high temperature were chosen to obtain a maximal adrenergic response from the erythrocytes. A control sample was taken from each cell suspension before the addition of noradrenaline solution, and the remainder of the erythrocyte suspension was divided in six subsamples. Noradrenaline was added to these subsamples so that the final concentrations were 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} mol l^{-1}. After a 10 min incubation at 20 °C in a shaking waterbath, samples were taken for ion and water content measurements.

To study the effects of extracellular pH on the adrenergic responses, every cell suspension was divided into 10 subsamples, and paired subsamples were incubated in salines with pH values of 6.8, 7.1, 7.4, 7.7 and 8.0. Noradrenaline (final concentration 10^{-5} mol l^{-1}, a concentration giving a maximal response in the dose–response study) was added to one of the subsamples at each pH, while the other served as a control. After a 10 min incubation at 20 °C, samples were taken for measurement of the ion and water content of erythrocytes.

The effects of acute temperature changes on the adrenergic response of erythrocytes were measured for Arctic charr and rainbow trout erythrocytes. The saline at pH 7.1 was either cooled or warmed to 2, 8, 14 and 21 or 26 °C. The erythrocytes were suspended in these salines and divided into two subsamples at each temperature. Noradrenaline (10^{-5} mol l^{-1} final concentration) was added to one of the subsamples at each temperature, the other subsample serving as a control. After a 10 min incubation at each experimental temperatures (2, 8, 14 and 21 or 26 °C), samples were taken for measurement of the ion and water content of red blood cells.

Measurement of intracellular water and ion content

To measure the intracellular water and ion contents, 1 and 0.5 ml samples, respectively, were placed into two preweighed Eppendorf tubes. The saline and erythrocytes were separated by centrifugation (10 000 ***g***, 2 min). The cell pellet in one of the tubes was weighed, dried at 105 °C for 24 h and reweighed, and the water content (%) of the packed cell pellet was calculated. The extracellular water in the pellet was not subtracted. The other tube was used for Na^+, K^+ and Cl^- measurements. The cell pellet was weighed, 300 µl of 0.6 mol l^{-1} perchloric acid was added to deproteinize the cells, and the samples were stored in liquid nitrogen until measurements were made. The Na^+ and K^+ contents (mmol l^{-1}) were measured using flame photometry (FLM3, Radiometer, Copenhagen), and the Cl^- content (mmol l^{-1}) using a Radiometer CMT 10 chloride titrator. The ion contents of the erythrocytes were calculated as mmol kg^{-1} dry cell mass. In the Results, the values are given as noradrenaline-induced changes in ion contents: in every case, paired samples were used, and the control value was subtracted from the catecholamine-treated value. In addition, the $[Na^+]/[Cl^-]$ accumulation ratio ($d[Na^+]_i/d[Cl^-]_i$) was calculated from the measured changes in ion contents.

Data handling

Dose–response curves, the EC_{50} value (the concentration giving 50 % maximal effect) for noradrenaline and the

maximal accumulation of Na^+ were obtained by fitting a four-parameter logistic equation (SigmaPlot 4.0, SPSS Inc.) to the dose–response data. The Kruskall–Wallis test was used to test for significant differences in the EC_{50} values and for the maximal accumulation of Na^+ between acclimation groups. Other data were analyzed by using multivariate analyses of variance (MANOVAs) followed by least-significant difference tests to determine whether the treatments (temperature or season) had a statistically significant effect on the magnitude of the adrenergic responses of Arctic charr and rainbow trout erythrocytes. $P<0.05$ was taken as the level of significance.

