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Intermediary metabolism of Arctic char *Salvelinus alpinus* during short-term salinity exposure

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Summary

The migration of Arctic char *Salvelinus alpinus* from freshwater to seawater requires a substantial reorganization of the osmoregulatory tissues to regulate plasma ion levels. These modifications have an inherent metabolic cost, which must be met through the upregulation of intermediary metabolism. Arctic char intermediary metabolism was monitored during the initial 96 h of seawater acclimation through measurement of key enzymes in gill, liver, red and white muscle as well as tissue and blood free amino acid (FAA) levels, and plasma glucose and non-esterified fatty acid content. In general, seawater exposure stimulated large changes in amino acid metabolism, but no change in lipid or carbohydrate metabolism. White muscle FAA content increased significantly following seawater exposure, with levels of essential FAAs doubling after 96 h. Similar increases were seen in the plasma, suggesting a rapid mobilization of FAAs to the circulation. These changes were accompanied by significant increases in the activities of enzymes involved in amino acid metabolism in the gill, liver, red and white muscle, suggesting seawater-acclimated fish have an enhanced capacity for energy production from amino acids. Increased energy requirements were evident in the gill of seawater-acclimated char, as citrate synthase activity increased significantly. The results of this study suggest a rapid upregulation of amino acid metabolism may be critical for the successful acclimation of Arctic char to seawater.

Key words: fish, salinity, metabolism, enzyme, amino acid, salmonid.

Introduction

Arctic char *Salvelinus alpinus* are the most northerly distributed anadromous fish species. Many make an annual seaward migration each spring to capitalize on the ocean's rich food resources before returning to freshwater every fall to over winter (Johnson, 1980). In freshwater, char actively take up ions from the dilute environment and combat a continual gain of water. Conversely, in the marine environment, they actively excrete excess ions and drink seawater to compensate for passive water loss. The successful acclimation of Arctic char from freshwater to seawater depends on a substantial reorganization of many physiological systems over a relatively short period of time. Upon exposure to seawater, gill chloride cells become larger, more numerous and their basolateral and apical membrane surface area is greatly increased to accommodate the placement of large numbers of newly synthesized ion transport proteins (e.g. Na^+,K^+-ATPase) (Eddy, 1982). The intestine is modified to enhance the uptake of sodium and chloride from ingested seawater to facilitate the passive absorption of water, and the kidney changes to produce a more concentrated urine to conserve water (Eddy, 1982). Taken together, these modifications to the osmoregulatory organs suggest the acclimation to seawater is an energetically costly task.

The high cost of osmoregulation is confirmed by reports of increased metabolic rate. Maxime et al. (Maxime et al., 1991) found a significant increase in oxygen consumption of rainbow trout in the first 24 h of seawater acclimation. Similar studies with rainbow trout (Rao, 1968) and tilapia (Farmer and Beamish, 1969; Febry and Lutz, 1987) show that oxygen consumption rates are ~27% higher in seawater than at isosmotic salinity (10‰). Leray et al. (Leray et al., 1981) found no change in rainbow trout oxygen consumption, but did report an immediate decrease in ATP levels, ATP:ADP ratio and adenylate energy charge in rainbow trout gill following seawater transfer.

The period immediately following seawater exposure is probably critical. During this time the osmoregulatory machinery is reorganized as the fish changes from actively accumulating sodium and chloride to actively secreting these ions. This period has been termed the 'initial crisis phase' and is characterized by increasing plasma ion concentrations as the fish struggles to osmoregulate (Gordon, 1959). For *Oncorhynchus* and *Salmo* species, this phase generally lasts up to 30 h (Finstad et al., 1988), but for *Salvelinus* species, including Arctic char, this critical phase is suspected to last much longer as they are considered poor osmoregulators when

compared to other anadromous salmonids (Gjedrem, 1975; Hoar, 1976; Wandsvik and Jobling, 1982; Delabbio et al., 1990). The osmoregulatory limit of this species may be linked to an inefficient upregulation of intermediary metabolism, which is required to pay for the increased cost of osmoregulation.

Although the energy metabolism of the osmoregulatory tissues is critical, the metabolism of the other 'supporting' tissues (e.g. liver, muscle) may be equally important. These 'support' tissues export substrates (e.g. amino acids, lipids) to the circulation, which can be picked up and oxidized by the gill and other osmoregulatory tissues for ATP production for the synthesis of macromolecules (e.g. proteins, membranes). Additionally, amino acids may be mobilized to serve as osmoregulatory intracellular solutes. No single study has made a comprehensive analysis of the importance of intermediary metabolism during seawater acclimation of fish. To assess this we monitored changes in carbohydrate, lipid and amino acid metabolism in the gill and in 'support' tissues during the first 96 h of seawater acclimation, a critical period when many of the major physiological changes required for successful acclimation occur. We considered the liver and white muscle as major 'support' tissues and monitored the metabolism of the red muscle for comparison. The maximal rates of several key enzymes, tissue and blood free amino acid (FAA) levels and plasma glucose and non-esterified fatty acid (NEFA) levels were determined and used to indicate changes in overall energy requirements and shifts in preferred substrate oxidation by different tissues. Plasma and red blood cell (RBC) FAA levels were also monitored to detect any change in inter-organ transfer of amino acids following seawater exposure. Owing to the importance of the gill during seawater acclimation, special emphasis was placed on its metabolism and how it may be reliant on the availability of circulating metabolites supplied by other tissues.

Materials and methods

Experimental animals

Arctic char *Salvelinus alpinus* L., obtained from the Alma aquaculture research station (Alma, Ontario, Canada), were reared at the University of Guelph Hagen Aqualab in 2000 l circular tanks containing freshwater at 10°C for 8 months under a simulated natural photoperiod that mimicked light conditions at 45° latitude. Char were fed to satiation daily during this period with trout chow pellets (Martin Feeds, Elmira, ON, Canada). Since char do not feed during the initial stages of salinity acclimation, food was withheld for 24 h prior to, and during the experiment. During the month of May, a period when char should be optimally prepared for a seaward migration, eight individuals were transferred to each of three tanks containing full-strength artificial seawater (32‰, 10°C; Instant Ocean sea salt, Aquarium Systems Inc., Mentor, OH, USA) with identical lighting conditions. An additional eight char were transferred to each of three identical freshwater tanks to serve as control fish. No mortalities occurred throughout the experiment. This research was approved by the Animal Care Committee at the University of Guelph. The gender of the fish sampled was not recorded. After 24, 48 and 96 h, eight freshwater (control) and eight seawater-acclimated char were sampled. Blood was sampled by caudal puncture using a heparinized (500 i.u. ml^{-1} sodium heparin) syringe. The fish were then killed by a sharp blow to the head. Samples of gill, liver, white muscle and red muscle were rapidly excised, frozen in liquid nitrogen and stored at –80°C. Blood was centrifuged at 3000 ***g*** for 5 min at 4°C. Plasma was removed and the remaining 'packed cells' (referred to from here on as red blood cells or RBCs) were frozen in liquid nitrogen and stored at –80°C for future analysis. The RBCs were not washed as this has been shown to significantly reduce their FAA content (Hagenfeldt and Arvidsson, 1980). The maximal activities of key enzymes involved in several pathways of intermediary metabolism were determined in each of the tissues collected from char exposed to seawater or freshwater (controls) for 96 h. FAA levels were determined in red and white muscle, gill, plasma and RBCs of fish exposed to freshwater or seawater for 24, 48 and 96 h. Plasma NEFA levels were determined for both seawater and freshwater fish following 24, 48 and 96 h exposure, as were plasma total osmolality, sodium and chloride levels and plasma glucose concentration. Finally, Na^+,K^+-ATPase activity was determined in gill samples from freshwater and seawater Arctic char after 24, 48 and 96 h exposure.

