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ENHANCED HYPO-OSMOREGULATION INDUCED BY WARM-ACCLIMATION IN ANTARCTIC FISH IS MEDIATED BY INCREASED GILL AND KIDNEY Na^+/K^+-ATPase ACTIVITIES

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Summary

Serum osmolality and serum inorganic ion concentrations were studied in two antarctic fish species, *Trematomus bernacchii* and *T. newnesi*, during 5 weeks of acclimation to 4 °C and compared with control values for groups acclimated to −1.5 °C. Acclimation to 4 °C significantly decreased the serum osmolality of both species, thereby increasing their seawater-to-extracellular fluid (ECF) osmotic gradient. The decline in osmolality with acclimation to 4 °C was accompanied by significant and rapid losses of Na^+ and Cl^- during the first 14 days of acclimation and was maintained throughout the study period. At day 35 of acclimation, the lipid composition and microsomal Na^+/K^+-ATPase specific activities at 4 °C and 37 °C were determined in membranes from gill, kidney, liver and muscle tissues. No warm-induced decrease in fatty acid unsaturation was found in the tissues of either species. In the gills and kidneys of both species, the Na^+/K^+-ATPase activities assayed at 4 °C were increased after acclimation to 4 °C. The Na^+/K^+-ATPase activities at 37 °C increased at the higher acclimation temperature in *T. newnesi* kidneys and *T. bernacchii* gills, but in both species there was no compensation to temperature in the liver, regardless of assay temperature. Muscle Na^+/K^+-ATPase activity decreased in response to warm-acclimation in *T. bernacchii* and *T. newnesi* assayed at 4 °C and 37 °C, respectively. During acclimation to 4 °C, the discontinuity in the Arrhenius plot of the Na^+/K^+-ATPase activities of *T. newnesi* gill moved to a lower temperature, whereas that of kidney remained unchanged. The results indicate that acclimation to 4 °C induced a decrease in serum osmolality which resulted from the positive compensation of Na^+/K^+-ATPase in osmoregulatory tissues. The enhancement in Na^+/K^+-ATPase activity at 4 °C suggests that energy expenditure in antarctic fish may be lessened, in part, by maintaining a reduced seawater-to-ECF osmotic gradient.

Key words: Na^+/K^+-ATPase, antarctic fish, osmoregulation, fatty acid composition, osmolality, *Trematomus bernacchii*, *Trematomus newnesi.*

Introduction

The antarctic marine environment of McMurdo Sound is characterized by chronic low temperatures that approach the freezing point of sea water, −1.9 °C (Littlepage, 1965). Temperatures of the Southern Ocean have been cold for the past 40 million years and have contributed to characteristics commonly ascribed to approximately 120 species of antarctic fish (order Notothenidae) such as endemism and marked stenothermy (Eastman and DeVries, 1986). Antarctic fishes are also unique in that their plasma osmolality is twice that of temperate marine fish (O'Grady and DeVries, 1982), and this relative hyperosmolality is attributed mainly to an increased serum NaCl concentration (Dobbs and DeVries, 1975). The elevated osmolality of antarctic fish plasma is, in turn, responsible for reducing the osmotic gradient between their extracellular fluid (ECF, 600 mosmol kg^{-1}) and the seawater environment (1000 mosmol kg^{-1}); the gradient that antarctic fish experience is half of that experienced by temperate and arctic marine teleosts (O'Grady and DeVries, 1982). Our preliminary studies showed that the serum osmolality of antarctic fish decreased significantly upon acclimation to 4 °C, thereby increasing the gradient between the ECF and the environment. In relation to plasma osmolality and acclimation temperature, there are no clear general trends that have emerged for teleost fish (Burton, 1986); however, similar

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osmolality responses to temperature have been reported in *Myoxocephalus scorpius* and *Myoxocephalus quadricornis*, whose serum osmolality, Na^+ and Cl^- concentrations were all significantly higher at −0.1 °C than at 10 °C (Oikari, 1975). In notothenioids, the nature of the enhanced ionic load to the ECF is unknown but, given that metabolic energy is at a premium for these fish and that ion transport requires ATP, the reduced ECF-to-seawater gradient experienced by these fish may result from reduced transport rates and conservation of energy by the osmoregulatory tissues (Prosser *et al.* 1970).

The pathways for ion transport in seawater fish and the key role of Na^+/K^+-ATPase in teleost osmoregulation have been widely recognized. In teleost gills, the Na^+/K^+-ATPase, located in the extensive tubular system of chloride cells, is indirectly responsible for Cl^- secretion by these cells (Hickman and Trump, 1969; Silva *et al.* 1977; Epstein *et al.* 1967; Prosser, 1986). In the renal proximal tubules of teleosts, fluid secretion is inhibited by ouabain, suggesting that the secreted fluid is driven by transport mechanisms indirectly dependent on the function of the Na^+/K^+-ATPase, possibly by an apically located Na^+/Mg^{2+} exchange (Beyenbach *et al.* 1986; Cliff *et al.* 1986). The proximal tubules are responsible for active secretion of divalent ions by the aglomerular kidneys of antarctic fish (Dobbs and DeVries, 1975). Na^+/K^+-ATPase activities in gill and kidney tissues of teleost fish have been shown to differ significantly between cold- and warm-acclimated states, making this enzyme a model for temperature-compensation studies. In kidney and gill tissues of the freshwater fish *Rutilus rutilus* and *Salvelinus alpinus*, the Na^+/K^+-ATPase activities increased with cold-acclimation (Schwarzbaum *et al.* 1991). In contrast, cold-acclimatization-induced decreases in Na^+/K^+-ATPase activity in gills have been reported in anadromous arctic char *Salvelinus alpinus*. Thomson *et al.* (1977) found that the increases in saturated fatty acid levels induced by higher temperatures in the gills of *Anguilla anguilla* resulted in a discontinuity in the Arrhenius plot Na^+/K^+-ATPase between 12 and 20 °C.

To investigate the capacity for osmoregulation of antarctic fish at different temperatures, the osmolality and ionic concentration were measured in the serum of control (−1.5 °C) and warm-acclimated (4 °C) groups of two species of notothenioids, *Trematomus bernacchii* and *Trematomus newnesi*. To explore the underlying mechanism for the enhanced hypo-osmoregulation induced by warm-acclimation, we investigated the hypothesis that the elevated serum osmolality of antarctic fishes may be the result of reduced excretion rates at the gills and/or kidneys by assaying the specific activities of Na^+/K^+-ATPase from fish in both temperature groups. Muscle and liver tissues, as representatives of non-osmoregulatory organs, served as control tissues in the enzyme studies. Furthermore, to investigate the physical state of the lipid membranes during acclimation, the fatty acid composition and the degree of saturation were determined in these same tissues.

Materials and methods

Fish collection

Trematomus bernacchii (Boulenger) and *Trematomus newnesi* (Boulenger) were caught by hook and line at a depth of 20 m and at a water temperature of −1.9 °C during mid-October to mid-November. The fish hole, located at 77°51′540″ S, 166°39′5530″ E, was adjacent to the rock jetty off McMurdo Station, Antarctica. Fish were placed in insulated coolers and carried 270 m to the aquarium. The fish were held at −1.8 °C in a 200 l holding tank for a period of 3–5 days until acclimation experiments were begun.

Temperature acclimation

Eight individuals of each species were transferred from the holding tank to individual 90 l opaque aquaria maintained at 4 °C and −1.5 °C (controlled to within 0.1 °C). The entire acclimation period was 5 weeks, during which the fish were not fed and no effort was made to control for photoperiod in the aquarium. For a second set of experiments, referred to in the Results section as ‘reversibility experiments’, eight individuals from each species were placed in aquaria at 4 °C for 8 days and serum osmolality was determined at 48 h intervals as described below. At day 8, the fish were placed in aquaria at −1.5 °C and measurement of serum osmolality was continued every 48 h for another 8 days.

Blood collection

Fish were weighed in a beaker of sea water on a tared balance at weekly intervals, and blood was collected from a caudal vessel into a non-heparinized 1 ml plastic syringe with a 30 gauge × 1.2 cm needle. The blood was placed in a polyethylene tube, capped and allowed to clot at 4 °C for 2 h. The clotted blood was centrifuged at 1000 ***g*** for 5 min and the serum was transferred to a second polyethylene tube for future analyses.

Serum osmolality and ionic concentrations

Serum osmolality was determined with a vapour pressure osmometer (Wescor Inc, Logan, UT, USA). Na^+ and K^+ concentrations were determined by flame photometry (Corning, NY, USA) and Cl^- concentrations were assessed employing a Buchler-Cotlove chloridometer. Ca^{2+} and Mg^{2+} concentrations were measured using atomic absorption spectroscopy (Perkin-Elmer, model 400, NY, USA).

