The Journal of Experimental Biology 199, 2467–2475 (1996)
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JEB0461

FOOD-DEPRIVATION AFFECTS SEAWATER ACCLIMATION IN TILAPIA: HORMONAL AND METABOLIC CHANGES

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Accepted 30 July 1996

Summary

We tested the hypothesis that nutritional state affects seawater acclimation by transferring either fed or food-deprived (2 weeks) male tilapia (*Oreochromis mossambicus*) from fresh water to full-strength sea water. Food-deprivation resulted in a significant increase in plasma concentrations of Na^+, Cl^-, cortisol, glucose, total amino acid, glutamate, serine and alanine, and in hepatic pyruvate kinase (PK) and lactate dehydrogenase (LDH) activities, whereas the prolactin-188 to prolactin-177 ratio ($tPRL_{188}$:$tPRL_{177}$) and plasma prolactin-188 ($tPRL_{188}$), lactate, arginine and hepatic glycogen content and hepatic alanine aminotransferase (AlaAT) and 3-hydroxyacyl-Coenzyme A dehydrogenase (HOAD) activities were lower than in the fed group. Seawater transfer significantly increased the $tPRL_{188}$:$tPRL_{177}$ ratio and plasma concentrations of Na^+, Cl^-, K^+, growth hormone (GH), glucose, aspartate, tyrosine, alanine, methionine, phenylalanine, leucine, isoleucine and valine levels as well as gill Na^+/K^+-ATPase activity and hepatic PK and LDH activities, whereas plasma $tPRL_{177}$, $tPRL_{188}$, glycine and lysine concentrations were significantly lower than in fish retained in fresh water. There was a significant interaction between nutritional state and salinity that affected the $tPRL_{188}$:$tPRL_{177}$ ratio and plasma concentrations of Cl^-, GH, glucose, aspartate, tyrosine, serine, alanine, glycine, arginine and hepatic PK, LDH, AlaAT, aspartate aminotransferase, glutamate dehydrogenase and HOAD activities. These results, taken together, indicate that food-deprived fish did not regulate their plasma Cl^- levels, despite an enhancement of plasma hormonal and metabolic responses in sea water. Our study also suggests the possibility that plasma prolactin and essential amino acids may be playing an important role in the seawater acclimation process in tilapia.

Key words: *Oreochromis mossambicus*, tilapia, stress, metabolism, food-deprivation, cortisol, growth hormone, prolactin, gill Na^+/K^+-ATPase, ion regulation.

Introduction

Tilapia (*Oreochromis mossambicus*) are commonly found in brackish water in estuaries around the world and can tolerate wide ranges of salinity because of their efficient ion regulation mechanisms (for a review, see Evans, 1993). Gills play a major role in ion regulation, and a direct correlation between gill chloride cell density and Na^+/K^+-ATPase activity has been established in this species (Perry and Walsh, 1989), supporting a link between chloride cell function and ion regulation (for reviews, see Jurss and Bastrop, 1995; McCormick, 1995).

Fish gills are highly oxidative tissues even in fresh water (FW) (Mommsen, 1984*a*,*b*), and the oxygen requirement increases even further when fish are transferred to sea water (SW) because of the metabolic cost associated with ion regulation (Kirschner, 1993). The energy requirement of the gills is thought to be maintained by oxidation of glucose and lactate obtained from the circulation (Mommsen, 1984*a*,*b*; Perry and Walsh, 1989). As the liver is the main site of glucose production in fish (see Suarez and Mommsen, 1987), it is likely that liver metabolism is enhanced in seawater-adapted fish, thereby providing energy substrates for gill metabolism. However, very little is known about the reorganization of liver metabolism and the mobilization of substrates during SW acclimation in fish.