Determination of maximal enzyme activities

Maximal enzyme activities were determined using a Hewlett Packard HP8452 diode array spectrophotometer (Hewlett Packard, Mississauga, ON, Canada), equipped with a thermostated cell changer maintained at 10°C ± 0.1°C with a Haake D8 circulating water bath (Haake Buchler Instruments Inc., Saddlebrook, NJ, USA). Tissue samples were homogenized in a known volume of 50 mmol l^{-1} imidazole buffer (pH 7.4). Homogenization was performed on ice using three ten second bursts from a Polytron PT10 unit (Kinematica Gmbh., Luzurn, Switzerland) interrupted by 30 s periods on ice. For assay of glutamate dehydrogenase (GDH) activity, Triton X-100 was added to a sub aliquot of the initial homogenate to a final concentration of 0.2% (v/v). All homogenates were then centrifuged at 8000 ***g*** for 5 min (4°C) and the resulting supernatant was used directly in the enzyme assays. Tissue preparation for assay of Na^+,K^+-ATPase activity is outlined later in this section. Maximal reaction rates of GDH, alanine aminotransferase (Ala-AT), aspartate aminotransferase (Asp-AT), 3-hydroxyacyl CoA dehydrogenase (HOAD), malic enzyme (ME), hexokinase (HK), pyruvate kinase (PK), lactate dehydrogenase (LDH), creatine phosphokinase (CK) and fructose 1,6-bisphosphatase (FBPase) were determined by a change in absorbance of reduced β-nicotinamide adenine dinucleotide (NADH) or β-nicotinamide adenine dinucleotide phosphate (NADP) at 340 nm (millimolar extinction coefficient ϵ_{340}=6.22). Citrate synthase (CS) and carnitine palmitoyl transferase (CPT) activities were monitored at 412 nm using 5,5′-dithiobis 2-nitrobenzoic acid (DTNB;

ϵ_{412}=13.6). Cytochrome *c* oxidase (CCO) activity was assayed at 550 nm by following the oxidation of reduced cytochrome *c* (ϵ_{550}=28.5). Maximal enzyme activities are expressed as nmol min^{-1} mg^{-1} protein. All substrates were prepared fresh daily and the conditions for each assay were optimized with respect to substrate and cofactor concentrations to give maximal enzyme activity. Reaction conditions were as follows.

Enzymes of oxidative metabolism

Citrate synthase (CS; E.C. 4.1.3.7): 50 mmol l^{-1} imidazole buffer, pH 8.0, at 10°C, 0.1 mmol l^{-1} DTNB, 0.3 mmol l^{-1} acetyl CoA, 0.5 mmol l^{-1} oxaloacetate (omitted for control).

Cytochrome *c* oxidase (CCO; E.C. 1.9.3.1): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 50 μmol l^{-1} cytochrome *c* (reduced) (omitted for control).

Enzymes of amino acid metabolism

Aspartate aminotransferase (Asp-AT; E.C. 2.6.1.1): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 7 mmol l^{-1} α-ketoglutarate, 0.2 mmol l^{-1} NADH, 0.025 mmol l^{-1} pyridoxal phosphate, 3 i.u. malate dehydrogenase, 40 mmol l^{-1} L-aspartate (omitted for control).

Alanine aminotransferase (Ala-AT; E.C. 2.6.1.2): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 10.5 mmol l^{-1} α-ketoglutarate, 0.2 mmol l^{-1} NADH, 0.025 mmol l^{-1} pyridoxal phosphate, 2 i.u. LDH, 200 mmol l^{-1} L-alanine (omitted for control).

Phosphate-dependent glutaminase (PDG; E.C. 3.5.1.2): Procedures were as described in Chamberlin et al. (Chamberlin et al., 1991).

Glutamate dehydrogenase (GDH; E.C. 1.4.1.3): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 250 mmol l^{-1} ammonium acetate, 0.1 mmol l^{-1} ethylenediaminetertraacetic acid disodium salt (EDTA), 0.1 mmol l^{-1} NADH, 0.1 mmol l^{-1} adenosine diphosphate (ADP), 14 mmol l^{-1} α-ketoglutarate (omitted for control).

Glutamine synthetase (GS; E.C. 6.3.1.2): Procedures were as described by Chamberlin et al. (Chamberlin et al., 1991).

Enzymes of lipid metabolism

Carnitine palmitoyltransferase (CPT; E.C. 2.3.1.21): 50 mmol l^{-1} imidazole buffer, pH 8.0, at 10°C, 0.2 mmol l^{-1} DTNB, 0.1 mmol l^{-1} palmitoyl CoA, 5 mmol l^{-1} L-carnitine (omitted for control).

3-hydroxyacyl CoA dehydrogenase (HOAD; E.C. 1.1.1.35): 50 mmol l^{-1} imidazole buffer, pH 8.0, at 10°C, 0.1 mmol l^{-1} NADH, 0.1 mmol l^{-1} acetoacetyl CoA (omitted for control).

Malic enzyme (ME; E.C. 1.1.1.40): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 1.0 mmol l^{-1} $MgCl_2$, 0.4 mmol l^{-1} NADP, 1.0 mmol l^{-1} malate (omitted for control).

Enzymes of carbohydrate metabolism

Hexokinase (HK; E.C. 2.7.1.1): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 5.0 mmol l^{-1} $MgCl_2$, 1.0 mmol l^{-1} glucose, 0.016 mmol l^{-1} NADP, 2 i.u. glucose-6-phosphate dehydrogenase (G6PDH), 1 mmol l^{-1} ATP (omitted for control).

Pyruvate kinase (PK; E.C. 2.7.1.40): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 0.15 mmol l^{-1} NADH, 5 mmol l^{-1} ADP, 10 mmol l^{-1} $MgCl_2$, 50 mmol l^{-1} KCl, 0.1 mmol l^{-1} fructose-1,6-bisphosphate (FBP), excess LDH, 5 mmol l^{-1} phosphoenol pyruvate (PEP) (omitted for control).

Lactate dehydrogenase (LDH; E.C. 1.1.1.27): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 0.2 mmol l^{-1} NADH, 1 mmol l^{-1} pyruvate (omitted for control).

Enzymes of gluconeogenesis

Fructose 1,6-bisphosphatase (FBPase; E.C. 3.1.3.11): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 15 mmol l^{-1} $MgCl_2$, 0.2 mmol l^{-1} NADP, 10 i.u. phosphoglucose isomerase (PGI), 2 i.u. G6PDH, 0.1 mmol l^{-1} FBP (omitted for control).

Other

Creatine kinase (CK; E.C. 2.7.3.2): 50 mmol l^{-1} imidazole buffer, pH 7.4, at 10°C, 1 mmol l^{-1} ADP, 10 mmol l^{-1} AMP, 0.2 mmol l^{-1} NADP, 4 mmol l^{-1} glucose, 5 mmol l^{-1} $MgCl_2$, 2 i.u. G6PDH, 5 i.u. HK, 50 mmol l^{-1} creatine phosphate (omitted for control).

Gill Na^+,K^+-ATPase activity

Gill filaments were homogenized on ice in SEI buffer (150 mmol l^{-1} sucrose, 10 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole; pH 7.5) by hand using a ground glass homogenizer. Homogenates were centrifuged for 30 s (4°C) at 5000 ***g*** to remove filaments and other insoluble material. The supernatant was used directly in the assay of enzyme activity. Na^+,K^+-ATPase activity was determined spectrophotometrically using a NADH-linked assay modified from the methods of Gibbs and Somero (Gibbs and Somero, 1990) and McCormick (McCormick, 1993). ADP formed from the hydrolysis of ATP by ATPases was enzymatically coupled to the oxidation of reduced NADH using commercial preparations of PK and LDH. Gill samples were assayed for ATPase activity in the presence and absence of the Na^+,K^+-ATPase-specific inhibitor ouabain (final concentration 1 mmol l^{-1}). Samples were run in triplicate with and without ouabain and the difference in the rate of NADH oxidation (ϵ_{340}=6.22) between the two conditions was used to calculate Na^+,K^+-ATPase activity. Optimal assay conditions to give maximal enzyme activity were determined as; 100 mmol l^{-1} NaCl, 20 mmol l^{-1} KCl, 5 mmol l^{-1} $MgCl_2$, 50 mmol l^{-1} imidazole, 3 mmol l^{-1} ATP, 2 mmol l^{-1} PEP, 0.2 mmol l^{-1} NADH, 4 i.u. LDH and 5 i.u. PK, pH 7.5. Na^+,K^+-ATPase activity is expressed as μmol ADP h^{-1} mg^{-1} protein.