Tissue collection

After acclimation at −1.5 °C or 4 °C for 5 weeks, fish were killed by a blow to the head. Whole gills (including filaments and gill arch), ‘trunk’ kidney, white muscle and liver tissues were then quickly excised and preserved for lipid and enzyme analyses. Half of each tissue sample was preserved for phospholipid analysis and the remainder for enzyme studies. White muscle tissue was sampled from each side of the fish, just below the first dorsal fin. Left gill arches were preserved for phospholipid analysis and right arches for enzyme assays. Different lobes of the liver were sampled for phospholipid and

enzyme determinations. Kidney tissue was taken from the trunk section of the organ, a well-defined area which makes up the nephron mass. The tissues (approximately 0.5 g wet mass) used for phospholipid analysis were excised and immediately placed in a cryotube containing a solution (3 ml) composed of chloroform:methanol (2:1, v/v). Autoxidation of the unsaturated fatty acids was minimized by the addition of 50 μl of butylated hydroxytoluene (2 % chloroform, w/v). The tubes were immediately placed in liquid nitrogen and stored at −80 °C until analysis. Tissues for enzyme assays were immediately wrapped in aluminium foil, flash-frozen between a pair of pliers previously chilled in liquid nitrogen and stored at −80 °C until analysis.

Urine collection

Bladder urine was sampled immediately after the fish had been killed. Urine samples were collected in a 1 ml non-heparinized plastic syringe with a 30 gauge × 1.2 cm needle and determinations of urine osmolality and electrolytes were made as described above.

Phospholipid analysis

Total lipids were extracted by homogenizing the tissues in 3 ml of chloroform:methanol (2:1, v/v), as described previously (Stanley-Samuelson and Dadd, 1983). For analysis of phospholipid fatty acids (FAs), total lipid extracts were applied to thin layer chromatography (TLC) plates (silica gel G, 20 cm × 20 cm, 0.25 mm thick; Sigma Chemical Co., St Louis, MO, USA). After developing the plates in petroleum ether:diethyl ether:acetic acid (80:20:1, v/v; Stanley-Samuelson and Dadd, 1983), bands corresponding to phospholipid standards were scraped into reaction tubes. The FAs in each fraction were then transmethylated to FA methyl esters by refluxing in acidified methanol for 90 min (Stanley-Samuelson and Dadd, 1983). The FA methyl esters were extracted from the reaction mixtures with petroleum ether, concentrated and then analyzed by gas chromatography, as described by Howard and Stanley-Samuelson (1990). They were then chromatographed isothermally at 190 °C on a Hewlett-Packard HP-5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a SP-2330 capillary column (0.25 mm × 30 m, 0.2 μm film thickness; Supelco, Bellefonte, PA, USA), a flame-ionization detector and an HP-3396A recording integrator. Injections were made in split mode (45:1), and separations were carried out with helium carrier at 0.6 ml min^{-1}. Components were identified by comparisons of retention times with authentic standards as reported elsewhere (Stanley-Samuelson and Dadd, 1983; Stanley-Samuelson and Loher, 1983; Ogg *et al.* 1991). The structures of the FA methyl esters were confirmed by capillary gas chromatography/mass spectrometry. Chemical ionization mass spectra were obtained using a Hewlett-Packard 5890 series II gas chromatograph (GC; Hewlett-Packard, Inc., San Fernando, CA, USA) coupled to a Hewlett-Packard 5971 mass selective detector (MSD) with G1072A chemical ionization accessory. The GC was equipped with a 30 m×0.32 mm DB-5 capillary column (J&W Scientific, Folsom, CA, USA), and all analyses utilized temperature-programming from 70 to 275 °C at 5 °C min^{-1}. Each run utilized an initial hold period of 2 min. The splitless injector port was set at 250 °C and the GC/MSD interface was set at 280 °C. Ultrapure helium was the carrier gas, with a column head pressure of 3.5 kg cm^{-3}, and ultrapure methane was the ionizing gas. Mass spectra were scanned from *m*/*z*=50 to *m*/*z*=400 and acquired data were collected and analyzed on a Hewlett-Packard Vectra 486/33T workstation using HP chemstation software. Chemical ionization mass spectra of fish-derived FA methyl esters were compared with authentic standards obtained from Sigma Chemical Co. (St Louis, MO, USA).

Determinations of Na⁺/K⁺-ATPase activity

Crude membrane preparation

The activity of Na^+/K^+-ATPase was determined in gill filaments (scraped from gill arches), 'trunk' kidney, liver and muscle tissues from three individuals of each species acclimated at each temperature (−1.5 °C and 4 °C) for 5 weeks. Each tissue was finely minced and 50 mg wet mass was homogenized per 1 ml of an ice-cold solution containing 1 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole buffer, pH 7.4, 5 mmol l^{-1} 2-mercaptoethanol and 250 mmol l^{-1} sucrose. Homogenization was carried out on ice, employing 15 complete strokes using an electric drill with rheostat set at 600 revs min^{-1}. Homogenized tissue was centrifuged at 4 °C and 2000 ***g*** for 15 min in a B20-A International Equipment Company centrifuge (Needham, MA, USA). The first pellets, containing large fragments and heavy cellular components, were discarded and the supernatants centrifuged at 19 000 ***g*** for 90 min. The final pellets were suspended in the original homogenizing medium to give a protein concentration of 0.5–2.0 mg ml^{-1} and stored in 0.75 ml samples at −80 °C. The supernatants obtained after the second centrifugation were not checked for ATPase activity because of our inability to detect a measurable protein concentration.

Protein determinations

The amount of protein was determined by the method of Lowry *et al.* (1951) using 0.2 mg ml^{-1} bovine serum albumin as a standard.

Enzyme assays

Enzyme assays were carried out at 4 °C and 37 °C using a reaction mixture consisting of 5 mmol l^{-1} Tris–ATP, pH 7.4, 50 mmol l^{-1} imidazole buffer, 100 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 5 mmol l^{-1} $MgCl_2$ and an appropriate amount of enzyme (usually 0.1–1.0 mg ml^{-1}) in the presence and absence of 2 mmol l^{-1} ouabain. The reaction volume was 0.5 ml for all mixtures. The reaction system, minus ATP, was equilibrated at the assay temperature for 10 min, and the reaction was started by adding ATP. The ATP (Tris salt, vanadate-free, 95–98 % purity) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Individual assays were run in duplicate for 15 or 20 min at 37 °C and for 30 min at 4 °C. Reactions were stopped

in ice by adding 100 μl of 25 % trichloroacetic acid. The mixture was then centrifuged at 2000***g*** for 10 min and a 0.5 ml sample of the supernatant was taken for the estimation of inorganic phosphate (P_i) by the method of Ames (1966). After 90 min of incubation at room temperature, the absorbance was read at 750 nm in an automated Bio-Tek Microplate reader (EL 340, Winooski, VT, USA), and the activity of the enzyme was expressed as micromoles of P_i released from a linear rate (between 15 and 30 min) per milligram of protein per hour (μmol P_i mg^{-1} protein h^{-1}). Na^+/K^+-ATPase activity was assayed as the ouabain-sensitive component of total ($Na^+/K^+/Mg^{2+}$-dependent) ATPase activity. The membrane preparation and the enzyme analyses for each tissue of fish acclimated at −1.5 °C and 4 °C were performed simultaneously.

The Arrhenius plots of the Na^+/K^+-ATPase enzyme were drawn by plotting the activity data over the 4–37 °C assay-temperature range. Each point on the graph was derived from three series of determinations (N=3) performed in duplicate at 4, 7, 11, 15, 20, 25, 33 and 37 °C. Measurements of Na^+/K^+-ATPase activation energy (E_a) were carried out using the Arrhenius equation:

$$\log V_{max} = -(E_a/2.3\boldsymbol{R}T) + \log A, \quad (1)$$

where $\boldsymbol{R}$ (8.314 J K^{-1} mol^{-1}) is the gas constant, T (K) is the absolute temperature, V_{max} (μmol P_i mg^{-1} protein h^{-1}) corresponds to the maximal activity rate measured at saturating substrate concentration and A represents the probability factor of useful collisions multiplied by the collision frequency.

Statistical analyses

For comparison of sample means from Na^+/K^+-ATPase assays and lipid analyses, unpaired Student's t-tests were performed. Statistical comparisons for the serum osmolality and ion concentration acclimation studies were made using one-way analysis of variance (ANOVA), followed by Tukey–Kramer multiple-comparisons tests to test for significance in the trend. A significance level of P<0.05 was chosen.

Results

Reversibility experiments

Fig. 1 shows that the serum osmolality and the Na^+ and Cl^- concentrations determined in *T. newnesi* and *T. bernacchii* decreased with acclimation to 4 °C from a holding temperature of −1.8 °C. It reveals a sharp reversibility in osmolality and Na^+ and Cl^- concentrations that takes place at day 8 of acclimation to 4 °C, the time at which the animals were placed in aquaria at −1.5 °C. The serum osmolality responses observed in both species of fish were well-defined and occurred within 48 h of a change in acclimation temperature. These results indicate that the acclimation temperatures (−1.5 °C and 4 °C) chosen in this study represent a relatively strong challenge to antarctic teleosts, thus making them unique models for studying the adaptation of serum osmolality to temperature.