Results

There was a clear effect of pH on the catecholamine response of Arctic charr erythrocytes (Fig. 1). When measured at constant temperature (20 °C), stimulation of the erythrocytes with 10^{-5} mol l^{-1} noradrenaline caused a far greater accumulation of Na^+ at pH 6.8 than at pH 8.0 ($P<0.001$). The effect of pH on Na^+ accumulation was similar at all seasons studied. Similarly, season did not affect Cl^- accumulation significantly. However, the $[Na^+]/[Cl^-]$ accumulation ratio, an indicator of the effect of adrenergic stimulation on the intraerythrocytic pH (Nikinmaa and Salama, 1998), was somewhat greater in early May, although not significantly ($P=0.133$), than in the other seasons studied, suggesting some seasonality in the response of fish acclimated to 8 °C.

Season strongly modifies the effects of acclimation temperature on the catecholamine response of Arctic charr erythrocytes (measured at a constant temperature). The data for the relationship between noradrenaline concentration and Na^+ accumulation were fitted to a four-parameter logistic equation:

$$y = y_0 + \frac{a}{1 + \left(\frac{x}{x_0}\right)^b},$$

where y is the change in Na^+ content, y_0 is the minimal response (unstimulated value), x is noradrenaline concentration, x_0 is the noradrenaline concentration giving a 50 % response, a is the maximal response and b is a constant (Fig. 2). To evaluate the statistical significance of differences in the concentration of noradrenaline required to give a 50 % response (EC_{50} value) and the maximal accumulation of Na^+ between acclimation groups, the dose–response data from each individual fish were fitted to the same equation as the averaged data (not shown). In February, prior acclimation to 2, 8 or 14 °C did not influence either the EC_{50} value or the maximal accumulation of Na^+. The calculated maximal changes in intracellular Na^+ content were 47.9 mmol kg^{-1} dry cell mass for the 2 °C-acclimated group, 44.3 mmol kg^{-1} dry cell mass for the 8 °C-acclimated group and 52.4 mmol kg^{-1} dry cell mass for the 14 °C-acclimated group. The EC_{50} values for noradrenaline were 7.45×10^{-9} mol l^{-1} for 2 °C-acclimated, 2.17×10^{-8} mol l^{-1} for 8 °C-acclimated and 3.66×10^{-8} mol l^{-1} for 14 °C-acclimated fish. Similar results were obtained for fish acclimated to the three temperatures in August (results not shown). In contrast, in early May, the EC_{50} value for noradrenaline was significantly affected by acclimation temperature ($P=0.042$); the dose–response curve was shifted to