Determination of free amino acid levels

Determination of FAAs levels in plasma, RBCs, gill, white and red muscle, using an HPLC (Hewlett-Packard, HP 1090 series II/L liquid chromatograph) equipped with a UV-visible series II diode array detector (DAD), an automatic injector, and a narrow bore (200×2.1 mm) reversed phase column (AminoQuant 79916AA-572, Hewlett-Packard), are as outlined for plasma in Barton et al. (Barton et al., 1995) and for tissues in Frick and Wright (Frick and Wright, 2002).

Briefly, frozen tissue samples (white muscle, red muscle, gill and RBCs; ~250 mg) or plasma (~250 μl) were homogenized and deproteinized simultaneously in 500 μl of 0.5% trifluoroacetic acid (TFA) in methanol in the presence of a known amount of two internal standards. Homogenization was performed on ice using three 10 s bursts from a Polytron PT10 interrupted by 30 s periods on ice. After centrifugation for 5 min (16 215 ***g***, 4°C), 1 mol l^{-1} sodium acetate and 100 mmol l^{-1} NaOH were added. This was followed by centrifugation for 25 min (16 215 ***g***, 4°C) and 1 μl of the resulting supernatant was injected into the column.

Internal and calibration standards were prepared from individual crystalline L-amino acids, to a final stock concentration of 2 mmol l^{-1}. Amino acid stock solutions were prepared in 0.1 mol l^{-1} HCl, with the exception of glutamine, asparagine, tryptophan and taurine, which were prepared in 0.1 mol l^{-1} sodium acetate buffer (pH 7.2). The internal standards for primary and secondary amino acids were norvaline and azetidine 2-carboxylic acid, respectively. Primary and secondary amino acids were derivatized with *o*-phthaldialdehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), respectively. Preparation and storage of OPA and FMOC reagents were as described (Barton et al., 1995). Amino acids were identified and quantified by comparing their retention times and peak areas to the prepared standard and internal standards. Concentrations of tissue FAAs are expressed as nmol g^{-1} wet mass tissue, while plasma FAA levels are expressed as nmol ml^{-1} plasma.

Determination of plasma non-esterified fatty acids

Specific methylation, to fatty acid methyl esters, and determination of plasma NEFAs, using a gas chromatograph (Hewlett-Packard, HP5890A) fitted with a flame ionization detector (FID), an automatic injector (Hewlett-Packard, 7673A) and a DB-225 megabore fused silica column (Chromatographic Specialities Inc., Brockville, ON, Canada), were as previously described by Singer et al. (Singer et al., 1990). Fatty acid methyl esters from plasma samples were identified by comparing their retention times to those of known standards and absolute amounts were quantified with the aid of the internal standard, heptadecanoic acid (17:0), added to the plasma samples prior to methylation.

Plasma osmolality was determined using a vapour pressure osmometer (Model 5500, Wescor, Utah, USA). Chloride levels were measured using a chloride titrator (Model CMT10, Radiometer, Copenhagen, Denmark). Sodium levels were measured using a flame photometer (Model FLM2, Radiometer, Copenhagen). Plasma glucose was determined using a Sigma diagnostic kit (Sigma, St Louis, MO, USA). Protein content of tissue homogenates was determined using the Bio-Rad standard protein assay (Bio-Rad Laboratories, Hercules, CA, USA), standardized with bovine serum albumin (BSA). All chemicals used were purchased from Sigma Chemical Co. (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) with the exception of the BSA (purchased from BioShop, Burlington, ON, Canada), HPLC grade methanol and acetonitrile (purchased from Fisher Scientific Ltd, Whitby, ON, Canada), and fatty acid standards (purchased from Nu Check Prep Inc., Elysian, MN, USA).

Statistical analysis

All data are presented as means ± s.e.m. Comparisons of maximal enzyme activities between control and seawater acclimated groups were performed using a two-tailed *t*-test (a=0.05). A one-way analysis of variance (ANOVA) (α=0.05) was used to establish differences between the control and treatment groups for total and individual plasma NEFAs, tissue and plasma FAAs and plasma osmolality, sodium, chloride and glucose levels. A Tukey HSD multiple comparison test was used to determine significance. Assumptions for normality, independence, and homeoscedasticity were verified by generating appropriate residual plots. Data transformations (log, square root, and inverse square root) were used when appropriate to meet the above assumptions. For all comparisons $P<0.05$ was considered significant.

Results

Plasma ions, osmolality and glucose

Plasma sodium, chloride, total osmolality and glucose of the freshwater (control) char were not different between the three sampling points. For this reason, data for these char plasma parameters (controls) were pooled for comparison with the seawater-acclimated conditions. Seawater-acclimated char had significantly higher plasma sodium, chloride and osmolality than control fish at all time points. These levels peaked after 48 h and remained high through the 96-h sampling period (Table 1). Plasma glucose did not change following seawater acclimation of Arctic char (Table 1).

Enzymes

Tissue protein content per gram of the white muscle was significantly higher (18%) in fish acclimated to seawater for 96 h whereas the protein content of liver, red muscle and gill were unchanged (data not shown). Gill Na^+,K^+-ATPase activity did not increase during the first 48 h of seawater exposure but was twofold higher than freshwater controls by 96 h (Table 1). Gill CS and Asp-AT activities significantly increased upon seawater exposure of Arctic char (Table 2). In the liver, GDH and Asp-AT increased significantly with seawater exposure (Table 2). Liver ME activity from seawater char was significantly higher than control fish when expressed per gram of tissue (data not shown) but not different when calculated per milligram of protein (Table 2). Red muscle GDH and Ala-AT and white muscle GDH activities were significantly higher in seawater-exposed fish (Table 2).

Tissue and plasma free amino acids

Freshwater char FAA levels (controls) were pooled for comparison with data from seawater-acclimated fish as there were no significant differences at the three sampling times for

Table 1. *Plasma sodium, chloride, osmolality and glucose levels and maximal gill Na^+,K^+-ATPase activity of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	FW	SW 24 h	SW 48 h	SW 96 h
Sodium (mequiv. l^{-1})	147.0±2.6	173.8±3.4*	181.1±2.9*,†	181.8±3.7*,†
Chloride (mequiv. l^{-1})	129.0±1.8	151.0±2.2*	164.6±3.3*,†	162.1±3.7*,†
Osmolality (mmol kg^{-1})	284.4±2.1	324.1±1.9*	343.3±4.4*,†	343.3±5.7*,†
Glucose (mmol l^{-1})	4.63±0.21	4.38±0.30	4.74±0.27	4.51±0.23
Gill Na^+,K^+-ATPase (μmol mg^{-1} protein h^{-1})	0.24±0.56	0.28±0.08	0.23±0.05	0.52±0.10*,†,‡

Levels for freshwater char are pooled since there were no significant differences between the 24, 48 and 96 h sample groups. Values are means ± s.e.m.; *N*=8 for SW 24, 48 and 96 h groups, *N*=24 for the pooled FW group.

*Significantly different from pooled freshwater (FW; control) value; †significantly different from seawater (SW; 32‰) 24 h group; ‡significantly different from SW 48 h group.

freshwater char in any of the tissues or plasma analyzed. The plasma of seawater acclimated char had significantly higher total essential (after 96 h), and significantly lower total non-essential (after 48 and 96 h), FAA levels when compared with those of control fish (Table 3). These changes summed to give a small but statistically significant increase in overall total FAAs in the plasma after 96 h. The increase in the total essential FAA levels was due to large increases in valine, isoleucine and leucine and more moderate increases in arginine and phenylalanine. Histidine levels were significantly lower in seawater char plasma. The non-essential amino acids asparagine, serine, glutamine, glycine, alanine and taurine were all significantly lower whereas tyrosine levels were significantly higher following seawater exposure (Table 3).