Serum osmolality

Table 1 shows that the total osmolality and the ionic concentrations of the aquarium sea water were not affected by acclimation temperatures. The serum compositions determined in thermally acclimated *T. bernacchii* and *T. newnesi* are listed in Tables 1 and 2 respectively. In *T. bernacchii* acclimated to −1.5 °C, the serum osmolality remained constant throughout the study (Table 1). Similarly, the osmolality measured immediately after fish capture, in order to test for natural variations before acclimation experiments began (Table 3), showed no significant osmolality variation with date of capture, suggesting that major changes in the serum osmolality did not seem to occur during the pre-acclimation of *T. bernacchii* to −1.8 °C. The concentrations of Na^+ and Cl^- in control *T. bernacchii* (both in those pre-acclimated to −1.8 °C and in those sampled immediately after capture) remained constant with time.

In contrast to *T. bernacchii*, the mean concentrations of Na^+ and Cl^- in control *T. newnesi* varied significantly after pre-acclimation to −1.8 °C (Table 2), although Na^+ and Cl^- concentrations did not change in fish sampled immediately after

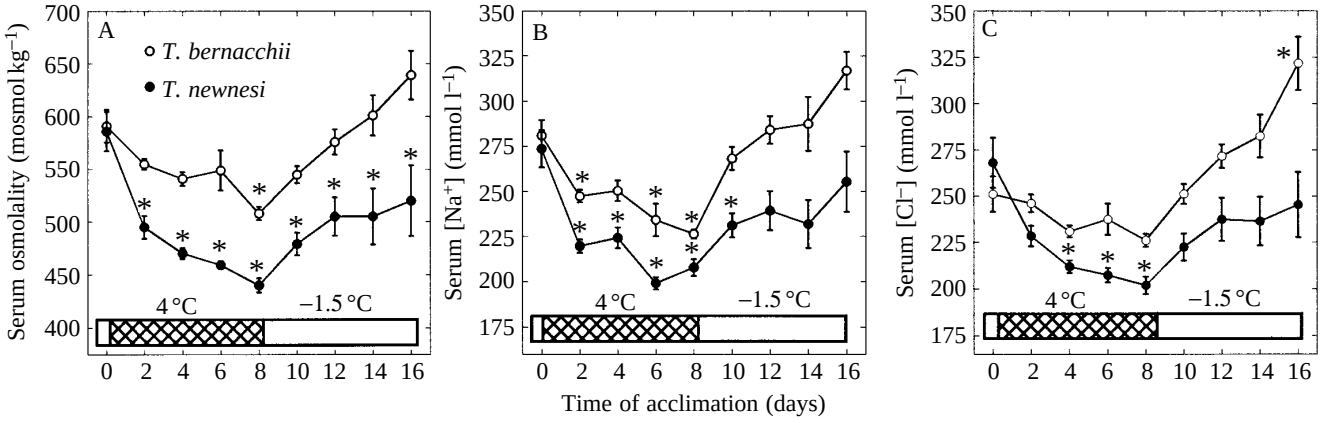

Fig. 1. Osmolality (A) and concentrations of Na^+ (B) and Cl^- (C) determined in the serum of *Trematomus bernacchii* (open symbols) and *T. newnesi* (filled symbols) acclimated to 4 °C from a holding temperature of −1.8 °C from day 0 to day 8, and transferred to −1.5 °C from day 8 to day 16. Each data point is the mean ± S.E.M. of eight fish. *P<0.05 with respect to values at day 0.

Table 1. *Osmolality and ion concentrations in the serum of* Trematomus bernacchii *acclimated to –1.5 °C (top) and 4 °C (bottom) for 35 days*

Acclimation time (day)	Osmolality (mosmol kg^{-1})	$[Na^+]$ (mmol l^{-1})	$[Cl^-]$ (mmol l^{-1})	$[K^+]$ (mmol l^{-1})	$[Mg^{2+}]$ (mmol l^{-1})	$[Ca^{2+}]$ (mmol l^{-1})
Acclimation to −1.5 °C						
0 (8)	564±4	263±4	237±3	2.4±0.2	0.70±0.1	2.9±0.1
7 (8)	561±4	265±3	231±4	2.7±0.3	0.70±0.1	3.4±0.1*
14 (8)	538±4	255±2	247±4	2.8±0.2	0.70±0.1	2.9±0.1
21 (8)	550±8	271±5	255±8	3.2±0.5	0.90±0.2	3.1±0.1
28 (8)	550±9	257±6	265±9*	4.0±0.6	0.70±0.1	3.1±0.1
35 (8)	522±6	270±5	249±2	4.1±1.3	0.83±0.1	3.2±0.1
SW (−1.5 °C)	1019	560	452	NA	1.08	9.01
Acclimation to 4 °C						
0 (8)	590±10	282±6	249±5	3.7±04	0.81±0.1	3.6±0.2
7 (8)	521±4*	247±2*	214±3*	2.9±0.1	0.82±0.1	3.4±0.3
14 (8)	488±4*	232±2*	217±3*	3.5±0.3	0.82±0.1	3.0±0.2
21 (8)	477±5*	244±3*	225±4*	2.6±0.3	0.91±0.1	3.1±0.2
28 (6)	462±7*	222±3*	220±7*	3.3±0.4	0.78±0.1	3.5±0.1
35 (6)	451±7*	248±6*	220±7*	2.7±0.6	0.86±0.1	3.2±0.4
SW (4 °C)	1018	552	458	NA	0.95	8.77

Values are means ± S.E.M. with the number of fish sampled given in parentheses.
*Indicates a significant difference from value on day 0 (P<0.05); NA indicates that a value was not measured.
The ionic composition of McMurdo Sound aquarium seawater (SW) determined at –1.5 °C and 4 °C is included for comparison.

capture (Table 3). The osmolality of serum from −1.5 °C-acclimated *T. bernacchii* was 80–100 mosmol kg^{-1} higher than that of *T. newnesi*.

Warm-acclimation was accompanied by significant decreases in serum osmolality of both species (Tables 1, 2). This decline in total solute content accounted for 139 mosmol kg^{-1} in *T. bernacchii* and 92 mosmol kg^{-1} in *T. newnesi*, decreases corresponding to 24 % and 20 % of their initial osmolality, respectively (based on the difference between the mean osmolalities at day 0 and day 35). The serum osmolality decrease for warm-acclimated fish of both species seemed to follow a similar pattern: a relatively large drop during the first 14 days of acclimation, followed by a more gradual decrease.

The Na^+ and Cl^- concentration changes observed in warm-acclimated fish paralleled the changes in serum osmolality. In *T. bernacchii*, the concentrations of Na^+ and Cl^- decreased by 34 mmol l^{-1} (12 % decrease) and 29 mmol l^{-1} (12 % decrease), respectively, during the study. As with osmolality, Na^+ and Cl^- concentrations decreased rapidly at first and became established at lower levels as acclimation time proceeded, suggesting that a 'new' state of hypo-osmoregulation may have been attained. Serum Na^+ and Cl^- concentrations in warm-acclimated *T. newnesi* displayed similar quantitative changes, decreasing by 27 mmol l^{-1} (12 % decrease) and 29 mmol l^{-1} (13 % decrease) over 35 days. The latter may explain, in part, the dramatic drop in osmolality during the first week of acclimation to 4 °C, since substantial amounts of Na^+ and Cl^- are lost within 48 h of warm-acclimation (note the rapid onset of Na^+ and Cl^- loss from day 0, Fig. 1).

As Tables 1, 2 and 3 show, serum K^+, Mg^{2+} and Ca^{2+} concentrations in both species were relatively insensitive to acclimation temperature.

Urine composition

Table 4 lists the urine composition for the thermally acclimated fish. The urine-to-plasma (U:P) ratios at day 35 of acclimation indicate that the urine osmolality in both species was nearly iso-osmotic to the serum. The urine osmolality for the warm-acclimated fish decreased in both species, although the U:P values were maintained near unity. Table 4 shows that Mg^{2+} is the dominant cation and Cl^- the dominant anion in the urine. The high U:P values for Mg^{2+} reflect the fishes' ability to concentrate this ion in the urine. Warm-acclimation significantly decreased Cl^- levels in the urine of both species. At day 35 of acclimation to 4 °C, the concentration of Na^+ in the urine of warm-acclimated *T. bernacchii* was significantly greater than that of the control (−1.5 °C-acclimated), while urine Na^+ concentration in *T. newnesi* appeared to be temperature-insensitive. There were no significant mass differences between warm-acclimated and control groups of both species (data not shown).