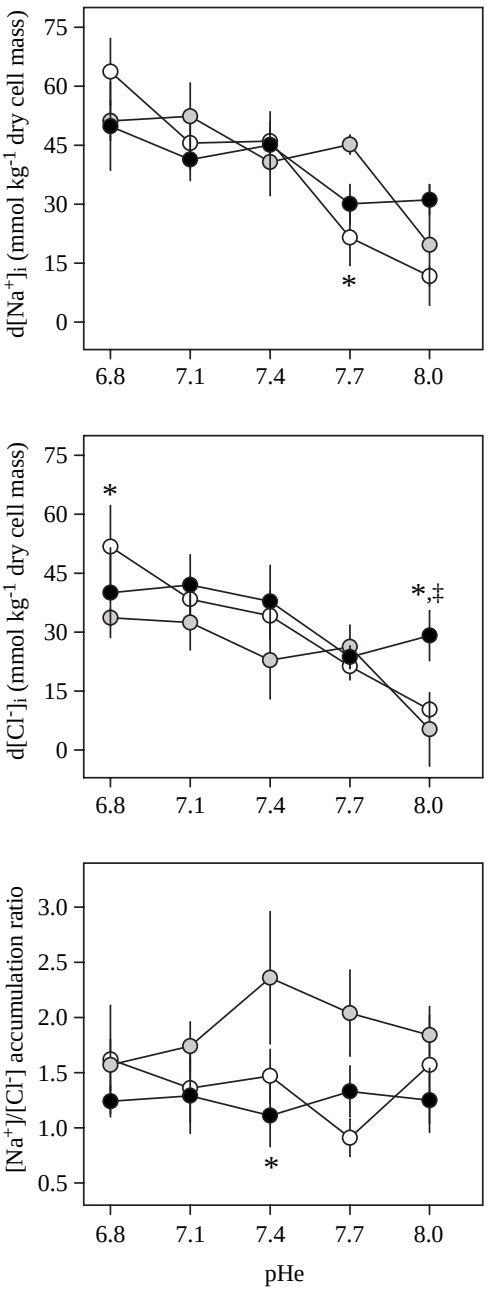

Fig. 1. Changes in intracellular Na^+ ($d[Na^+]_i$) and Cl^- ($d[Cl^-]_i$) content and $[Na^+]/[Cl^-]$ accumulation ratio during a 10 min incubation with 10^{-5} mol l^{-1} noradrenaline as a function of extracellular pH (pHe) in Arctic charr erythrocytes acclimated to 8 °C in February (open circles), in early May (shaded circles) or in August (filled circles). Measurements were performed at 20 °C, and values are means ± S.E.M. from 4–6 experiments. ‡ and * indicate a significant difference from the value in February and May, respectively ($P<0.05$; MANOVA). The increase in $d[Na^+]_i$ and $d[Cl^-]_i$ with decreasing extracellular pH from 8.0 to 6.8 was significant in each acclimation groups ($P<0.001$; MANOVA).

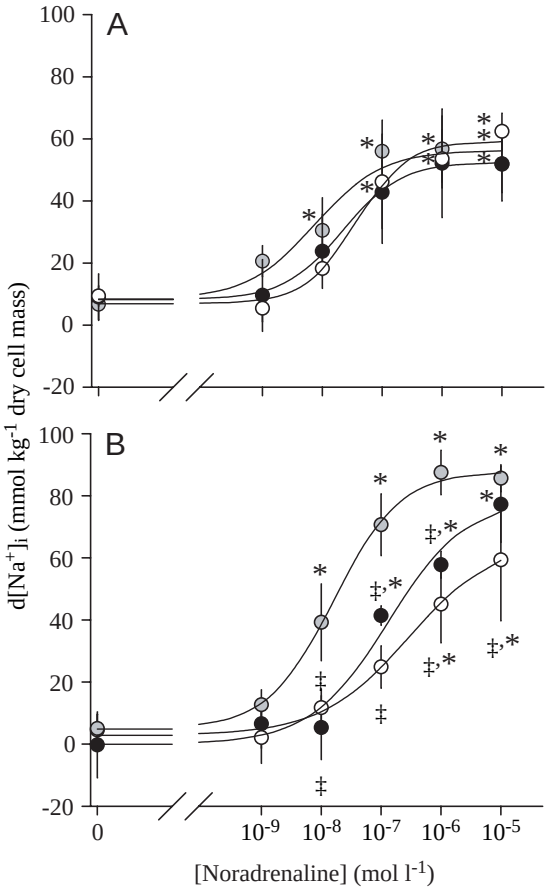

Fig. 2. The increase in intracellular Na^+ content ($d[Na^+]_i$) of Arctic charr erythrocytes after a 10 min noradrenaline stimulation at extracellular pH 7.1 and at 20 °C in February (A) and in early May (B). Charr were acclimated at 2 °C (shaded circles), 8 °C (filled circles) or 14 °C (open circles). Values are means ± S.E.M., N=4–6. The dose–response curves were obtained by fitting a four-parameter logistic equation (see text) to the data. An asterisk (*) indicates a significant difference from the unstimulated value (P<0.05; MANOVA), and a double dagger (‡) indicates a significant difference from the corresponding value at an acclimation temperature of 2 °C (P<0.05; MANOVA).

the left in the cold-acclimated specimens. Similarly, maximal Na^+ accumulation after noradrenaline stimulation was affected by prior thermal acclimation in early May (P=0.049): it increased with decreasing acclimation temperature of the fish. The EC_{50} values for noradrenaline were 1.62×10^{-8}, 1.33×10^{-7} and 2.95×10^{-7} mol l^{-1}, and the calculated maximal increases in intracellular Na^+ content were 83.1, 79.1 and 63.4 mmol kg^{-1} dry cell mass, respectively, for erythrocytes from 2 °C-, 8 °C- and 14 °C-acclimated Arctic charr. The temporal variation may change rapidly since, in late May, the most pronounced catecholamine response was observed for 8 °C-acclimated animals, followed by 2 °C- and 14 °C-acclimated fish (Fig. 3B).