Table 2. *Maximal enzyme activities in gill, liver, red muscle and white muscle of Arctic char Salvelinus alpinus exposed to freshwater or seawater for 96 h*

	Maximal enzyme activity (nmol min^{-1} mg^{-1} protein)							
	Gill		Liver		Red muscle		White muscle	
Enzyme	FW	SW	FW	SW	FW	SW	FW	SW
Oxidative metabolism								
CCO	4.0±0.5	4.0±0.4	9.6±0.7	10.4±0.6	55.1±6.7	49.7±7.3	1.1±0.3	1.0±0.2
CS	24.1±0.8	26.0±0.8*	11.8±0.36	12.1±0.3	349.6±19.1	325.7±6.4	17.7±1.2	17.6±1.0
Amino acid metabolism								
GDH	116.7±9.8	106.7±8.3	279.7±13.6	312.9±16.1*	159.3±11.2	180.9±7.9*	9.1±0.7	11.6±1.2*
Ala-AT	49.1±9.2	54.0±9.0	343.8±17.9	343.9±8.9	99.2±11.3	136.2±19.6*	2.4±0.2	2.9±0.2
Asp-AT	83.8±8.0	106.0±10.2*	256.9±24.7	354.6±18.0*	724.6±46.4	717.2±26.7	187.3±10.1	182.4±9.3
GS	0.2±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	bld	0.1±0.0	0.1±0.0
PDG	3.4±0.3	3.1±0.4	2.4±0.2	2.1±0.2	2.5±0.1	2.7±0.1	bld	bld
Lipid metabolism								
CPT	0.3±0.0	0.2±0.0	1.3±0.1	1.2±0.1	3.4±0.4	3.8±0.6	bld	bld
HOAD	5.4±0.6	5.5±0.4	1.9±0.1	2.1±0.1	19.1±1.6	20.7±1.1	1.1±0.2	1.0±0.1
ME	0.7±0.2	0.7±0.3	22.8±2.0	25.4±1.2	13.4±1.3	16.0±1.8	2.3±0.1	2.6±0.3
Carbohydrate metabolism								
HK	1.6±0.2	1.4±0.2	0.5±0.0	0.5±0.0	0.4±0.1	0.5±0.1	0.2±0.0	0.2±0.0
PK	146.0±12.0	160.9±9.2	23.7±2.9	23.2±1.4	1130.0±256.5	1015.7±177.0	1811.0±273.8	2281.9±91.8
LDH	212.8±17.9	247.9±11.1	1318.7±115.4	1351.5±51.5	2937.9±197.9	3308.7±222.9	4705.6±216.2	5073.6±210.3
Gluconeogenesis								
FBPase	bld	bld	6.5±0.6	5.2±0.5	bld	bld	bld	bld
Other								
CK	164.5±14.6	152.1±18.1	14.0±0.7	14.1±0.6	736.9±42.8	721.7±53.1	2917.8±42.0	2978.3±102.5

CCO, cytochrome *c* oxidase; CS, citrate synthase; GDH, glutamate dehydrogenase; Ala-AT, alanine aminotransferase; Asp-AT, aspartate aminotransferase; GS, glutamine synthetase; PDG, phosphate-dependent glutaminase; CPT, carnitine palmitoyl transferase; HOAD, 3-hydroxyacyl CoA dehydrogenase; ME, malic enzyme; HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; FBPase, fructose 1,6-bisphosphatase; CK, creatine phosphokinase. SW, seawater, 32‰; bld, below level of detection.

Values are means ± s.e.m.

*Significantly different from freshwater (FW) control group.

Table 3. *Absolute amounts of free amino acids in the plasma of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	Free amino acid concentration (nmol ml^{-1} plasma)			
Amino acid	FW	SW 24 h	SW 48 h	SW 96 h
Essential				
Histidine	111.1±4.8	102.4±11.8	59.2±4.7*,†	57.7±4.9*,†
Arginine	165.0±7.6	189.5±20.6	148.2±17.3	224.1±13.9*,‡
Threonine	393.7±13.4	345.5±26.4	305.4±34.1	365.6±38.4
Valine	486.2±24.6	679.9±62.4*	744.6±64.1*	883.7±46.7*,†
Methionine	167.7±9.0	189.2±17.5	180.5±19.6	176.8±8.2
Tryptophan	47.9±4.9	40.5±3.3	37.8±4.4	43.5±4.0
Phenylalanine	99.2±3.5	136.0±15.8*	128.4±4.6*	145.6±8.1*
Isoleucine	207.3±10.0	272.3±24.0	357.7±36.1*,†	542.8±36.9*,†,‡
Leucine	333.3±9.0	593.6±52.9*	584.0±31.3*	836.4±37.6*,†,‡
Lysine	472.6±29.1	406.3±51.2	322.9±27.0*	482.3±34.3‡
Total essential	2484.0±67.7	2955.3±192.5	2868.6±178.8	3758.5±163.2*,†,‡
Non-essential				
Aspartate	66.2±4.6	69.5±9.4	70.7±5.9	67.9±6.8
Glutamate	21.0±2.4	29.5±9.0	22.2±3.8	35.8±3.9
Asparagine	115.7±11.9	108.3±18.1	57.1±6.0*,†	60.0±5.9*,†
Serine	117.5±7.0	87.9±12.0	54.5±5.4*	78.0±16.3*
Glutamine	347.2±14.5	267.1±30.9*	186.8±13.9*,†	201.9±10.6*,†
Glycine	315.8±13.7	255.6±19.0	168.1±16.0*,†	168.7±19.9*,†
Alanine	479.5±27.6	563.9±66.8	301.9±19.0*,†	408.5±45.0 †
Taurine	410.6±29.2	181.2±13.8*	156.0±20.9*	187.2±16.5*
Tyrosine	112.1±7.4	157.6±14.5	169.7±18.2*	243.4±20.2*,†,‡
Hydroxyproline	427.9±46.9	503.0±114.7	389.3±105.1	341.0±61.7
Proline	37.9±5.6	38.2±7.0	32.6±10.9	33.0±9.2
Total non-essential	2451.3±89.3	2261.7±183.5	1609.0±117.1*,†	1825.4±82.8*
Total FAA	4935.3±108.7	5216.9±282.7	4477.5±163.9 †	5583.9±202.5*,‡

Values are means ± s.e.m.; N=8 for SW 24, 48 and 96 h groups, N=24 for the pooled FW group.

Free amino acid (FAA) levels for FW char are pooled since there were no significant differences between the 24, 48 and 96 h sample groups.

SW, seawater, 32‰.

*Significantly different from pooled freshwater (FW) control group; †significantly different from SW 24 h group; ‡significantly different from SW 48 h group.

Total essential FAA levels in white muscle nearly doubled in seawater-exposed fish due to large increases in arginine, threonine, valine, isoleucine, leucine and lysine and smaller increases in methionine and phenylalanine (Table 4). There was a small but statistically significant increase in total non-essential FAA levels after 96 h in seawater, mainly because of a more than two-fold increase in alanine content and a near doubling of asparagine and serine concentrations. Glutamine, taurine and tyrosine levels also increased significantly but to a lesser degree. These changes led to total FAA levels in white muscle of seawater-exposed char increasing by approximately one third after 96 h. The predominant FAA in white muscle was glycine (35–45%) in both acclimation groups.

Total FAA levels in red muscle were relatively similar between freshwater and seawater-acclimated Arctic char (Table 5). The essential amino acids valine, phenylalanine, isoleucine and leucine all increased significantly following seawater exposure. Taurine made up the majority of the total red muscle FAA content (63–69%). In gill, histidine, tryptophan and phenylalanine all decreased significantly with seawater acclimation while alanine and glutamate levels increased (Table 6). Transient increases in gill isoleucine, glutamine and glycine were seen after 48 h in seawater but returned to control levels by 96 h. As a percentage of the total FAAs, taurine content decreased significantly from 60% in control fish to 52% following seawater acclimation, but changes in absolute levels were not found to be statistically significant.

In RBCs there was a general increase in essential FAAs, but no change in non-essential or total FAA content following seawater acclimation (Table 7). Valine, phenylalanine, isoleucine, leucine and tyrosine levels were all higher in seawater char. Again, the predominant FAA in the RBCs was taurine (~55%).

Plasma non-esterified fatty acid levels

Plasma NEFA levels were not different between the three

sampling points for freshwater char and were pooled for comparison with seawater fish. Exposure to seawater did not alter circulating levels of plasma NEFAs (Table 8). The predominant fatty acids found were 18:1 and 16:0 followed by 22:6n3, 20:5n3, 18:2n6 and 16:1 in both acclimation groups.