Phospholipid analyses

Table 5 lists the fatty acid (FA) composition of phospholipids isolated from total lipids in the thermally acclimated *T. bernacchii* and *T. newnesi* tissues. Over the experimental period, there were no significant changes in kidney, gill, muscle and liver FA patterns due to temperature, except for two FAs in the kidney of *T. newnesi*. The significant

Table 2. *Osmolality and ion concentrations in the serum of* Trematomus newnesi *acclimated to –1.5 °C (top) and 4 °C (bottom) for 35 days*

Acclimation time (day)	Osmolality (mosmol kg^{-1})	[Na^+] (mmol l^{-1})	[Cl^-] (mmol l^{-1})	[K^+] (mmol l^{-1})	[Mg^{2+}] (mmol l^{-1})	[Ca^{2+}] (mmol l^{-1})
Acclimation to −1.5 °C						
0 (8)	458±4	228±2	213±2	2.0±0.1	0.71±0.1	2.9±0.1
7 (8)	485±4*	248±2*	217±2	2.0±0.4	0.70±0.1	3.2±0.1*
14 (8)	480±7*	238±4*	223±5	2.1±0.4	0.70±0.1	3.4±0.2
21 (8)	473±4	242±2*	233±2*	1.4±0.1	0.57±0.0	3.4±0.2
28 (8)	463±4	247±2*	225±2	3.0±0.2	0.55±0.0	3.4±0.0
35 (8)	454±3	240±3*	222±3	1.5±0.1	0.69±0.1	3.4±0.1
Acclimation to 4 °C						
0 (7)	470±3	232±3	215±3	1.4±0.1	0.72±0.1	2.9±0.1
7 (8)	428±5*	225±2	197±3*	2.0±0.2	0.70±0.1	2.9±0.1
14 (5)	397±1*	208±5*	188±3*	1.6±0.1	0.74±0.1	3.4±0.2
21 (5)	398±1*	201±1*	187±1*	1.3±0.2	0.71±0.7	2.9±0.1
28 (5)	380±3*	209±2*	182±1*	2.1±0.2	0.97±0.1	2.9±0.2
35 (5)	378±3*	205±1*	186±2*	2.1±0.1	0.73±0.0	3.7±0.4

Values are means ± S.E.M. with the number of fish sampled given in parentheses.
*Indicates a significant difference from values on day 0 (P<0.05).

changes in *T. newnesi* kidney included a warm-promoted increase in 18:1 and a warm-promoted decrease in 22:6(n-3). Except for these, no major warm-promoted changes in FA composition were detected: levels of long-chain polyunsaturated FAs such as 18:3(n-3) and 20:5(n-3) in both species did not change, and there was no significant change in 22:6(n-3) in *T. bernacchii* tissues. The FA composition determined in the species studied followed a consistent abundance pattern regardless of acclimation temperature: the relative levels of polyunsaturated fatty acids (PUFAs) were significantly greater than those of saturated FAs (SFAs) and/or mono-unsaturated FAs (MUFAs). Table 5 also shows that the PUFAs of antarctic fish fit the general observation for marine teleosts that PUFAs of the (n-3) series are more abundant than PUFAs of the (n-6) series; in freshwater teleosts the opposite is seen.

Na^+/K^+-ATPase activity

Table 6 shows the total ATPase activities (TAs) calculated in the absence of the Na^+/K^+-ATPase inhibitor ouabain, the Na^+/K^+-ATPase (ouabain-inhibitable ATPase) specific activities (SAs) and the percentage of SA with respect to TA of the tissues studied (%*I*=inhibition). Several aspects of these data are particularly important. First, the mean activities (TA and SA) obtained in the osmoregulatory tissues (gill and kidney) of both species increased significantly with

Table 3. *Osmolality and ion concentrations in the serum of* Trematomus bernacchii *and* T. newnesi *determined immediately after fish capture to test for natural variations before acclimation experiments began*

Date of capture	Osmolality (mosmol kg^{-1})	[Na^+] (mmol l^{-1})	[Cl^-] (mmol l^{-1})	[K^+] (mmol l^{-1})	[Mg^{2+}] (mmol l^{-1})	[Ca^{2+}] (mmol l^{-1})
T. bernacchii						
Nov 2nd (7)	559±7	270±5	254±6	6.0±1.7	1.10±0.3	3.2±0.2
Nov 4th (8)	572±5	274±3	270±4	3.9±0.4	0.84±0.1	4.0±0.3
Nov 6th (8)	557±4	263±3	262±6	3.5±0.5*	0.67±0.1	3.4±0.1
Nov 8th (8)	562±5	263±2	251±4	1.5±0.3	0.77±0.1	3.6±0.3
T. newnesi						
Nov 2nd (6)	485±2	247±1	223±2	1.1±0.1	1.10±0.3	3.2±0.2
Nov 4th (8)	481±4	246±3	225±3	3.3±0.9	0.84±0.1	4.0±0.3
Nov 6th (8)	481±2	242±2	235±2	1.9±0.3	0.67±0.1	3.4±0.1
Nov 8th (8)	481±3	244±5	235±5	1.8±0.3	0.77±0.1	3.6±0.3

Reported values are means ± S.E.M.; the number of specimens sampled is given in parentheses.
*Indicates a significant difference from values on Nov 2nd (P<0.05).

Table 4. *Urine osmolality (mosmol kg⁻¹), urine electrolyte concentrations (mmol l⁻¹) and urine-to-plasma (U:P) ratios determined on day 35 of acclimation in* Trematomus bernacchii *and* T. newnesi *acclimated to –1.5 °C and 4 °C*

	T. newnesi		*T. bernacchii*	
	–1.5 °C	4 °C	–1.5 °C	4 °C
Urine osmolality	459±2	381±3*	514±6	444±8*
(U:P)	1.0±0.001	1.0±0.013	0.97±0.003	0.98±0.001
Urine [Na^+]	12±4	3.0±0.4	4.0±0.1	76±10*
(U:P)	0.05±0.01	0.01±0.02	0.01±0.01	0.19±0.28
Urine [Ca^{2+}]	4.3±0.3	4.4±0.3	4.4±0.5	3.5±0.4
(U:P)	1.2±0.07	1.6±0.15	1.6±0.1	0.24±0.01
Urine [Cl^-]	219±5	179±2*	301±3	89±30*
(U:P)	0.98±0.04	0.95±0.01	1.2±0.01	0.42±0.14
Urine [Mg^{2+}]	230±6	194±7	247±28	173±7
(U:P)	320±21	237±22	274±63	198±24

Values are means ± S.E.M., N=3.
*Indicates significant differences from values for fish acclimated to –1.5 °C (P<0.05).

acclimation temperature when assayed at 4 °C. Second, TAs and SAs from kidney and gill preparations in both species were significantly greater than those of tissues not involved in teleostean osmoregulation. Third, the SAs from kidney tissues in both species were significantly higher than that in gills. Fourth, the %I values in *T. newnesi* kidneys and gills from 4 °C assays were significantly greater than those in *T. bernacchii*. Fifth, there was a only small degree of variability in ATPase assays within individual groups of fish (indicated by the relatively low S.E.M. values). This finding is not surprising, in view of the invariant temperature fluctuation of coastal antarctic waters, and it also suggests that the acclimation time was sufficiently long to ensure a homogeneous sample.

In assays carried out at 4 °C, warm-acclimation resulted in 43 % and 27 % increases in kidney SA of *T. bernacchii* and *T. newnesi* compared with −1.5 °C-acclimated fish, respectively. In gill preparations, the Na^+/K^+-ATPase activity in warm-acclimated *T. bernacchii* was nearly doubled (47 % increase), whereas in *T. newnesi*, warm-acclimation resulted in a 25 % increase in SA compared with −1.5 °C-acclimated fish.

Assay temperature is directly correlated to enzymic activity, as the rate of many enzyme-catalyzed reactions generally doubles or triples for every 10 °C increment (Q_{10}). Accordingly, for antarctic fishes, the SAs obtained in assays at 37 °C were eight- to tenfold higher than those at 4 °C. In assays at 37 °C, the gills of warm-acclimated *T. bernacchii* and kidneys of warm-acclimated *T. newnesi* showed significant increases in SA and TA compared with −1.5 °C-acclimated fish (55 % and 48 %, respectively). At 37 °C, however, the activities from warm-acclimated *T. bernacchii* kidney and warm-acclimated *T. newnesi* gill tissues were not significantly different from those for the −1.5 °C-acclimated fish.

The results obtained in assays from liver tissues support the hypothesis that the lower serum osmolality may result from the higher Na^+/K^+-ATPase activity upon warm-acclimation. Comparisons of liver microsomal ATPases (TA and SA) between acclimation temperatures showed no significant differences in both species. Furthermore, the SA at either assay temperature was not significantly different between species.

The SA from muscle tissues of both species was variable. In contrast to the osmoregulatory tissues, warm-acclimation in *T. bernacchii* resulted in a significant decrease in SA when assayed at 4 °C (32 % decrease). The SA in the muscle of warm-acclimated *T. newnesi* assayed at 37 °C decreased significantly by 30 %. Interestingly, the TA in *T. newnesi* showed an increase in response to an increase in acclimation temperature when assayed at 37 °C. From muscle assays performed at 37 °C in *T. bernacchii* and at 4 °C in *T. newnesi*, warm-acclimation had no significant effects on either TA and/or SA, yielding values of SA that were virtually identical.