The acute effects of temperature on the adrenergic response of charr erythrocytes were also affected by season (P<0.01). In February, there was an increase in adrenergic Na^+ accumulation with increasing measurement temperature over the temperature range 2–14 °C; the response was slightly reduced in the samples measured at 26 °C (Fig. 3). The effect of temperature was independent of prior temperature acclimation except at the lowest incubation temperature (2 °C), where the response was smaller in the 14 °C-acclimated fish than in other acclimation groups. In contrast, in late May, prior temperature acclimation clearly affected the acute temperature response: the fish acclimated to 2 °C showed increasing Na^+ accumulation only over the temperature range 2–8 °C; at higher temperatures of acclimation, the accumulation remained constant. Fish acclimated to 8 °C showed a clear increase in the response throughout the temperature range studied, but with a slight reduction in the rate of increase in Na^+ accumulation between 14 and 26 °C. In contrast, 14 °C-acclimated fish showed an increased rate of Na^+ accumulation between 14 and 26 °C. Comparisons of the acute temperature responses of Arctic charr erythrocytes and rainbow trout erythrocytes to adrenergic stimulation (measurements made in February) indicated that the adrenergic accumulation of Na^+ was somewhat greater in rainbow trout than in Arctic charr (P<0.01) and that, whereas in rainbow trout the accumulation increased more or less linearly with temperature throughout the

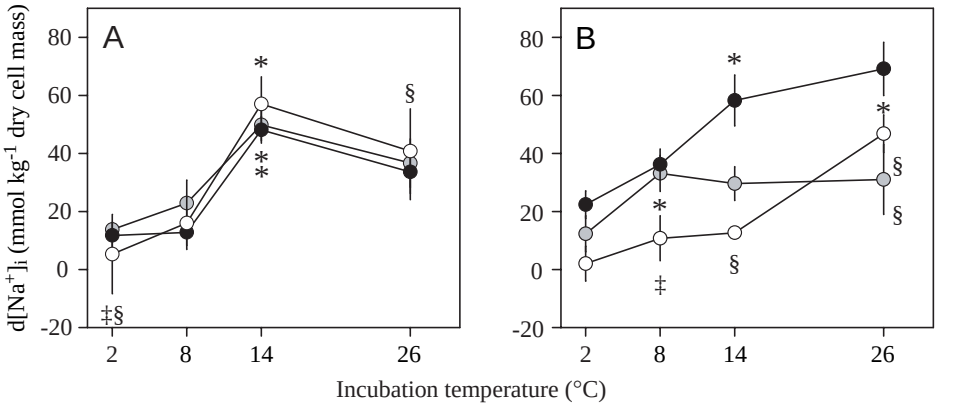

Fig. 3. The effects of temperature *in vitro* on the adrenergic increase in cellular Na^+ content ($d[Na^+]_i$) of Arctic charr erythrocytes. Fish were acclimated to 2 °C (shaded circles), 8 °C (filled circles) or 14 °C (open circles). Measurements were performed at extracellular pH 7.1 in February (A) or in late May (B) after a 10 min noradrenaline incubation (10^{-5} mol l^{-1}). An asterisk (*) indicates a significant increase in intracellular Na^+ content from the value at the preceding incubation temperature (P<0.05; MANOVA). ‡ and § indicate a significant difference from the corresponding value at acclimation temperatures of 2 and 8 °C, respectively (P<0.05; MANOVA). Values are means ± S.E.M., N=5–6.