Discussion

Osmoregulation

An initial rise in plasma sodium, chloride and total osmolality is typical of the early acclimation period of euryhaline fish to seawater (Gordon, 1959). During this stage of seawater acclimation the fish struggles to osmoregulate, as it has not yet modified its osmoregulatory tissues to efficiently excrete excess salts from the plasma. Plasma ion and osmolality levels appear to plateau between 48 and 96 h suggesting the Arctic char are acclimating to seawater and have improved their osmoregulatory capacity. The active excretion of plasma sodium and chloride by the gill is powered by ion gradients maintained by Na^+,K^+-ATPase (Eddy, 1982). Gill Na^+,K^+-ATPase activity remained low for the first 48 h of seawater exposure before increasing twofold by 96 h. Many studies report similar changes in Arctic char plasma ion content and gill Na^+,K^+-ATPase activity during salinity acclimation (e.g. Arnesen et al., 1992; Nilssen et al., 1997). The observed rise in gill Na^+,K^+-ATPase activity is very characteristic of the acclimation of euryhaline fish to seawater (McCormick, 1996). Na^+,K^+-ATPase is known to account for 20% of the standard metabolic rate in mammals (Rolfe and Brown, 1997) and turtles (Land and Hochachka, 1994) and likely represents a similar overall contribution to metabolic rate in fishes. Any change in Na^+,K^+-ATPase activity would have a profound effect on total ATP energy demand, especially in the gill (Eddy, 1982) and intestine (Colin et al., 1985), where Na^+,K^+-ATPase activity is known to increase with seawater exposure. The ongoing energy requirements of Na^+,K^+-ATPase must also be added to the cost

Table 4. *Absolute amounts of free amino acids in the white muscle of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	Free amino acid concentration (nmol g^{-1} wet mass tissue)			
Amino acid	FW	SW 24 h	SW 48 h	SW 96 h
Essential				
Histidine	592.3±55.2	706.1±71.5	653.6±50.8	882.0±51.9*,‡
Arginine	50.6±3.7	83.6±9.9*	94.6±8.5*	151.6±13.9*,†
Threonine	385.7±18.3	488.5±42.2	567.6±27.2*	709.0±55.6*,†
Valine	197.0±9.5	247.9±17.9	299.3±12.4*	397.9±31.0*,†
Methionine	211.3±6.8	249.7±10.9*	241.2±12.8	281.6±12.6*,‡
Tryptophan	232.9±11.0	247.9±20.9	231.5±25.2	267.1±24.2
Phenylalanine	55.4±2.1	70.2±2.9*	76.4±4.1*	87.5±5.0*,†,‡
Isoleucine	106.9±5.4	135.8±8.9	186.5±9.8*,†	252.1±19.5*,†,‡
Leucine	148.6±4.7	248.2±14.5*	277.0±13.9*	390.9±31.4*,†,‡
Lysine	165.4±12.8	275.3±33.6	418.0±52.6*	711.8±81.6*,†
Total essential	2146.0±79.9	2753.2±175.2*	3045.6±141.2*	4131.5±268.3*,†,‡
Non-essential				
Aspartate	819.9±99.0	754.9±90.5	758.2±103.0	831.8±121.4
Glutamate	246.2±24.0	354.4±39.1	330.9±32.5	350.6±26.3
Asparagine	515.0±36.0	756.2±116.8*	781.5±53.2*	919.8±69.1*
Serine	541.6±50.9	697.7±127.0	774.7±81.5	912.8±135.6*
Glutamine	285.7±19.8	310.5±26.5	376.2±34.7	385.6±24.9*
Glycine	7535.1±415.1	8675.4±816.2	9367.4±734.9	8324.0±705.2
Alanine	1200.9±54.2	1772.7±129.6*	2112.1±113.6*	2919.8±286.4*,†,‡
Taurine	993.4±113.5	962.5±119.3	1107.2±167.5	1137.2±358.9*
Tyrosine	109.1±7.1	145.2±9.1	169.5±22.2*	226.6±23.5*,†,‡
Hydroxyproline	2800.0±447.4	1818.5±560.2	2199.8±509.1	2815.2±749.3
Proline	193.6±36.9	184.9±32.2	204.5±45.5	279.4±71.8
Total non-essential	15241.3±799.0	16432.9±960.6	18181.9±887.3	19102.9±716.2*
Total FAA	17387.2±851.3	19186.1±1071.8	21227.5±944.9	23234.3±963.8*,†

Values are means ± s.e.m.; N=8 for SW 24, 48 and 96 h groups, N=24 for the pooled FW group.

Free amino acid (FAA) levels for FW char have been pooled since there were no significant differences between the 24, 48 and 96 h sample groups. SW, seawater, 32‰.

*Significantly different from pooled freshwater (FW) control value; †significantly different from SW 24 h group; ‡significantly different from SW 48 h group.

Table 5. *Absolute amounts of free amino acids in the red muscle of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	Free amino acid concentration (nmol g^{-1} wet mass tissue)			
Amino acid	FW	SW 24 h	SW 48 h	SW 96 h
Essential				
Histidine	178.8±24.0	218.7±51.9	255.4±38.0	211.4±50.2
Arginine	12.8±3.3	12.7±2.7	19.2±2.5	25.6±2.9
Threonine	227.7±18.0	247.9±25.3	312.2±17.9	282.1±39.1
Valine	90.6±6.9	96.3±13.7	123.6±11.0	149.2±9.8*,†
Methionine	93.7±5.8	100.1±14.0	108.2±8.1	107.1±6.9
Tryptophan	483.0±26.3	470.2±31.2	528.0±47.2	535.6±92.4
Phenylalanine	12.6±2.9	16.2±4.9	24.7±3.0	30.4±4.7*,†
Isoleucine	37.4±3.8	43.0±6.6	64.9±8.1*,†	89.4±5.6*,†,‡
Leucine	72.9±5.5	83.3±10.1	117.3±14.1*,†	147.4±6.2*,†,‡
Lysine	76.4±12.7	67.6±10.8	90.1±16.1	85.6±11.8
Total essential	1285.9±90.9	1356.2±147.0	1643.7±105.7	1663.7±210.5
Non-essential				
Aspartate	426.8±43.2	415.4±124.3	414.2±68.9	413.6±91.4
Glutamate	520.2±32.3	576.4±72.1	540.7±42.7	587.7±51.6
Asparagine	197.7±21.6	337.2±140.0	324.7±45.3	236.4±61.1
Serine	78.6±10.3	139.7±39.2	135.5±21.7	100.9±11.8
Glutamine	194.2±13.1	268.7±44.7	303.5±22.3*	241.5±34.5
Glycine	444.5±103.5	525.7±170.1	471.8±96.9	408.7±80.5
Alanine	723.1±46.3	912.3±141.2	978.8±132.5	829.4±102.1
Taurine	12814.2±449.1	12199.9±913.0	12681.2±565.4	13303.1±1407.8
Tyrosine	70.7±5.9	83.1±9.7	79.7±15.3	81.2±11.3
Hydroxyproline	1372.4±200.4	2202.3±543.9	2069.8±635.5	2869.3±1396.8
Proline	430.5±133.1	823.2±441.6	332.8±155.5	674.9±318.5
Total non-essential	17272.9±532.6	18483.9±1772.5	18332.8±1220.4	19746.6±1649.2
Total FAA	18558.8±582.3	19840.1±1914.2	19976.5±1302.0	21410.3±1809.6

Values are means ± s.e.m.; *N*=8 for SW 24, 48 and 96 h groups, *N*=24 for the pooled FW group.

Free amino acid (FAA) levels for FW char have been pooled since there were no significant differences between the 24, 48 and 96 h sample groups. SW, seawater, 32‰.

*Significantly different from pooled freshwater (FW; control) value; †significantly different from SW 24 h group; ‡significantly different from SW 48 h group.

of reorganizing the osmoregulatory tissues for work in the marine environment, including *de novo* synthesis of ionoregulatory proteins (e.g. Na^+,K^+-ATPase) and the enlargement of membrane surface areas (e.g. gill basolateral membrane). These energetic pressures suggest the initial period following seawater exposure is especially critical for successful acclimation. Higher energy requirements are evident from reports of increased oxygen consumption during seawater acclimation in several fish species (Rao, 1968; Farmer and Beamish, 1969; Febry and Lutz, 1987; Maxime et al., 1991) and from the heightened catabolism of carbohydrates, fats and proteins in salmonids migrating to sea (Hoar, 1988). The cost of osmoregulation may also be indicated by changes in the intermediary metabolism of individual tissues by monitoring the activity of several key enzymes and the levels of important tissue and blood metabolites. Both FAAs and NEFAs are known to be important energy sources in fish (Walton and Cowey, 1982), and their oxidation by individual tissues may be increased during seawater acclimation. Tissues with limited pools of FAAs and NEFAs may rely on a supply of these metabolites from the blood, originally mobilized from muscle and liver stores.