Arrhenius plots

The Arrhenius plots of gill and kidney tissues in acclimated *T. newnesi* are shown in Fig. 2. The SA ($100\log V_{max}$) of the enzyme was plotted from 4 to 37 °C, and it is clear that warm-acclimation resulted in a greater SA (the upper curves) throughout the entire assay temperature range, compared with fish acclimated to −1.5 °C. The plots from warm-acclimated and control (−1.5 °C-acclimated) *T. newnesi* kidney (Fig. 2B) displayed a T_d at 7 °C, leading to different activation energies above (E_{a1}) and below (E_{a2}) the break (E_{a2}=114.5 and E_{a1}=53 kJ mol^{-1} in warm-acclimated fish; E_{a2}=123 and E_{a1}=47 kJ mol^{-1} in −1.5 °C-acclimated fish). The SA in the kidney of *T. newnesi* decreased at higher assay temperatures (temperatures exceeding 30 °C), suggesting a loss of enzyme activity.

Over the 4–37 °C temperature range, the SA in the gills of acclimated *T. newnesi* (Fig. 2A) was never inactivated, suggesting that the Na^+/K^+-ATPase in the gills of this species is less thermosensitive than that in the kidney. As opposed to

Table 5. *Fatty acid compositions as percentages of total fatty acids, in phospholipids prepared from total lipid extracts of the tissues indicated, isolated from* Trematomus bernacchii *and* T. newnesi *acclimated to –1.5 °C and 4 °C*

	Kidney		Gill		Liver		Muscle	
Fatty acid	−1.5 °C	4 °C	−1.5 °C	4 °C	−1.5 °C	4 °C	−1.5 °C	4 °C
T. bernacchii								
SFA								
14:0	0.4±0.2	0.6±0.4	1.6±0.8	0.3±0.1	0.2±0.0	0.3±0.0	0.8±0.9	0.9±0.8
16:0	14.3±1.2	14.7±0.9	12.5±0.8	11.7±1.4	13.7±1.7	17.4±3.1	22.6±5.4	18±1.8
18:0	3.6±1.1	4.5±0.5	3.8±0.2	4.7±0.7	2.1±0.1	3.1±0.0	3.2±1.0	2.8±0.1
Total	18.3	19.8	17.9	16.7	16.0	20.8	26.6	21.7
MUFA								
16:1	8.3±2.3	5.8±0.8	9.3±0.9	7.1±0.3	8.2±0.1	6.7±1.4	4.8±1.8	4.7±1.3
18:1	16.0±0.1	16.6±1.9	20.0±0.4	20.8±1.8	13.6±2.3	20.0±0.4	13.5±3.4	10.5±0.4
20:1	1.9±1.0	1.5±1.2	T	1.5±1.1	1.3±0.6	2.0±0.4	T	T
22:1	2.4±0.5	2.5±0.1	2.6±0.4	2.7±0.2	1.5±0.0	1.5±0.2	1.3±0.2	1.5±0.3
Total	28.6	26.4	31.9	32.1	21.5	23.8	18.3	19.7
PUFA								
18:2	0.8±0.7	0.6±0.8	0.7±0.4	0.6±0.4	1.0±0.1	0.8±0.5	1.8±2.0	1.0±0.3
18:3(n-6)	T	T	T	T	T	T	T	T
18:3(n-3)	T	0.6±0.9	2.5±0.7	0.9±1.3	T	T	1.4±0.3	1.9±0.5
20:4(n-6)	3.7±0.5	3.7±0.1	4.2±0.5	4.1±0.4	2.7±0.3	2.2±0.5	1.9±0.8	2.1±1.5
20:5(n-3)	19.9±1.3	20.3±2.1	16.0±1.5	15.6±1.6	15.5±0.8	19.6±1.0	16.3±9.5	21.5±3.1
22:5(n-3)	2.2±0.8	1.9±0.4	1.8±0.7	1.7±0.2	1.6±1.0	1.2±0.1	1.9±0.6	2.1±1.1
22:6(n-3)	21.4±7.7	24.2±1.1	20.0±1.9	21.6±0.6	34.4±0.4	27.8±3.6	23.5±11	25.0±3.7
Total	48.0	51.3	45.2	44.5	55.2	51.6	46.8	53.5
T. newnesi								
SFA								
14:0	0.6±0.5	1.0±0.7	0.2±0.1	2.1±1.6	1.6±1.5	1.3±1.0	0.2±0.1	1.5±2.2
16:0	19.1±2.0	18.7±0.6	14.2±1.2	12.2±0.1	20.3±5.5	18.5±2.7	19.1±1.0	20.2±2.8
18:0	3.6±0.6	4.4±0.4	3.6±0.3	3.5±0.1	2.7±0.2	3.2±0.2	1.8±0.3	2.7±0.3
Total	23.3	24.1	18.0	17.8	24.6	23.0	21.1	24.4
MUFA								
16:1	3.9±0.6	3.8±0.6	7.8±0.6	6.5±0.5	5.9±1.0	5.2±0.6	2.9±1.0	3.3±0.4
18:1	16.3±0.2	17.3±0.3*	24.0±0.7	20.1±0.6	18.0±3.0	19.7±2.1	15.1±1.8	13.5±2.2
20:1	T	T	2.7±0.1	3.2±0.3	T	T	T	T
22:1	2.9±1.0	3.6±0.4	1.8±0.1	0.9±1.1	1.0±0.8	0.7±1.0	0.7±0.6	0.6±0.4
Total	23.1	24.7	36.3	30.7	24.9	25.6	18.7	18.1
PUFA								
18:2	1.4±0.6	1.3±0.4	1.0±0.1	1.2±0.3	0.2±0.2	0.8±0.2	1.2±0.1	1.4±0.2
18:3(n-6)	T	T	T	T	T	T	T	T
18:3(n-3)	1.9±0.4	2.1±0.9	T	T	0.8±0.3	0.9±0.1	0.5±0.4	T
20:4(n-6)	2.5±0.3	2.5±0.9	3.3±0.1	3.3±0.6	1.7±0.3	1.9±0.2	2.1±0.1	2.1±0.3
20:5(n-3)	13.1±0.5	12.2±1.5	13.2±1.0	12.1±1.5	13.0±0.7	14.2±1.7	21.2±0.2	19.9±1.5
22:5(n-3)	T	T	T	2.8±3.4	T	T	T	T
22:6(n-3)	31.8±1.9	28.1±1.2*	24.0±1.4	24.2±5.4	30.5±8.0	30.1±2.7	29.0±7.6	29.0±1.1
Total	50.7	46.4	41.5	43.6	46.2	47.9	54.0	52.6

Values are means ± S.D.; T (trace) signifies <0.1 %; N=3.
*Indicates a significant difference between values at different acclimation temperatures (P<0.05).
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

the T_d (7 °C) observed in kidney preparations, the Arrhenius plot for gill tissue of warm-acclimated *T. newnesi* displayed a T_d at 15 °C, whereas in −1.5 °C-acclimated fish T_d was at 20 °C. In *T. newnesi* gills, E_{a2} was 40 kJ mol^{-1} and E_{a1} was 31 kJ mol^{-1} in warm-acclimated fish; E_{a2} was 47 kJ mol^{-1} and E_{a1} was 44 kJ mol^{-1} in −1.5 °C-acclimated fish.

Discussion

Our results show that *T. bernacchii* and *T. newnesi* acclimated to 4 °C have an enhanced capacity to hyper-osmoregulate. The temperature-induced decline in serum osmolality observed during warm-acclimation appears to have resulted from the positive compensation of Na^+/K^+-ATPase

Table 6. *Total ATPase activity (TA), Na⁺/K⁺-ATPase activity (SA) and SA as a percentage of TA (%I) determined in kidney, gill, liver and muscle tissues from* Trematomus bernacchii *and* T. newnesi, *both acclimated to –1.5 °C and 4 °C for 35 days*

		T. bernacchii		*T. newnesi*	
Tissue		−1.5 °C	4 °C	−1.5 °C	4 °C
Assay temperature 4 °C					
Kidney	TA	0.297±0.03	0.367±0.01*	0.336±0.04	0.574±0.02*
	SA	0.230±0.02	0.314±0.01*	0.306±0.02	0.540±0.05*
	%*I*	81	85	92	94
Gill	TA	0.181±0.04	0.282±0.05*	0.103±0.01	0.146±0.006*
	SA	0.070±0.01	0.131±0.01*	0.079±0.006	0.105±0.004*
	%*I*	39	46	76	72
Liver	TA	0.0405±0.005	0.0423±0.006	0.053±0.007	0.051±0.009
	SA	0.0177±0.003	0.0150±0.002	0.016±0.002	0.018±0.003
	%*I*	43	37	30	35
Muscle	TA	0.059±0.01	0.073±0.01	0.053±0.007	0.044±0.004
	SA	0.019±0.001	0.013±0.01*	0.022±0.001	0.021±0.003
	%*I*	32	21	41	48
Assay temperature 37 °C					
Kidney	TA	3.16±0.20	3.97±0.40	2.96±0.16	5.33±0.61*
	SA	2.25±0.09	2.93±0.25	2.42±0.09	4.62±0.75*
	%*I*	71	74	83	87
Gill	TA	1.28±0.34	2.30±0.10*	1.16±0.14	1.42±0.20
	SA	0.56±0.01	1.29±0.04*	0.64±0.06	0.74±0.03
	%*I*	44	56	55	52
Liver	TA	0.13±0.01	0.58±0.01	0.303±0.06	0.290±0.05
	SA	0.133±0.01	0.130±0.01	0.130±0.01	0.110±0.01
	%*I*	22	22	43	38
Muscle	TA	0.00±0.05	0.65±0.01	1.1±0.01	1.17±0.02*
	SA	0.130±0.01	0.128±0.02	0.24±0.01	0.17±0.01*
	%*I*	18	21	24	15