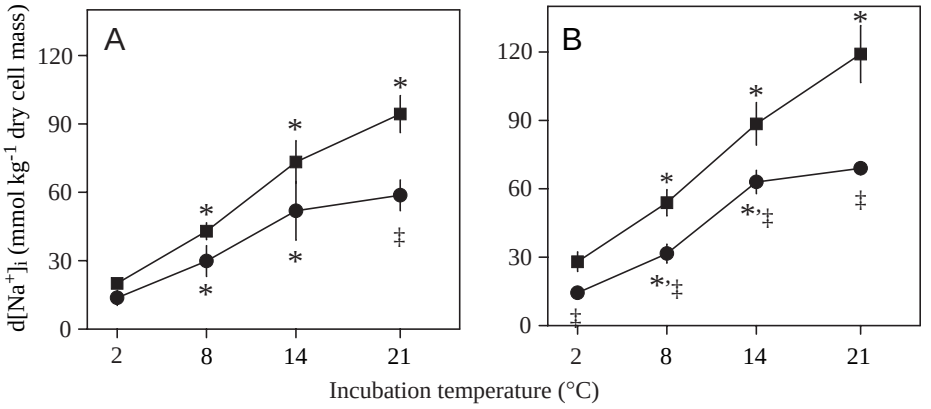

Fig. 4. The effects of temperature *in vitro* on the adrenergic increase in cellular Na^+ content ($d[Na^+]_i$) of Arctic charr (circles) or rainbow trout (squares) acclimated at 2 °C (A) or 14 °C (B) in February. Measurements were performed at extracellular pH 7.1 after a 10 min noradrenaline incubation (10^{-5} mol l^{-1}). An asterisk (*) indicates a significant change in intracellular Na^+ content with an increase in incubation temperature, and a double dagger (‡) indicates a significant difference between species (P<0.05; MANOVA). Values are means ± S.E.M. of four fish.

temperature range studied (2–21 °C), in charr the accumulation did not increase between 14 and 21 °C (Fig. 4).

Discussion

The adrenergic responses of Arctic charr erythrocytes show properties similar to those of other fishes. The response increases with a decrease in pH, as in other species studied (Nikinmaa et al., 1987; Salama and Nikinmaa, 1989; Cossins and Kilbey, 1991). The EC_{50} values for the noradrenaline response of charr erythrocytes measured in the present study (between 7×10^{-9} and 10^{-7} mol l^{-1} depending on the acclimation temperature and season) are within the range observed in other species studied, e.g. rainbow trout *O. mykiss* (1.3×10^{-8}, Tetens et al., 1988; 2.3×10^{-8}, Cossins and Kilbey, 1989), Atlantic cod *Gadus morhua* (1.4×10^{-7}, Berenbrink and Bridges, 1994), yellowfin tuna *Thunnus albacares* (8.9×10^{-8}, Lowe et al., 1998) and skipjack tuna *Katsuwonus pelamis* (4.8×10^{-8}, Lowe et al., 1998). Although the catecholamine response of Arctic charr is relatively large, at a given temperature, it is less pronounced than in rainbow trout under similar conditions.

Cossins and Kilbey (1990) reported that the adrenergically stimulated Na^+ influx into rainbow trout red cells was highly temperature-dependent, with a Q_{10} value of 4.4 over the temperature range 0–20 °C. The acute effect of temperature on the catecholamine response of Arctic charr erythrocytes was similar to that of rainbow trout at low temperatures. However, the lower optimum temperature of Arctic charr than of rainbow trout (Elliott, 1981) is reflected in the effects of acute temperature change on the adrenergic responses at higher temperatures. Whereas the accumulation of Na^+ over a 10 min period in rainbow trout showed a linear increase within the temperature range 2–21 °C, in Arctic charr the accumulation of Na^+ was similar at 14 and 21 °C. It is, however, obvious that in May, during which the temperature increases, prior acclimation to high temperature (14 °C) is able to shift the profile for acute temperature response such that an increase in response is observed between 14 and 26 °C.