Changes in plasma concentrations of several hormones, including cortisol, growth hormone and prolactin, have been associated with the process of ion regulation and, consequently with SW acclimation in fish (for reviews, see Wendelaar Bonga, 1993; McCormick, 1995). In addition, these hormones have been shown to play a role in the mobilization of energy substrates in fish (see Sheridan, 1986; Leung *et al.* 1991;

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Vijayan *et al.* 1996*a*,*b*). Consequently, some of the effects of these hormones on SW acclimation may be mediated indirectly by providing substrates for gill metabolism. However, no study has addressed the effects of prior nutritional state on plasma hormonal and metabolite profiles, hepatic metabolic status and the associated seawater acclimation process in fish.

In the present study, we deprived male tilapia of food for 2 weeks with the specific objectives of (i) examining plasma Na^+, Cl^- and K^+ concentrations and gill Na^+/K^+-ATPase activity in FW and full-strength SW as a measure of ionic regulation in these animals; (ii) assessing whether food-deprivation modulates plasma cortisol, growth hormone (GH), prolactin-177 ($tPRL_{177}$) and prolactin-188 ($tPRL_{188}$) concentrations in FW and full-strength SW; and (iii) examining plasma glucose, lactate, total and free amino acid concentrations and liver glycogen content, as well as several liver metabolic enzyme activities, in order to shed light on the substrate mobilization associated with SW acclimation in this species. Tilapia (*Oreochromis mossambicus*) was used for this study because the mechanisms of ion regulation, including the role of hormones, have been extensively studied in this species (for reviews, see Evans, 1993; McCormick, 1995). Furthermore, it is known that tilapia can acclimate to full-strength SW, as indicated by plasma ion concentrations and Na^+/K^+-ATPase activity, when fish are held at a salinity of 12‰ for 24 h prior to increasing the salinity to 34‰ (Hwang *et al.* 1989).

Materials and methods

Animals

Male tilapia (*Oreochromis mossambicus* Peters) were maintained in outdoor tanks with flowing fresh water under a natural photoperiod (14 h:10 h light: dark) at 25 °C for at least a month prior to the start of the experiment. The fish were separated into two groups; one group was fed Purina trout chow once daily to satiety, whereas the other group was deprived of food for 2 weeks prior to experimentation.

Experimental protocol

Groups of ten tilapia from either the fed group or the food-deprived group were lightly anaesthetized with buffered MS222 (10 p.p.m.; to prevent the stress response associated with handling) and transferred to either FW or brackish water (12‰; obtained by mixing FW and SW). Food-deprived tilapia had a significantly lower body mass (152±8 g) than fed fish (216±11 g; means ± S.E.M., N=10). The FW tanks for both the fed and food-deprived groups acted as the control, while FW inflow was shut off in the brackish-water tanks after 24 h and the salinity gradually rose to 34‰ within 24 h. These fish were held at 34‰ salinity for another 24 h prior to sampling. This protocol has previously been shown to acclimate tilapia to full-strength SW within 3 days (Hwang *et al.* 1989). Both groups were deprived of food during the experiment.

Three days after transfer, eight fish from each group were quickly sampled (at 10:00 h) after giving them an overdose of buffered MS222 (400 p.p.m.). Blood was removed by caudal puncture and the plasma obtained after centrifugation (10 000 revs min^{-1} for 2 min) was stored frozen at −80 °C for hormone and metabolite analysis (see below). The liver was rapidly dissected out and frozen between blocks of dry ice and was then stored at −80 °C until glycogen content and the activities of several metabolic enzymes (see below) could be measured. Gill filaments were removed from the second gill arch, placed in 1 ml of ice-cold SEI buffer (0.3 mol l^{-1} sucrose, 0.02 mol l^{-1} sodium EDTA, 0.1 mol l^{-1} imidazole, pH 7.1) and stored at −80 °C until assayed for Na^+/K^+-ATPase activity (see below).

Plasma cortisol, growth hormone, $tPRL_{177}$ and $tPRL_{188}$ concentrations

Plasma cortisol concentration was measured using a commercially available radioimmunoassay kit (Incstar Corp., Stillwater, Minnesota, USA) according to Iwama *et al.* (1989), while plasma GH, $tPRL_{177}$ and $tPRL_{188}$ concentrations were measured using a homologous radioimmunoassay according to Ayson *et al.* (1993).