Reported values are means ± S.E.M.; N=3 indicates the number of fish tissues sampled.
*Indicates a significant difference between values at different acclimation temperatures (P<0.05).
ATPase activities are measured in μmol P_i mg^{-1} protein h^{-1}; %*I* is SA as a percentage of TA.

activity in gill and kidney tissues of these fish. The increases in SA of the enzyme at day 35 of acclimation, coupled to the constancy in the mass of the fish, suggest that the decline in serum osmolality did not involve an enhanced hydration state of the fish, but an increased excretion of NaCl. Previous studies have shown that temperature acclimatization can affect serum osmolality. Arctic teleosts of Labrador, Canada, increase their serum osmolality in the winter when water temperatures may be as low as −1.7 °C (Gordon *et al.* 1962). Staurnes (1993) reported that the Cl^- concentration of anadromous Arctic char *Salvelinus alpinus* acclimatized to 5 °C increased by 30 mmol l^{-1} compared with that of 10 °C-acclimatized fish, whereas the activity of gill Na^+/K^+-ATPase was simultaneously halved, which agrees well with the present findings. Umminger (1969), working on supercooled killifish (*Fundulus heteroclitus*), measured a 20% increase in serum osmolality when the acclimation temperature was lowered from 20 to −1.5 °C, also agreeing with the present findings. In the same study, serum Na^+ and Cl^- concentrations of fish at −1.5 °C increased by 12% and 17%, respectively, when compared with fish at 20 °C. However, Franklin *et al.* (1991) showed that acute increases in temperature (from 0 to 10 °C) for 10 min resulted in an increase in serum osmolality and Cl^- concentration in the antarctic teleost *Pagothenia borchgrevinki*. They suggested that the osmoregulatory mechanisms of this fish could not compensate for the large influx of ions that resulted from greater infusion rates at 10 °C. In the present study, however, we have shown that prolonged exposure to warmer temperatures (35 days at 4 °C) lowered the osmolality and ion concentrations in the serum of two species and increased Na^+/K^+-ATPase activity in the osmoregulatory tissues. Presumably, therefore, antarctic teleosts are able to maintain ionic and osmotic homeostasis at 4 °C by means of enhanced osmoregulatory mechanisms.

The serum osmolality recorded in both species of antarctic fish at the start of the acclimation period is consistent with previous observations that antarctic fishes have a higher serum osmolality than arctic and temperate marine teleosts (Dobbs and DeVries, 1975; O'Grady and DeVries, 1982). The osmolality measured in *T. bernacchii* sampled immediately

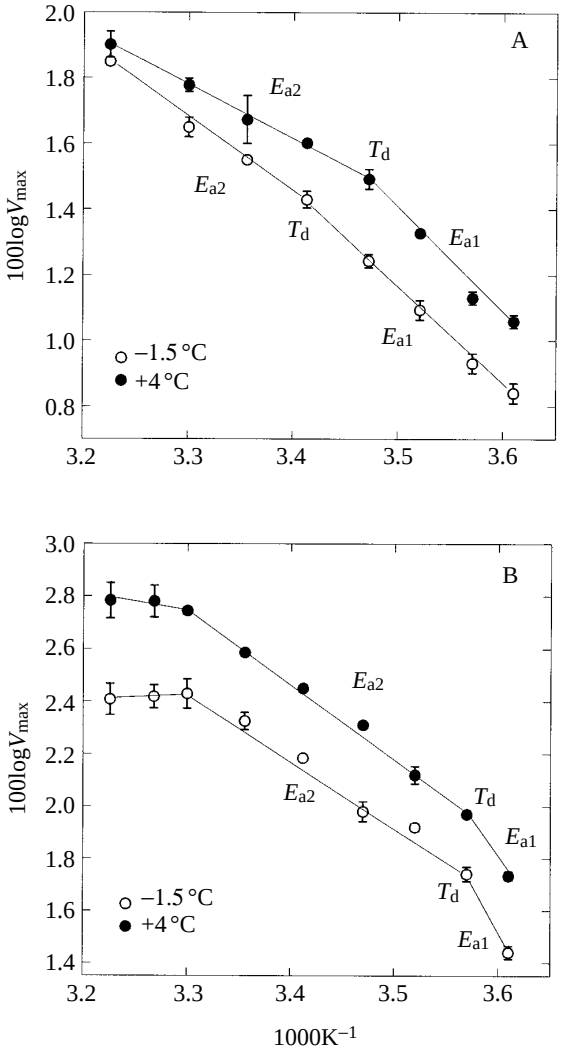

Fig. 2. Arrhenius plots ($100\log_{10}V_{max}$ *versus* $1000K^{-1}$) calculated from the Na^+/K^+-ATPase specific activity in gill (A) and kidney (B) tissues from *T. newnesi* acclimated to −1.5 °C (open symbols) and 4 °C (filled symbols) for 35 days. Each data point represents the mean ± S.E.M. of (N=3) assays performed at 4, 7, 11, 15, 20, 25, 33 and 37 °C. The energy of activation (E_a, kJ mol^{-1}) is indicated above (E_{a1}) and below (E_{a2}) the temperature of discontinuity (T_d).

after line-capture (Table 3) was not significantly different from that at the start of acclimation to −1.5 °C (Table 1), suggesting that no major change occurred in the serum during the pre-acclimation period to −1.8 °C. For *T. newnesi*, the differences in mean serum osmolalities between fish immediately after capture and those pre-acclimated to −1.8 °C were significant (Tables 2, 3); however, the osmolality of the former remained virtually unchanged with time, suggesting that the differences observed may reflect intraspecific variation.

The electrolyte contribution to total osmolality in the serum of an organism can serve as an indirect measure of the relative proportions of organic and/or inorganic molecules that constitute the serum. In antarctic fish, the increased serum osmolality is due primarily to increased concentrations of Na^+ and Cl^- (Dobbs and DeVries, 1975). These electrolytes in control fish accounted for at least 90 % of the total solute content, consistent with data available for other antarctic fish (Dobbs and DeVries, 1975; O'Grady and DeVries, 1982). During warm-acclimation, the percentage electrolyte contribution to total osmolality was similar to that of −1.5 °C-acclimated fish, providing evidence that the observed decline in osmolality involved losses in Na^+ and Cl^-. In addition, the decline in Na^+ and Cl^- concentrations measured for 4 °C-acclimated fish was not indicative of osmoregulatory failure, as both species appeared to establish new Na^+ and Cl^- levels that were maintained as long as the fish were alive. Umminger (1969) reported similar results for *Fundulus heteroclitus* acclimated to −1.5 and 4 °C.

Teleost blood concentrations of K^+, Mg^{2+} and Ca^{2+} are generally low (Hickman and Trump, 1969; Dobbs and DeVries, 1975). Antarctic fish are no exception; levels of K^+, Mg^{2+} and Ca^{2+} are low and remained constant with acclimation temperature.

The urine of *T. bernacchii* and *T. newnesi* also fits the reported pattern for marine teleosts, with Mg^{2+} and Cl^- being the dominant ions present (Dobbs and DeVries, 1975). High urine Mg^{2+} concentrations reflect renal secretion of this ion, which enters the intestine by active transport (Prosser, 1973). The U:P ratios for Mg^{2+} and Ca^{2+} of both species greatly exceeded unity; this is in agreement with the concept that the primary role of the marine teleost kidney in osmoregulation is the active secretion of divalent ions entering the bloodstream from ingested sea water (Hickman and Trump, 1969; Dobbs and DeVries, 1975).

Antarctic fish normally have little Na^+ in the urine, because this ion leaves the body extrarenally, mainly by excretion at the gills. The urine Na^+ levels in both groups of acclimated fish are similar to the literature values, although the mean Na^+ concentration of *T. bernacchii* did increase with warm-acclimation. Dobbs and DeVries (1975) reported urine Na^+ levels as high as 100 mmol l^{-1} for several antarctic species; the increased Na^+ content of the urine of warm-acclimated *T. bernacchii* may therefore be unrelated to warm-acclimation.

It is impractical to incorporate the urine data from this study in an explanation of the changes that took place during acclimation to 4 °C. First, the urine analysis was performed by sampling urine from the bladder, an organ lined with an epithelium involved in the reabsorption of Na^+ and Cl^- from the secreted fluids (Beyenbach and Baustian, 1989). These authors report that ureter urine must be sampled in order to evaluate the transport rates in the kidney tubules, a technique which would exclude any reabsorption of salts. Second, the relationship between transport rates in the tubular kidney and reabsorptive capacity in the bladder of antarctic fish has not been documented.