The major finding of the present study was the pronounced seasonality of the adrenergic responses and, particularly, the effects of season on the temperature acclimation of the adrenergic responses. Nikinmaa and Jensen (1986) could detect no adrenergic responsiveness of rainbow trout erythrocytes during early winter. Cossins and Kilbey (1989) observed that the capacity of the Na^+/H^+ exchanger was minimal in winter and increased during the early spring. The increase in the capacity of the catecholamine-stimulated Na^+/H^+ exchanger of rainbow trout red blood cells in spring may be caused by activation of erythropoiesis (Cossins and Kilbey, 1989). However, the transport properties of mature and immature red blood cells have hitherto not been compared. In addition to the catecholamine-stimulated Na^+/H^+ exchange, other transport systems also exhibit seasonal variation in activity. The activity of the Na^+ pump has been shown to be greatest in summer (Gabbianelli et al., 1996). The activities of amino acid carriers (ASC and asc) vary according to a seasonal cycle, showing maximal activity in winter and spring (Gallardo et al., 1997). Although seasonal effects have been demonstrated for many transporters in fish erythrocytes, the present study is the first in which the interactions between season and acclimation temperature have been studied in detail. It is clear from the present studies that, in winter, prior acclimation temperature does not affect the maximal Na^+ accumulation (over 10 min), the dose–response curve or the effects of acute temperature change on the transporter. In contrast, in spring (early May), cold-acclimated specimens had the highest Na^+ accumulation (during the 10 min measuring time) and lowest EC_{50} value. This indicates a pronounced upregulation of the adrenergic system in cold-acclimated specimens. It is unlikely that the upregulation could be due to erythropoiesis, since after the haematocrit had been decreased by 50 %, there was no indication of the activation of erythropoiesis in cold-acclimated fish in May (Lecklin and Nikinmaa, 1998). Other possibilities include an increase in the number of functional adrenergic receptors, e.g. *via* recycling of internalized receptors (Reid and Perry, 1991), or an increase in the number of Na^+/H^+ exchangers (Reid et al., 1993) in existing erythrocytes. Such upregulation would probably require hormonal signals. At present, the role of other hormones in regulating the adrenergic system of fish erythrocytes is poorly understood. Notably, Reid and Perry (1991) have observed an interaction between corticosteroids and adrenergic receptors. Steroid hormone levels were not measured in the present study. However, since

sexually mature Arctic charr were used in this study, the effects of annual changes in the reproductive system on adrenergic responsiveness cannot be excluded.

The upregulation of the erythrocytic adrenergic system of Arctic charr at low temperature corresponds to the observation that charr continue eating and growing even at temperatures as low as 1–3 °C (Wandsvik and Jobling, 1982; Jensen, 1985). Our data suggest that the responses of Arctic charr to temperature acclimation change rapidly with an increase in the 'expected' ambient temperature. In early May, when the normal ambient temperature is 2 °C, the adrenergic responses of charr erythrocytes were most pronounced in 2 °C-acclimated fish, whereas in late May, when the ambient temperature is normally approximately 8 °C, the most pronounced responses were seen in the 8 °C-acclimated group. Furthermore, at that time, the 2 °C-acclimated fish failed to show an increase in the adrenergic response at temperatures above 8 °C. However, since our experiments were not specifically designed to investigate this point, definite conclusions require a more thorough study using exactly the same protocol throughout the time during which ambient temperature increased.

In conclusion, our results show that season markedly influences the effects of prior temperature acclimation on the adrenergic responses of Arctic charr erythrocytes. The results, furthermore, clearly show that both the affinity of the adrenergic system and the capacity of the Na^+/H^+ transporter can be influenced by temperature acclimation and season. Also, the enhancement of the response in cold-adapted Arctic charr in spring demonstrates that the species is physiologically cold-adapted.

These studies were supported by grant 40830 from the Academy of Finland. T.L. is a recipient of Ministry of Education graduate scholarship in the National Graduate Program on Fish Biology and Fisheries.

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