Metabolic capacity

The gill is a highly aerobic tissue and has been shown to account for 7% a fish's total oxygen consumption (Mommsen, 1984). CCO, the terminal enzyme in the electron transport chain, and CS, a limiting enzyme in the Krebs cycle, are good indicators of overall metabolic rate. In this study, gill CS activity increased by 8% whereas CCO activity remained constant following seawater exposure. An increase in CS activity indicates an increased capacity for energy production from a variety of substrates that feed into the Krebs cycle. As CS activity was determined in whole gill homogenates, it may be reasonable to suggest that much of the observed increase in activity may be specific to gill chloride cells. Perry and Walsh

Table 6. *Absolute amounts of free amino acids in the gill of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	Free amino acid concentration (nmol g^{-1} wet mass tissue)			
Amino acid	FW	SW 24 h	SW 48 h	SW 96 h
Essential				
Histidine	95.2±4.0	91.5±7.0	84.9±15.4	53.1±4.1*,†
Arginine	241.4±8.8	235.4±18.1	241.7±34.8	220.3±11.6
Threonine	491.3±34.6	513.7±58.8	629.0±109.8	599.9±51.0
Valine	272.2±13.5	302.5±20.7	355.2±44.4	312.6±29.6
Methionine	321.5±13.0	303.0±21.9	334.7±36.3	292.2±20.1
Tryptophan	571.0±23.6	615.8±20.2	549.9±38.3	428.8±41.0*,†
Phenylalanine	197.7±11.5	176.2±18.6	202.0±33.3	128.0±13.4*
Isoleucine	123.1±8.4	144.0±11.8	191.8±29.3*	178.1±20.3
Leucine	349.9±18.7	382.3±29.3	452.7±62.3	282.7±57.6
Lysine	239.4±10.8	240.1±39.5	219.0±30.5	233.6±19.4
Total essential	2902.6±118.7	3004.5±181.9	3260.8±413.6	2729.3±109.1
Non-essential				
Aspartate	330.7±37.9	372.1±32.8	502.4±25.1	413.6±48.7
Glutamate	1468.6±66.2	1980.8±82.0*	2460.0±134.1*	2114.2±150.5*
Asparagine	207.3±10.0	240.8±20.8	236.8±17.6	195.6±33.2
Serine	398.4±18.5	379.2±26.9	427.8±41.7	315.5±24.4
Glutamine	627.0±25.5	678.2±26.3	830.8±75.3*	641.9±52.6
Glycine	1123.0±37.5	1266.1±71.7	1431.0±96.4*	1150.4±106.0
Alanine	862.2±20.8	1057.5±71.4*	1221.7±83.5*	1256.0±80.6*
Taurine	15434.5±501.8	15626.5±762.1	14884.6±1217.6	12940.3±769.4
Tyrosine	326.9±20.5	272.0±37.5	415.8±45.5	330.1±33.4
Hydroxyproline	1435.0±127.6	1893.2±305.5	1735.7±241.9	2536.3±819.6
Proline	273.1±64.2	291.2±155.9	439.1±132.5	225.1±80.6
Total non-essential	22486.7±597.1	24057.5±858.5	24585.7±1601.7	22119.1±615.4
Total FAA	25389.3±694.7	27062.0±969.0	27846.5±1977.7	24848.3±694.4

Values are means ± s.e.m.; *N*=8 for SW 24, 48 and 96 h groups, *N*=24 for the pooled FW group.

Free amino acid (FAA) levels for FW char have been pooled since there were no significant differences between the 24, 48 and 96 h sample groups. SW, seawater, 32‰.

*Significantly different from pooled freshwater (FW; control) value; †significantly different from SW 24 h group; ‡significantly different from SW 48 h group.

(Perry and Walsh, 1989) suggested that although chloride cells only account for up to about 13% of the total gill cell population, their contribution to the overall metabolic rate of the gill is far more substantial. If examined specifically, the change in gill chloride cell CS activity may actually be much higher than our reported 8% increase following seawater exposure. McCormick et al. (McCormick et al., 1989) found no change in gill CCO or CS activity of Atlantic salmon smolts following seawater transfer, but did report a decline in CS activity when non-smolts were exposed to seawater. The poor performance of parr during salinity acclimation in that study may be due to a decreased capacity for energy production in the gill. The activity of succinate dehydrogrenase (SDH), also a limiting enzyme in the Krebs cycle, has been shown to increase in eel gill (Sargent et al., 1975), and increase (Langdon and Thorpe, 1984) or remain unchanged (Conte, 1969) in Atlantic salmon gill following seawater exposure, which may suggest an increased requirement for energy production.

Amino acid metabolism

The increase in gill Asp-AT activity following salinity exposure suggests the char gill has an enhanced ability to utilize aspartate. Transdeamination of aspartate and alanine are known to be important pathways for energy production in fish (Walton and Cowey, 1982). The oxidation of aspartate and alanine by their respective aminotransferases can lead to the accumulation of glutamate if it is not deaminated by GDH (Mommsen, 1984). GDH levels did not increase in Arctic char gill but have been reported to increase in tilapia gill following seawater exposure (Kultz and Jurss, 1993). This may explain the significant increase in gill glutamate levels found in seawater-acclimated Arctic char, and supports the idea of increased energy production from aspartate and possibly from other amino acids *via* transdeamination. Oxidation of branched chain amino acids (BCAAs; leucine, isoleucine and valine) by BCAA transaminase also produces glutamate. Levels of all three BCAAs rise in the plasma following seawater transfer and

Table 7. *Absolute amounts of free amino acids in the RBCs of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	Free amino acid concentration (nmol g^{-1} wet mass tissue)			
Amino acid	FW	SW 24 h	SW 48 h	SW 96 h
Essential				
Histidine	55.9±7.9	55.1±6.4	33.7±12.8	35.0±8.8
Arginine	81.3±6.8	97.1±8.6	70.2±11.0	96.0±12.8
Threonine	463.0±23.2	435.9±42.4	448.1±56.1	439.8±40.0
Valine	216.9±10.1	281.8±21.1	309.0±29.7*	405.1±37.9*,†
Methionine	141.0±10.6	134.8±18.8	142.7±21.9	151.6±20.5
Tryptophan	684.5±60.1	903.5±144.5	572.8±56.4	704.5±116.6
Phenylalanine	91.7±4.2	113.9±8.7	107.4±8.8	122.9±9.6*
Isoleucine	134.8±5.5	175.3±17.4	220.6±18.7*	283.4±20.3*,†,‡
Leucine	234.5±7.9	368.9±35.6*	337.9±37.5*	481.3±37.9*,‡
Lysine	108.1±7.2	130.0±24.0	104.1±29.6	106.2±15.3
Total essential	2211.8±75.7	2696.2±190.6*	2346.4±144.7	2825.8±170.4*
Non-essential				
Aspartate	845.0±76.1	750.3±81.8	707.1±83.8	650.5±105.6
Glutamate	307.2±19.2	335.2±35.9	315.8±46.5	283.8±26.8
Asparagine	80.5±5.9	107.2±17.5	71.6±11.6	64.5±11.1
Serine	101.3±6.5	105.7±12.0	79.5±10.0	73.0±11.4
Glutamine	3157.5±145.9	3810.4±324.7	3479.9±408.5	3264.7±297.4
Glycine	993.9±99.2	904.6±108.5	1013.6±212.6	687.9±57.7
Alanine	415.9±27.7	544.4±70.4	455.1±85.5	452.4±56.7
Taurine	11616.9±488.3	13341.7±805.3	12301.3±798.0	13082.0±1024.9
Tyrosine	197.3±14.0	190.5±27.1	307.2±36.1*,†	334.2±16.1*,†
Hydroxyproline	901.5±94.2	794.5±139.1	737.2±91.9	827.8±194.7
Proline	87.9±25.9	82.9±16.1	50.6±10.8	57.5±18.5
Total non-essential	18704.6±559.6	20967.3±959.3	19519.0±1029.9	19778.3±1195.2
Total FAA	20916.4±571.1	23663.5±1087.5	21865.4±1081.8	22604.1±1279.0

Values are means ± s.e.m.; *N*=8 for SW 24, 48 and 96 h groups, *N*=24 for the pooled FW group.