In response to temperature changes, lipid membranes of poikilotherms show increased unsaturated fatty acid levels during cold-acclimation, whereas the opposite is seen during warm-acclimation (Hazel, 1984). Modifications in the degree of unsaturation of acyl chains ensure that the membrane

components present are those best suited to functioning within the constraints imposed by the thermal environment. The constancy in FA composition between *T. bernacchii* and *T. newnesi* acclimated to −1.5 and 4 °C may reflect an evolutionary adaptation to ensure that fluid–gel transitions occur only at temperatures below the physiological range (Hazel, 1988). If this were the case, one would expect a marked difference in FA composition between poikilotherms of extremely different, but relatively constant, temperatures. For instance, warm-adapted marine fish contain higher levels of saturated FAs and thus greater saturated-to-unsaturated FA ratios than fish from colder waters (Dey *et al.* 1993). Consistent with these observations are our findings that the lipid membranes of *T. bernacchii* and *T. newnesi* possess relatively large percentages of polyunsaturated FAs, presumably to ensure membrane homeoviscosity at subzero temperatures. Morris and Schneider (1969) have previously shown that the brain of *T. bernacchii* contains small quantities of short-chain (C_{12}–C_{14}) FAs, but relatively high proportions of long-chain C_{24} polyunsaturated FAs. In addition to the role of polyunsaturated FAs in maintaining membrane fluidity at low temperatures, the relatively large amounts of corporeal lipid stores in antarctic fish are associated with buoyancy adaptation (Eastman and DeVries, 1981) and are also a reflection of the preferential catabolism of FAs (especially unsaturated FAs) over carbohydrate substrates for aerobic metabolism (Sidell *et al.* 1988).

The overall constancy of FA pattern in the gills and kidneys of *T. bernacchii* and *T. newnesi* indicates that the warm-promoted changes in Na^+/K^+-ATPase activity were not modulated by changes in membrane unsaturation. Therefore, other mechanisms that may alter the composition of the enzyme microenvironmental lipids, irrespective of FA pattern, may account for the positive compensation of Na^+/K^+-ATPase activity to temperature. Alterations in the protein–lipid microenvironment may include the rearrangement of pre-existing acyl chains (Hazel and Landrey, 1988), altered ratios of phospholipid classes (Hazel, 1990) or modified cholesterol content (Wodtke, 1978). Temperature acclimation may even imply the prevalence of alternative enzyme forms in fish acclimated to different temperatures, as shown by the well-known polymorphism of Na^+/K^+-ATPase (Takeyasu *et al.* 1990). Furthermore, different Na^+/K^+-ATPase activities in different tissues may involve alterations in enzyme turnover rates (activity per enzyme unit) as well as quantitative changes in Na^+/K^+-ATPase pump sites. For instance, Towle *et al.* (1977) showed a decrease in the number of binding sites following transfer of *Fundulus heteroclitus* to 30 % sea water, associated with a rise in Na^+/K^+-ATPase activity. Similarly, Na^+/K^+-ATPase activity in gills of cold-acclimated *Rutilus rutilus* (5 °C) was 1.5- to twofold higher than that in warm-acclimated fish (15 °C), although B_{max} (the maximal number of binding sites per milligram of protein) of the enzyme was lower in the former condition (Schwarzbaum *et al.* 1991). In the present study, the possibility that enhanced ATPase activity in the osmoregulatory tissues of warm-acclimated fish could result from an increased number of transport sites cannot be discounted. However, rapid modulation of Na^+/K^+-ATPase activity, as seems to be the case from the reversibility experiments, has been suggested to be due to alterations in the catalytic rate of the enzyme (Sargent and Thomson, 1974; Karnacky *et al.* 1976; Towle *et al.* 1977).

Discontinuous Arrhenius plots of Na^+/K^+-ATPase activity for poikilotherms are consistent with previous findings (Thomson *et al.* 1977; Prosser, 1986; Ventrella *et al.* 1993), although some linear plots have been reported (McMurchie *et al.* 1973). In *T. newnesi*, the T_d in the gill plot (Fig. 2A) showed a decrease (from 20 to 15 °C) with warm-acclimation. Thomson *et al.* (1977) reported an increase in T_d (from 12 to 20 °C) in warm-acclimated eels *Anguilla anguilla*, which was attributed to an increase in levels of saturated FAs. In the present study, however, the FA compositions remained constant between groups of fish acclimated to different temperatures. The T_d for *T. newnesi* kidney (Fig. 2B) from both temperature groups was at 7 °C. This is interesting because the upper lethal temperature for antarctic fish is 6 °C (Somero and DeVries, 1967). A T_d close to the upper lethal temperature (5 °C) was reported by Somero *et al.* (1968) for *T. bernacchii* succinic dehydrogenase. Another feature of interest in the kidney data (Fig. 2B) is the thermal inactivation observed in both groups of *T. newnesi* at assay temperatures exceeding 30 °C, which may reflect the thermal lability of the enzyme at high assay temperatures. This thermal inactivation is not surprising, since antarctic fish are likely to have evolved to attain the highest catalytic efficiency at low temperature. Somero *et al.* (1968) showed that the succinic dehydrogenase activity exhibited a similar inactivation in −1.9 °C-acclimated *T. bernacchii*. The absence of inactivation at higher assay temperatures for gill tissue in our study shows that, in contrast to the kidney, the gill enzymes are less thermosensitive.

The E_a of an enzyme may be used as an index of catalytic efficiency. The E_a values for the kidney Na^+/K^+-ATPase were similar for *T. newnesi* acclimation groups both below and above the T_d, although below T_d there was a slight decrease in E_a of warm-acclimated fish, suggesting the production of more efficient enzyme. A similar E_a decrease was measured for the gill Na^+/K^+-ATPase enzyme of warm-acclimated *T. newnesi*. The E_a obtained for the gill tissue of *T. newnesi* was similar to that reported for *T. bernacchii* leucine aminopeptidase (31.4 J mol^{-1}) and maltase (30.5 J mol^{-1}; Maffia *et al.* 1993; 1 cal=4.18 J). These authors found no detectable Na^+/K^+-ATPase activity in the intestinal membranes of *T. bernacchii*.

In antarctic fish held at different salinities (50–200 ‰), no change in serum osmolality was found with altered salinity (O'Grady and DeVries, 1982). It is not surprising, then, to find that *T. bernacchii* and *T. newnesi* can regulate Na^+ and Cl^- content over the temperature range from −1.5 to 4 °C. However, it is difficult to attribute any ecological significance to these regulatory abilities because antarctic fishes do not experience significant fluctuations in seawater temperature and/or salinity (O'Grady and DeVries, 1982).

The data presented in this study agree with the hypothesis

by Prosser *et al.* (1970) with regard to the osmotic gradient between the seawater environment and the fishes' ECF. The increased energy expenditure by the osmoregulatory tissues brought about by warm-acclimation suggest that antarctic fishes maintain a relatively small osmotic gradient between the ECF and sea water in order to save energy.

Somero *et al.* (1968) showed that warm-acclimation of *T. bernacchii* to 4 °C led to an increase in iodoacetate sensitivity, which may have reflected a reorganization of metabolism. As a result, the authors hypothesized that the pentose-shunt pathway was favoured over glycolysis in these animals. In addition, they suggested that elevated levels of NADPH that would result from pentose-shunt activity could be used in the reductive steps of lipid biosynthesis. Data from Sidell *et al.* (1988) indicate that antarctic fish utilize FAs as fuels (beta-oxidation) instead of the anaerobic glycolysis of carbohydrates to obtain maximal rates of ATP generation. The production of more efficient enzymes, as found in the present study, may be a concomitant to the increase in energy production to drive the pump. Thus, the high lipid content of antarctic fishes may be associated with the temperature-induced decrease in serum osmolality brought about by an increase in Na^+/K^+-ATPase activity at 4 °C.

This study investigated the hypothesis that the activity of Na^+/K^+-ATPase may be increased during warm-acclimation in antarctic fish. The enhanced hypo-osmoregulation found in warm-acclimated fish raises further questions that remain to be answered. For example, what are the specific change or changes in the membrane that lead to the positive compensation of Na^+/K^+-ATPase activity in gills and kidneys? Is the enhanced activity of the enzyme due to an increase in efficiency of pre-existing enzymes or to an increased number of pump sites, and are hormonal changes coupled to higher transport rates?

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References

AMES, B. N. (1966). Assay of inorganic phosphate, total phosphate and phosphatases. *Meth. Enzymol*. **8**, 115–118.

BEYENBACH, K. AND BAUSTIAN, M. (1989). Comparative physiology of the proximal tubule from the perspective of aglomerular urine formation. *Structure and Function of the Kidney*, vol. 1 (ed. R. K. H. Kinne), pp. 103–134. Basel: Publisher.

BEYENBACH, K., PETZEL, D. AND CLIFF, W. (1986). Renal proximal tubule of flounder. I. Physiological properties. *Am. J. Physiol.* **250**, R608–R615.

BURTON, R. F. (1986). Ionic regulation in fish: the influence of acclimation temperature on plasma composition and apparent set points. *Comp. Biochem. Physiol.* **85**A, 23–28.