Plasma metabolite and ion concentrations

Plasma glucose and lactate concentrations were measured colorimetrically using commercially available kits (Sigma, St Louis, Missouri, USA). Plasma Na^+ and K^+ concentrations were measured using an ion chromatograph (Shimadzu, model HIC-6A, Shimadzu Corp., Kyoto, Japan), while plasma Cl^- concentration was determined by coulometric titration (Haale Buchler Instruments, digital chloridometer). The free amino acid concentration in plasma was measured after reverse-phase high-performance liquid chromatography (HPLC) using pre-column derivatization and fluorometric detection (Puchata *et al.* 1994).

Liver glycogen content and enzyme activities

The glycogen content of the liver was measured after amyloglucosidase hydrolysis according to Keppler and Decker (1974). Measurements of hepatic phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), lactate dehydrogenase (LDH), alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), glutamate dehydrogenase (GDH), 3-hydroxyacyl-Coenzyme A dehydrogenase (HOAD) and malate dehydrogenase (MDH) activities were carried out using a homogenization buffer according to Henriksson *et al.* (1986). The enzyme activities were measured according to Mommsen *et al.* (1980) at 22 °C by continuous spectrophotometry (at 340 nm) on a microplate reader (ThermoMax, Molecular Devices Corp., Menlo Park, California, USA). The activities are expressed as μmoles of substrate consumed or product liberated per minute per gram wet mass of liver.

Gill Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity (μmoles of ADP liberated per hour per milligram protein) in crude gill homogenates was

determined at 25 °C using the microplate reader mentioned above according to McCormick (1993). The protein content in the gill homogenate was determined using the bicinchoninic acid procedure (Smith *et al.* 1985).

Statistical analyses

The data were analyzed using two-way analysis of variance (ANOVA); where *P* values were significant ($P<0.05$), multiple comparisons were carried out using the Student–Newman–Keuls test. Log-transformed data were used wherever necessary to satisfy homogeneity of variance, although non-transformed data are shown in the tables and figures.

Results

Plasma ion concentrations and gill Na^+/K^+-ATPase activity

Food-deprivation resulted in significantly higher plasma Na^+ and Cl^- concentrations, but had no significant effect on plasma K^+ concentration or gill Na^+/K^+-ATPase activity in tilapia (Fig. 1A–D; $P<0.05$, two-way ANOVA, $N=7$). Seawater transfer caused significantly higher plasma Na^+, Cl^- and K^+ concentrations and gill Na^+/K^+-ATPase activity regardless of the nutritional state of the animal (Fig. 1A–D; two-way ANOVA, $P<0.05$). There was a significant interaction between nutritional state and seawater transfer only for plasma Cl^- concentration, but not for plasma Na^+ and K^+ levels or gill Na^+/K^+-ATPase activity; plasma Cl^- concentration in the food-deprived fish in SW was significantly higher than that in all the other groups (Fig. 1B).

Plasma hormonal changes

Food-deprivation significantly increased plasma concentrations of cortisol, but had no significant effect on plasma GH or $tPRL_{177}$ concentrations, whereas plasma $tPRL_{188}$ concentration and the $tPRL_{188}$:$tPRL_{177}$ ratio were significantly lower in the food-deprived fish compared with the fed fish (Figs 2A,B, 3A–C; two-way ANOVA, $P<0.05$). Seawater transfer resulted in significantly higher plasma concentrations of GH and significantly lower concentrations of $tPRL_{177}$ and $tPRL_{188}$; the $tPRL_{188}$: $tPRL_{177}$ ratio was significantly higher in the SW fish compared with the FW fish (Figs 2B, 3A–C; two-way ANOVA, $P<0.05$). There was no significant effect of either SW transfer or the interaction of salinity and nutritional state on plasma cortisol concentration in the present study (Fig. 2A; two-way ANOVA, $P<0.05$). There were significant interactions between nutritional state and seawater transfer on plasma GH concentration, but not on plasma $tPRL_{177}$ or $tPRL_{188}$ levels, GH levels in the food-