Free amino acid (FAA) levels for FW char have been pooled since there were no significant differences between the 24, 48 and 96 h sample groups. SW, seawater, 32‰.

*Significantly different from pooled freshwater (FW; control) value; †significantly different from SW 24 h group; ‡significantly different from SW 48 h group.

isoleucine is accumulated in the gill where it may be broken down to glutamate. Increased gill glutamate levels may have also originated in the plasma, as Walton and Cowey (Walton and Cowey, 1977) have shown rainbow trout gills are capable of taking up glutamate from the blood.

Increased alanine concentrations in the gills of Arctic char following seawater transfer correspond to decreased levels in the plasma. This suggests the gill may also accumulate alanine for use as an energy source. Mommsen et al. (Mommsen et al., 1980) proposed that alanine may be a preferred carrier of amino acid nitrogen for inter-tissue transport, as several amino acids can be converted to alanine, released to the blood and used as a fuel source in other tissues. In addition to alanine, glutamine and glycine levels also increase in the gills of seawater char with a corresponding drop in plasma levels. Non-essential FAAs are preferentially catabolized over essential FAA (Ballantyne, 2001), which may explain why levels of non-essential FAAs decline in the plasma of seawater-exposed Arctic char. Similarly, Kaushik and Luquet (Kaushik and Luquet, 1979) have also shown an increased proportion of essential FAAs in the plasma of seawater acclimated rainbow trout.

The essential FAAs, histidine, tryptophan and phenylalanine all decreased in the gills of seawater-exposed char. Fish gills have high rates of protein synthesis (Lyndon and Houlihan, 1998) and decreases in these essential FAAs may be a result of their increased incorporation into newly synthesized proteins required during seawater acclimation. Lyndon and Houlihan (Lyndon and Houlihan, 1998) also report an increase in gill mitochondrial protein synthesis in tilapia following seawater exposure. Some essential FAAs are also important energy sources in fish. For example, 35–40% of leucine is oxidized in rainbow trout, with the rest converted to protein (Fauconneau and Arnal, 1985). Histidine may also be an important energy source in Arctic char gill as it can be converted to glutamate and oxidized. Histidine has been found to be rapidly utilized

Table 8. *Absolute amounts of individual non-esterified fatty acids in the plasma of Arctic char Salvelinus alpinus acclimated to freshwater or seawater for up to 96 h*

	NEFA concentration (nmol ml^{-1} plasma)			
Fatty acid	FW	SW 24 h	SW 48 h	SW 96 h
Saturates				
14:0	27.12±2.62	30.12±4.95	29.62±4.22	34.99±3.15
16:0	251.09±24.89	269.98±36.92	265.62±23.95	303.53±19.77
18:0	42.99±4.28	51.98±10.49	60.18±9.04	57.88±6.67
20:0	0.90±0.68	0.00±0.00	1.35±1.35	0.42±0.31
22:0	4.52±3.26	7.69±7.69	bld	bld
24:0	2.33±1.86	1.37±1.37	0.15±0.15	0.39±0.24
Monoenes				
14:1	1.61±0.73	0.21±0.21	2.46±1.60	4.32±1.48
16:1	95.14±11.33	93.67±15.69	107.60±16.12	128.79±15.02
18:1	246.33±26.27	237.60±28.64	277.50±37.78	299.39±28.62
20:1	36.45±4.58	32.55±3.99	37.51±6.98	37.16±5.83
22:1	18.06±3.09	14.49±3.79	11.08±3.60	26.14±6.77
24:1	0.37±0.37	1.37±1.37	0.99±0.99	bld
Polyenes				
18:2n6	92.10±11.16	80.85±11.43	126.88±30.72	114.93±21.17
18:3n3	14.40±2.75	14.10±5.91	27.28±8.53	19.82±5.35
18:4n3	17.98±3.10	16.37±6.49	8.00±4.42	30.00±7.92
20:2n6	1.96±0.58	0.55±0.55	1.62±1.08	1.69±0.70
20:3n6	0.12±0.09	bld	bld	0.23±0.23
20:4n6	10.40±1.73	10.72±2.69	9.31±3.24	15.12±1.96
20:3n3	1.85±1.71	bld	bld	bld
20:4n3	1.77±0.46	2.29±0.50	1.88±0.86	2.72±0.42
20:5n3	131.37±17.76	139.19±24.57	126.49±20.35	181.77±24.08
22:2n6	0.48±0.48	bld	bld	bld
22:4n6	0.23±0.23	bld	1.69±1.69	2.27±1.42
22:5n6	5.43±1.94	4.04±2.22	0.15±0.15	1.88±1.19
22:5n3	13.28±2.00	10.08±2.03	9.11±4.22	11.77±1.91
22:6n3	196.02±20.64	212.45±32.83	190.50±22.66	216.31±17.08
Total	1215.30±116.20	1230.57±178.77	1297.70±37.17	1492.34±126.69
Total saturates	329.93±30.13	361.41±52.82	357.64±37.17	398.04±25.99
Total monoenes	397.97±41049	378.53±46.22	437.15±57.11	495.81±48.48
Total polyenes	487.40±50.81	490.64±81.07	502.91±55.96	598.50±59.13
n3 polyenes	376.68±42.38	394.48±68.82	363.26±50.02	462.38±42.24
n6 polyenes	110.73±12.01	96.16±15.13	139.66±27.77	136.12±22.32
n3:n6	3.78±0.39	4.15±0.52	3.59±1.12	4.40±0.70
Monoene:polyene	0.89±0.05	0.82±0.06	0.87±0.06	0.86±0.05
Unsaturation index	215.13±5.48	218.75±7.98	206.80±7.13	217.23±4.77
Mean chain length	18.41±0.04	18.40±0.06	18.27±0.06	18.33±0.03

Non-esterified fatty acid (NEFA) levels for FW char have been pooled since there were no significant differences between the 24, 48 and 96 h sample groups. FW, freshwater (control); SW, seawater, 32‰; bld, below level of detection.

Values are means ± s.e.m.; *N*=8 for SW 24, 48 and 96 h groups, *N*=24 for the pooled FW group. No significant differences found.

by salmon during their spawning migration (Mommsen et al., 1980). With the exception of methionine, tryptophan and threonine, all of the essential FAAs increased in the plasma of seawater acclimated char, making them available for use by tissues that require them.

The increase in essential FAAs in the plasma of seawater Arctic char suggests a stimulation of proteolysis, as fish cannot synthesize these amino acids and the fish were not fed during the experimental period. The source of these FAAs is likely the white muscle, and to a lesser degree, the liver. White muscle is by far, the largest FAA pool in fish and is known to export FAAs to the circulation (Ballantyne, 2001). Levels of most FAAs increased significantly in the white muscle of Arctic char following seawater exposure. The observed increases in FAA

levels cannot be explained simply by the dehydration of the white muscle, as increases in FAA levels far exceed the effects of tissue concentration. During the initial phase of seawater acclimation, white muscle is allowed to dehydrate so that plasma osmolality is maintained within an acceptable range (Eddy, 1982).

The fish liver is an important site of lipid synthesis and gluconeogenesis and plays a significant role in regulating circulating levels of glucose, lipid and FAAs in the blood (Walton and Cowey, 1982; Ballantyne, 2001). The fish liver relies mainly on amino acid catabolism for its own energy requirements (Ballantyne, 2001). The main pathway for amino acid catabolism in fish liver is through transdeamination of several amino acids to form glutamate and its further deamination by GDH (Ballantyne, 2001). In this study, liver GDH and Asp-AT activities increased in seawater-acclimated char suggesting increased amino acid catabolism. This may be of great importance as several other amino acids including glutamine, proline, arginine, histidine and asparagine, can be funnelled indirectly through these reactions *via* conversion to glutamate or aspartate. Plasma levels of glutamine, alanine and asparagine all decrease following seawater exposure and may also be important energy sources for the liver. In other studies, Assem and Hanke (Assem and Hanke, 1983) reported increases in liver Asp-AT and Ala-AT activities in tilapia acclimating to seawater, whereas Jurss et al. (Jurss et al., 1983) found no change in either Asp-AT or Ala-AT activity in rainbow trout liver following seawater exposure. Interestingly, Aas-Hansen et al. (Aas-Hansen et al., 2005) report increased liver Ala-AT and Asp-AT activities during downstream migration of Arctic char prior to seawater exposure, suggesting the observed changes in amino acid metabolism are an important preparation for life in seawater.