CLIFF, W. H., SAWYER, D. B. AND BEYENBACH, K. W. (1986). Renal proximal tubule of flounder. II. Transepithelial Mg secretion. *Am. J. Physiol*. **250**, R616–R624.

DEY, I., BUDA, C., WIIK, T., HALVER, J. E. AND FARKAS, T. (1993). Molecular and structural composition of phospholipid membranes in livers of marine and freshwater fish in relation to temperature. *Proc. natn. Acad. Sci. U.S.A.* **90**, 7498–7502.

DOBBS, G. AND DEVRIES, A. L. (1975). Renal function in Antarctic teleost fishes: serum and urine composition. *Mar. Biol*. **29**, 59–70.

EASTMAN, J. T. AND DEVRIES, A. L. (1981). Buoyancy adaptations in a swim-bladderless Antarctic fish. *J. Morph*. **167**, 91–102.

EASTMAN, J. T. AND DEVRIES, A. L. (1986). Antarctic fishes. *Scient. Am*. **254**, 106–114.

EPSTEIN, F. H., KATZ, A. I. AND PICKFORD, G. E. (1967). Sodium- and potassium-activated adenosine triphosphatase of gills: role in adaptation of teleosts to salt water. *Science* **156**, 1245–1247.

FRANKLIN, C. E., DAVISON, W. AND CAREY, P. W. (1991). The stress response of an Antarctic teleost to an acute increase in temperature. *J. therm. Biol*. **16**, 173–177.

GORDON, M. S., AMBDUR, B. H. AND SCHOLANDER, P. F. (1962). Freezing resistance in some northern fishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **122**, 52–62.

HAZEL, J. R. (1984). Effects of temperature on structure and metabolism of cell membranes in fish. *Am. J. Physiol*. **246**, R460–R470.

HAZEL, J. R. (1988). Homeoviscous adaptation in animal cell membranes. In *Physiological Regulation of Membrane Fluidity*, vol. 3 (ed. A. Aloia, C. C. Curtain and L. Gordon), pp. 150–189. New York: Alan R. Liss, Inc.

HAZEL, J. R. (1990). Adaptation to temperature: phospholipid synthesis in hepatocytes of rainbow trout. *Am. J. Physiol*. **258**, R1495–R1501.

HAZEL, J. R. AND LANDREY, S. R. (1988). Time course of thermal adaptation in plasma membranes of trout kidney. II. Molecular species composition. *Am. J. Physiol*. **255**, R628–R634.

HICKMAN, C. AND TRUMP, B. (1969). The kidney. In *Fish Physiology*, vol. 1 (ed. W. Hoar and D. Randall), pp. 91–139. New York: Academic Press.

HOWARD, R. W. AND STANLEY-SAMUELSON, D. (1990). Phospholipid fatty acid composition and arachidonic acid metabolism in selected tissues of adult *Tenebrio molitor*. *Ann. ent. Soc. Am*. **83**, 975–981.

KARNAKY, K. J., JR, ERNST, S. A. AND PHILPOTT, C. W. (1976). Teleost chloride cell. I. Response of gill Na^+/K^+-ATPase and chloride cell fine structure to various environments. *J. Cell Biol*. **70**, 144–156.

LITTLEPAGE, J. L. (1965). Oceanographic investigations in McMurdo Sound, Antarctica. In *Biology of the Antarctic Seas*, vol. 2 (ed. M. O. Lee), pp. 1–37. Washington DC: American Geophysical Union.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem*. **193**, 264–275.

MAFFIA, M., ACIERNO, R., DECEGLIE, G., VILELLA, S. AND STORELLI, C. (1993). Adaptation of intestinal cell membrane enzymes to low

temperatures in the Antarctic teleost *Pagothenia bernacchii*. *J. comp. Physiol.* B **163**, 265–270.

McMurchie, E. J., Raison, J. K. and Cairncross, K. O. (1973). Temperature-induced phase changes in membranes of heart: a contrast between the thermal response of poikilotherms and homeotherms. *Comp. Biochem. Physiol*. **44**B, 1017–1026.

Morris, R. W. and Schneider, M. J. (1969). Brain fatty acids of an Antarctic fish, *Trematomus bernacchii*. *Comp. Biochem. Physiol*. **28**, 1461–1465.

O'Grady, S. M. and DeVries, A. L. (1982). Osmotic and ionic regulation in polar fishes. *J. exp. mar. Biol. Ecol*. **57**, 219–228.

Ogg, C. L., Howard, R. W. and Stanley-Samuelson, D. W. (1991). Fatty acid composition and incorporation of arachidonic acid into phospholipids of hemocytes from the tobacco hornworm *Manduca sexta*. *Insect Biochem*. **21**, 809–814.

Oikari, A. (1975). Hydromineral balance in some brackish-water teleosts after thermal acclimation, particularly at temperatures near zero. *Ann. zool. Fenn*. **12**, 215–229.

Prosser, C. L. (1973). *Comparative Animal Physiology*. Philadelphia: Saunders Co. 3rd edition.

Prosser, C. L. (1986). *Adaptational Biology Molecules to Organisms*. New York: John Wiley & Sons.

Prosser, C. L., Mackay, W. and Kato, K. (1970). Osmotic and ionic concentration in some Alaskan fish and goldfish from different temperatures. *Physiol. Zool*. **43**, 81–89.

Sargent, J. R. and Thomson, A. J. (1974). Th e nature and properties of the inducible sodium-plus-potassium ion dependent adenosine triphosphatase in the gills of eels (*Anguilla anguilla*) adapted to fresh water and sea water. *Biochem. J*. **144**, 69–75.

Schwarzbaum, P. J., Wieser, W. and Niederstatter, H. (1991). Contrasting effects of temperature acclimation on mechanisms of ionic regulation in eurythermic and a stenothermic species of freshwater fish (*Rutilus rutilus* and *Salvelinus alpinus*). *Comp. Biochem. Physiol*. **98**A, 483–489.

Sidell, B. D., Crockett, E. L. and Driedzic, W. (1988). Metabolic characteristics from muscle tissues of Antarctic fishes. *Antarctic J*. **23**, 138–140.

Silva, P., Solomen, R., Spokes, K. and Epstein, F. H. (1977). Ouabain inhibition of gill Na^+/K^+-ATPase: relationship to active chloride transport. *J. exp. Zool*. **199**, 419–426.

Somero, G. N. and DeVries, A. (1967). Temperature tolerance of some Antarctic fish. *Science* **156**, 257–258.

Somero, G. N., Giese, A. C. and Wohlschlag, D. E. (1968). Cold adaptation of the Antarctic fish *Trematomus bernacchii*. *Comp. Biochem. Physiol*. **26**, 223–233.

Stanley-Samuelson, D. and Dadd, R. (1983). Long chain polyunsaturated fatty acids: patterns of occurrence in insects. *Insect Biochem*. **13**, 549–558.

Stanley-Samuelson, D. and Loher, W. (1983). Arachidonic acid and other long chain polyunsaturated fatty acids in spermatophores and spermatheca of *Teleogryllus comodus*: significance in prostaglandin-mediated reproductive behavior. *J. Insect Physiol*. **29**, 41–45.

Staurnes, M. (1993). Difference between summer and winter in gill Na^+/K^+-ATPase activity and hypoosmoregulatory ability of seafarmed anadromus Arctic charr (*Salvelinus alpinus*). *Comp. Biochem. Physiol*. **105**A, 475–477.

Takesayu, K., Lemas, V. and Fambrough, M. (1990). Stability of Na^+/K^+-ATPase alpha-subunit isoforms in evolution. *Am. J. Physiol*. **259**, C619–C630.

Thomson, H. J., Sargent, J. R. and Owen, J. M. (1977). Influence of acclimatization temperature and salinity on (Na^++$K^{+)}$-dependent adenosine triphosphatase and fatty acid composition in the gills of the eel (*Anguilla anguilla*). *Comp. Biochem. Physiol.* **56**B, 223–228.

Towle, D. W., Gilman, M. E. and Hempel, J. D. (1977). Rapid modulation of gill Na- and K-dependent ATPase activity during acclimation of the killifish *Fundulus heteroclitus* to salinity change. *J. exp. Zool*. **202**, 179–186.

Umminger, B. M. (1969). Physiological studies on supercooled killifish (*Fundulus heteroclitus*). I. Serum inorganic constituents in relation to osmotic and ionic regulation at subzero temperatures. *J. exp. Zool*. **172**, 283–302.

Ventrella, V., Pagliarani, A., Pirini, M., Trigari, J., Trombeti, F. and Borgatti, A. R. (1993). Lipid composition and microsomal ATPase activities in gills and kidneys of warm- and cold-acclimated sea bass (*Dicentrarchus labrax*). *Fish Physiol. Biochem*. **12**, 293–304.

Wodtke, E. (1978). Lipid adaptation in liver mitochondrial membranes of carp acclimated to different environmental temperatures – phospholipid composition, fatty acid patterns and cholesterol content. *Biochim. biophys. Acta* **640**, 698–709.