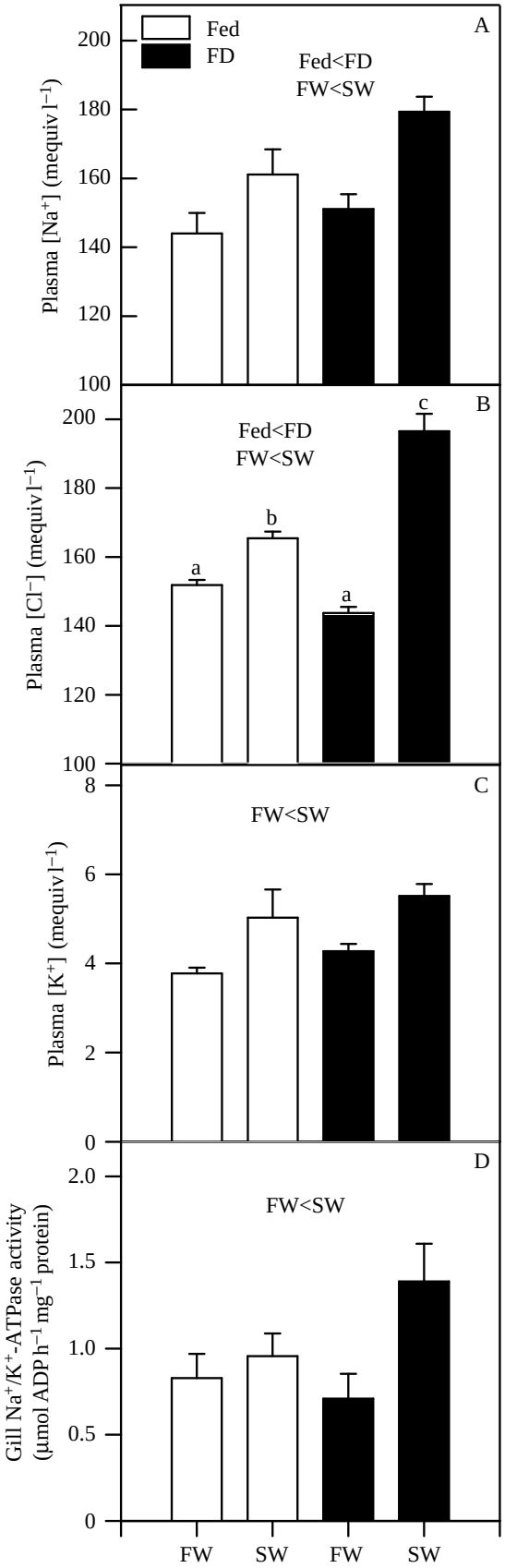

Fig. 1. Plasma Na^+ (A), Cl^- (B) and K^+ concentrations (C) and gill Na^+/K^+-ATPase activity (D) in *Oreochromis mossambicus* that had been either fed or food-deprived (FD) for 2 weeks and transferred to either fresh water (FW) or sea water (SW) and sampled 3 days later. Values represent means + S.E.M. ($N=7$ fish). Treatments that resulted in significantly different values ($P<0.05$, two-way ANOVA) are noted above each histogram; a significant interaction is shown by letters above bars; values with the same letters are not significantly different ($P<0.05$, two-way ANOVA).