The observation of increased amino acid metabolism following seawater acclimation is also evident in char red muscle as Ala-AT increased following seawater exposure. In white muscle, GDH activity increased by 27% following seawater exposure. Although white muscle GDH levels are low compared to other tissues, changes in its activity must be considered important because of the large size of the white muscle and its important role in amino acid metabolism.

Non-essential FAAs also act as compatible solutes for cell volume regulation in fish (King and Goldstein, 1983). This may explain the drop in plasma asparagine, serine, glutamine, glycine, alanine and taurine following seawater exposure as tissues may accumulate these amino acids to regulate intracellular osmolality and maintain cell volume. The 18% increase in white muscle protein concentration (due to dehydration) was accompanied by a 33% increase in total FAA levels. This increase in FAA concentration may offset some of the osmotic stress on white muscle cells and aid in their maintaining their cell volume. However, the osmotic difference only amounts to about 6 mmol l^{-1}, suggesting the role of FAAs in cell volume regulation is not that significant during acclimation to higher salinity in Arctic char. Several other studies have also shown increased total FAA levels in the muscle of rainbow trout (Kaushik and Luquet, 1979; Leray et al., 1981; Jurss et al., 1983) and tilapia (Venkatachari, 1974) acclimating to seawater. Interestingly, total FAA levels did not rise in the red muscle or gill to any significant degree and their overall protein content remained constant. This suggests a major difference in the role of red and white muscle during salinity acclimation, as the white muscle appears to be acting as a major supplier of amino acids for use in other tissues.

Taurine is suspected to be an important osmotic effector as it is found in such high concentrations in fish tissues. Taurine levels only increased slightly in white muscle after 96 h of seawater acclimation. Red muscle and RBC taurine levels remained stable while gill taurine content actually decreased (on a percentage basis) with seawater exposure. Taurine levels did not change in various chum salmon tissues (Sakaguchi et al., 1988) or eel muscle (Huggins and Colley, 1971) and appear to decrease in guppy muscle (Daikoku and Sakaguchi, 1983) during seawater acclimation. This is in contrast to other studies that show increased tissue taurine levels in flounder heart (Vislie and Fugelli, 1975; Fugelli and Zachariassen, 1976) and in rainbow trout intestinal mucosa (Auerswald et al., 1997) following seawater exposure. Taurine was by far the predominant amino acid found in gill, red muscle and RBCs. The role of taurine in fish is still unclear but several functions have been suggested (for a review, see Huxtable, 1992). Interestingly, the most common FAA found in white muscle was glycine. Similar observations have been made in sticklebacks, where the glycolytic axial muscle is also high in glycine and the oxidative pectoral fin muscle is high in taurine (Schaarschmidt et al., 1999), and in chum salmon (Sakaguchi et al., 1988) where white muscle taurine levels are very low.

The function of the gill during salinity acclimation may be reliant on the supply of several substrates from the blood. FAAs can be utilized as oxidative substrates are needed for protein synthesis and can act as compatible solutes for cell volume regulation. Seawater exposure induced significant changes in FAA levels in each of the tissues studied as well as enzymes involved in amino acid metabolism. The mobilization of amino acids to the blood for transport to tissues with more limited FAA stores may be very important during salinity acclimation. We have already discussed the relevant changes in the plasma but we should not underestimate the importance of RBCs for inter-organ transport of FAAs. RBC and plasma FAA pools are known to be equally important for amino acid transport between tissues in humans and other mammals (Felig et al., 1973; Proenza et al., 1994). The RBCs of seawater-acclimated char contained significantly higher levels of total essential FAAs, especially valine, isoleucine and leucine. This may indicate that RBCs are particularly important for transport of some essential FAAs. The levels of several FAAs are found in much higher concentrations in RBCs when compared to the plasma. Of note is the high level of glutamine found in both freshwater- and seawater-acclimated char. Glutamine is thought to be an important oxidative substrate in fish RBC metabolism and is effectively transported into RBCs (Nikinmaa and Tiihonen, 1994). High levels of RBC glutamine

may also serve as an important substrate for purine and pyrimidine synthesis, a mechanism for inter-organ glutamine transport or perhaps, the result of ammonia detoxification.

Lipid and carbohydrate metabolism

Levels of non-esterified fatty acid (NEFAs) in the plasma did not change during seawater acclimation nor did the activity of HOAD in any of the tissues examined suggesting that lipid metabolism was unchanged. This is in contrast to the findings of Aas-Hansen et al. (Aas-Hansen et al., 2005) who reported an increase in liver HOAD activity during the downstream migration of Arctic char (prior to moving into seawater), suggesting an enhanced capacity for oxidizing lipid at least in that tissue. We hypothesized that upon exposure of Arctic char to seawater there would be a greater utilization of lipid by certain tissues for oxidative fuels and membrane synthesis. If this is in fact occurring, levels of plasma NEFAs must remain stable due to increased mobilization of NEFAs to the circulation, thereby masking any increase in utilization.

Plasma glucose levels were also found to remain constant following seawater exposure, suggesting the reliance on this fuel source is unchanged in this study. Activities of enzymes involved in carbohydrate metabolism also did not change following seawater exposure in any of the tissues examined. Even though glucose is considered an important fuel source for fish gills (Mommsen, 1984), we did not find any evidence of any change in its utilization in Arctic char following seawater exposure. BY contrast, other studies have shown evidence of an increased reliance on glucose during seawater acclimation of Salmonids. Aas-Hansen et al. (Aas-Hansen et al., 2005) report decreased levels of liver glycogen and glucose in Arctic char migrating downstream, with a concomitant rise in liver PK and PEPCK activities and plasma glucose levels, suggesting an increased reliance on carbohydrate as a fuel source. Similarly, Soengas et al. (Soengas et al., 1995a; Soengas et al., 1995b) report decreased liver and gill glycogen levels, increased blood glucose levels and increased gill HK, PFK and PK activities in rainbow trout during seawater acclimation.

Other considerations

Several studies have suggested CK plays a pivotal role in regulating cellular ATP concentration through a phosphocreatine circuit (Blum et al., 1991; Kultz and Somero, 1995). The phosphocreatine circuit is very important in muscle during swimming where ATP must be replenished quickly, but it may also play an important role in other tissues with high-energy demands. The high need for ATP by gill Na^+,K^+-ATPase may be supplied by CK via a phosphocreatine circuit (Kultz and Somero, 1995). Studies have even shown that CK is localized in close structural association to several ATPases, including Na^+,K^+-ATPase (Blum et al., 1991; Krause and Jacobus, 1992; Korge et al., 1993). Although levels of CK do not change during seawater acclimation, CK activity is high in red and white muscle and moderate in gill tissues and may be adequate to supply sufficient ATP to meet cellular requirements.

Another important consideration that should be discussed is the influence of feeding on seawater acclimation. It is known that wild Arctic char experience a prolonged fasting during the winter months preceding their spring seaward migration (Boivin and Power, 1990). Therefore our experimental design in which the fish used were fed a commercial diet up to 2 days prior to the start of the experiment does not mimic the natural condition. Other studies have indeed shown that nutritional status does influence intermediary metabolism during seawater acclimation (Vijayan et al., 1996; Polakof et al., 2006). In fact, Vijayan et al. reported that food-deprived tilapia have greater difficulty regulating plasma chloride levels following seawater exposure, and Polakof et al. (Polakof et al., 2006) showed that an increase in gill Na^+,K^+-ATPase activity following seawater exposure is limited or abolished in food-deprived gilthead seabream. Therefore it is important to take nutritional status into account; thus our findings may be more valuable under an aquaculture scenario where feeding is maintained, than in predicting the specific changes occurring in wild migrating Arctic char.

In conclusion, Arctic char appear to upregulate some aspects of their intermediary metabolism during salinity acclimation. Significant increases in amino acid metabolism, as indicated by tissue and blood FAA levels and tissue enzyme activities, suggest, that following seawater exposure, these fish have an enhanced capacity for energy production from amino acids. This may offset the cost of osmoregulation during salinity acclimation. These early modifications to intermediary metabolism may be critical in determining whether Arctic char successfully acclimate to seawater.

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