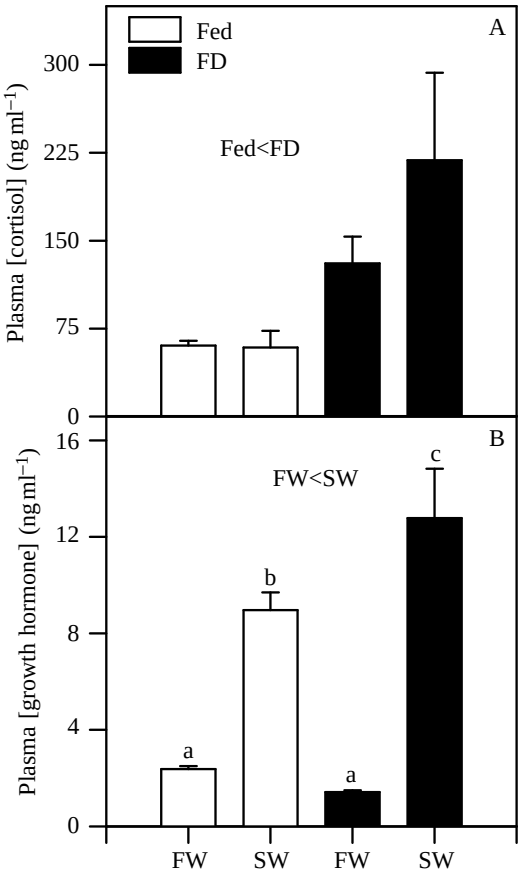

Fig. 2. Plasma cortisol (A) and growth hormone (GH) (B) concentration in *Oreochromis mossambicus* that had been either fed or food-deprived (FD) for 2 weeks and transferred to either FW or SW and sampled 3 days later. Values represent means + S.E.M. (*N*=7 for cortisol; *N*=5 for GH). Treatments resulting in significantly different values ($P<0.05$, two-way ANOVA) are noted above each histogram; a significant interaction is shown by letters above bars; values with the same letters are not significantly different ($P<0.05$, two-way ANOVA).

deprived fish in SW being significantly higher than in all the other groups (Fig. 2B). The $tPRL_{188}$:$tPRL_{177}$ ratio, however, was significantly higher in SW than in FW in the fed fish; this effect was abolished in the food-deprived fish (Fig. 3C).

Plasma metabolite, liver glycogen and hepatic enzyme activities

Food-deprivation resulted in significantly higher plasma glucose and total amino acid concentrations, while plasma lactate and liver glycogen concentrations were significantly lower compared with the fed fish (Fig. 4A–D; two-way ANOVA, $P<0.05$). Seawater transfer resulted in significantly higher plasma glucose concentration, but had no significant effect on plasma lactate, total amino acid concentrations or liver glycogen content compared with the FW fish (Fig. 4A–D; two-way ANOVA, $P<0.05$). Plasma glucose concentration was significantly higher in the food-deprived fish in SW compared with all the other groups, whereas there were no interactive

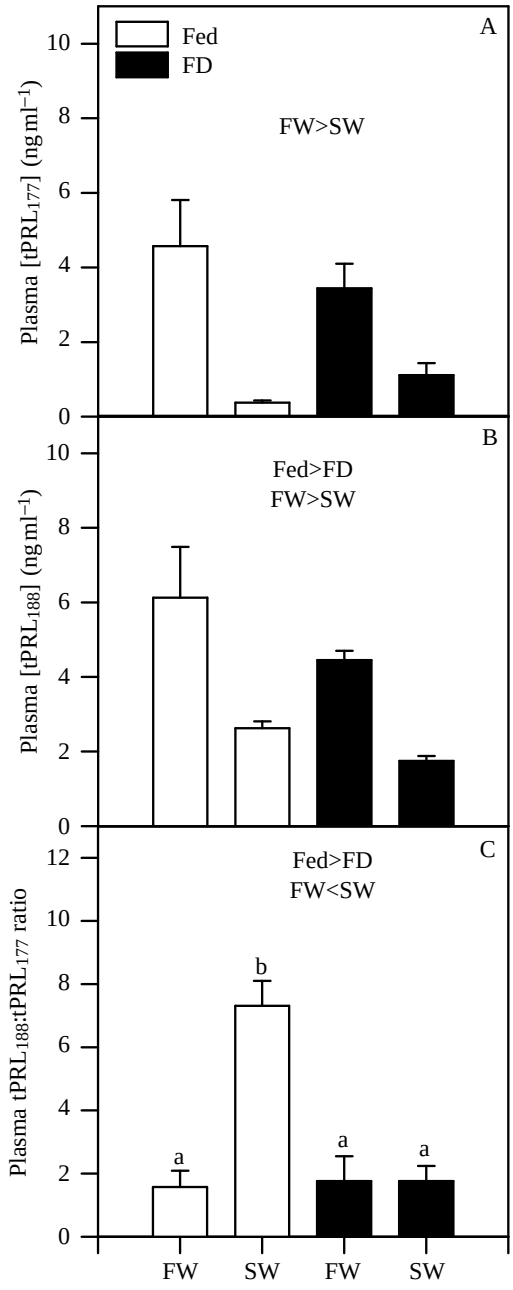

Fig. 3. Plasma prolactin 177 ($tPRL_{177}$; A) and prolactin 188 ($tPRL_{188}$; B) concentrations and plasma prolactin 188:prolactin 177 ratio ($tPRL_{188}$:$tPRL_{177}$; C) in *Oreochromis mossambicus* that had been either fed or food-deprived (FD) for 2 weeks and transferred to either FW or SW and sampled 3 days later. Values represent means + S.E.M. (*N*=4 for $tPRL_{177}$; *N*=7 for $tPRL_{188}$). Treatments resulting in significantly different values ($P<0.05$, two-way ANOVA) are noted above each histogram; a significant interaction is shown by letters above bars; values with the same letters are not significantly different ($P<0.05$, two-way ANOVA).

effects between nutritional state and salinity for lactate, total amino acids or liver glycogen content.

With the exception of histidine and threonine, the concentration of free amino acids in the plasma showed significant treatment effects (Table 1). Food-deprivation alone

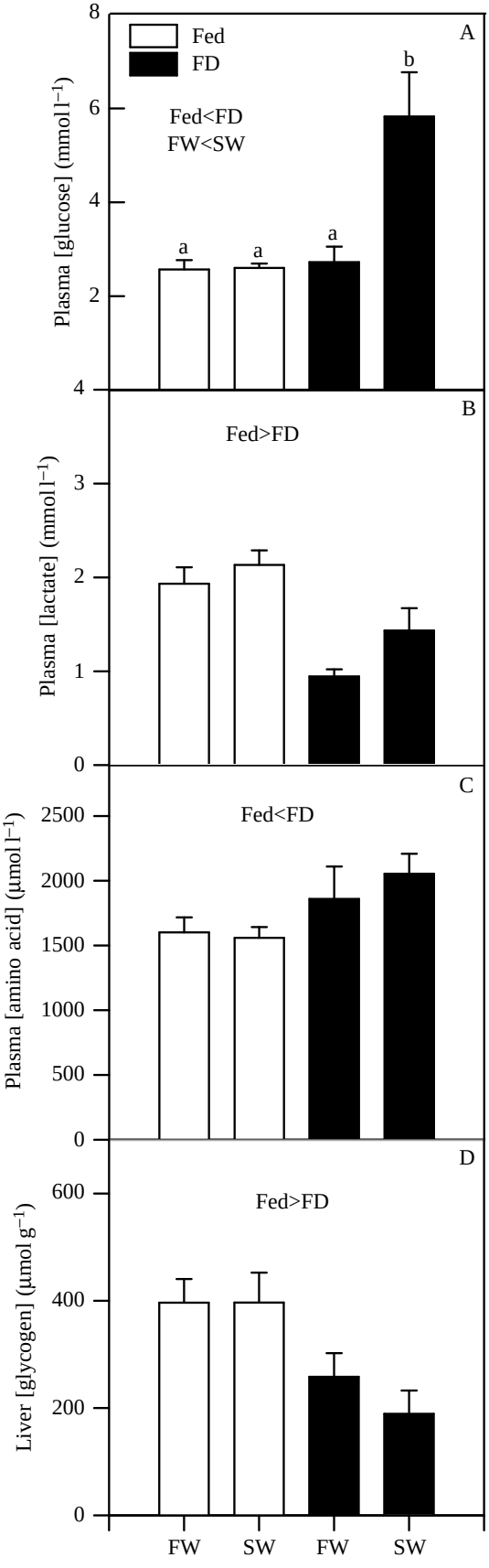

Fig. 4. Plasma glucose (A), lactate (B) and total amino acid (C) concentrations and liver glycogen content (D) in *Oreochromis mossambicus* that had been either fed or food-deprived (FD) for 2 weeks and transferred to either FW or SW and sampled 3 days later. Values represent means + S.E.M. (N=6–8 fish). Treatments resulting in significantly different values (P<0.05, two-way ANOVA) are noted above each histogram; a significant interaction is shown by letters above bars; values with the same letters are not significantly different (P<0.05, two-way ANOVA).

(regardless of the medium salinity) significantly increased plasma glutamate, serine and alanine concentrations, whereas arginine concentration was significantly lower compared with the fed fish; none of the other free amino acids showed any significant effects of food-deprivation compared with fed fish (Table 1). Seawater transfer (regardless of the nutritional state) significantly increased plasma aspartate, tyrosine, alanine, methionine, phenylalanine, leucine, isoleucine and valine concentrations, while plasma glycine and lysine concentrations were significantly lower compared with the FW fish, regardless of the nutritional state of the animal (Table 1). Food-deprivation, however, resulted in significantly higher concentrations of aspartate, tyrosine, serine and alanine, and significantly lower concentration of arginine in SW compared with the other groups (Table 1; interactive effects). Plasma glycine concentration was significantly lower in SW in the fed group and not in the food-deprived group, while none of the other free amino acids showed any interactive effects (Table 1).

There were no significant treatment effects on hepatic PEPCK or MDH activities in the present study (Table 2). Food-deprivation (regardless of the medium salinity) significantly increased hepatic PK and LDH activities, whereas hepatic AlaAT and HOAD activities were significantly lower compared with the fed group (Table 2). Seawater transfer (regardless of the nutritional state of the animal) resulted in significantly higher hepatic PK and LDH activities; the high values in SW were due to the significantly higher activity of these two enzymes in the food-deprived fish (Table 2; interactive effects). There was no significant effect of either food-deprivation or SW transfer on any of the other hepatic enzyme activities (Table 2). Hepatic AlaAT, AspAT, GDH and HOAD activities in FW were significantly lower in the food-deprived fish compared with the fed fish. Seawater transfer, however, resulted in significantly higher hepatic AlaAT, AspAT and GDH activities only in the food-deprived fish, while the fed fish showed either no change (AlaAT, AspAT) or had significantly lower hepatic GDH and HOAD activities (Table 2).

Discussion

Ion regulation

Our results clearly demonstrate that food-deprivation affects SW acclimation processes in tilapia. Regulation of plasma Na^+ and Cl^- concentration is important for successful acclimation

Table 1. *Plasma free amino acid concentrations in* Oreochromis mossambicus *that had been either fed or food-deprived for 2 weeks and transferred to either fresh water (FW) or sea water (SW) and sampled 3 days later*

	Fed		Food-deprived		
Amino acid	FW	SW	FW	SW	$P<0.05$*
Aspartate	3±0.49[a,†]	ND	9.8±2.9[b,†]	16.3±2.6[c]	FW<SW
Tyrosine	52.8±3.6[a]	46.6±1.9[a]	38.1±2.8[b]	63.3±9.7[c]	FW<SW
Glutamate	10±1.7	7.3±1.7	14.9±3.3	17±3.0	Fed<FD
Serine	64.5±7.0[a,b]	45±2.6[a]	54.3±4.8[a]	84.5±16.3[b]	Fed<FD
Alanine	128±12[a]	149±4[a]	144±11[a]	335±66[b]	Fed<FD; FW<SW
Glycine	405±49[a]	210±12[b]	393±77[a]	386±72[a]	FW>SW
Arginine	78.4±3.2[a,b]	87.1±7.5[a]	64.0±5.9[b]	33.7±5.4[c]	Fed>FD
Methionine	22.1±.8	43.4±3.4	26.9±3.9	34.5±6.8	FW<SW
Phenylalanine	54.5±2.5	81±2.3	58.7±5.7	87.2±13.5	FW<SW
Leucine	81.6±10.1	171±16	88.3±7.6	212±41	FW<SW
Isoleucine	32.5±6.2	84.4±6.9	34.5±3.9	99±16.8	FW<SW
Valine	82.5±9.4	152±9	88.7±9	185±34	FW<SW
Lysine	166±13	163±15	218±37	129±23	FW>SW
Histidine	302±49	212±29	388±83	258±41	
Threonine	120±5	106±7	138±16	115±19	

Amino acid concentrations (in $\mu mol\,l^{-1}$) represent means ± S.E.M. (N=6–8 fish).

†3–4 values below detectable levels; ND, values below detectable levels.

*Significant treatment effects and the interaction are shown as superscripts (two-way ANOVA); values with the same superscripts are not significantly different between treatments ($P<0.05$, two-way ANOVA).

Table 2. *Liver enzyme activities in* Oreochromis mossambicus *that had been either fed or food-deprived for 2 weeks and transferred to either fresh water (FW) or sea water (SW) and sampled 3 days later*

	Fed		Food-deprived		
Enzymes	FW	SW	FW	SW	$P<0.05$*
PEPCK	0.39±0.07	0.63±0.21	0.58±0.14	0.39±0.1	
PK	2.6±0.15[a]	2.6±0.2[a]	2.4±0.18[a]	3.7±0.25[b]	Fed<FD; FW<SW
LDH	17.8±1.6[a,b]	21.3±3.3[a]	12.8±1.1[b]	43.5±5.4[c]	Fed<FD; FW<SW
AlaAT	32.6±2[a]	30.5±1.1[a]	17.1±1.8[b]	23.2±1.8[c]	Fed>FD
AspAT	62.1±3.8[a]	54.4±2.2[a,b]	44.3±5[b]	60.4±4[a]	
GDH	66.2±4.2[a]	49.5±1.9[b]	45.8±5.1[b]	55.4±3.8[a,b]	
HOAD	4.3±0.25[a]	3.6±0.17[b]	2.9±0.21[c]	3.3±0.14[b,c]	Fed>FD
MDH	118±4.3	112±3	107±4.2	106±5	

Values represent means ± S.E.M. (N=8 fish).

*Significant treatment effects and the interaction are shown as superscripts (two-way ANOVA); values with the same superscripts are not significantly different ($P<0.05$, two-way ANOVA).

Phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), lactate dehydrogenase (LDH), alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), glutamate dehydrogenase (GDH), 3-hydroacyl-Coenzyme A dehydrogenase (HOAD) and malate dehydrogenase (MDH).

Enzyme activities are expressed as $\mu mol\,min^{-1}\,g^{-1}$ wet $mass^{-1}$.

to SW, and the plasma ion concentrations in the fed fish (Fig. 1A–C) were within the ranges reported for SW-acclimated *O. mossambicus* (Hwang *et al.* 1989; Yada *et al.* 1994). The higher plasma Cl^- concentration in the food-deprived fish in SW may be due to several factors including a higher drinking rate, increased branchial permeability and/or decreased ion excretion. Previous studies, however, have shown that food-deprivation decreased the drinking rate in Atlantic salmon (*Salmo salar*) (Usher *et al.* 1988) and also the gill permeability to ions in rainbow trout (*Oncorhynchus mykiss*) (Nance *et al.* 1987), thereby implicating modulations in Cl^- excretion processes as the possible cause for the ionic imbalance. Although body size has been shown to affect salinity-tolerance in fish, all the food-deprived fish in this study were larger than the size at which fish have been shown to reach a maximum salinity tolerance (Watanabe *et al.* 1990), suggesting that the changes in body mass (30 % lower in the food-deprived fish) were probably not the cause of the impaired Cl^- regulation.

NaCl excretion in SW is thought to be carried out

exclusively by the chloride cells, and the present model for ion regulation underscores the importance of Na^+/K^+-ATPase activity (or the sodium pump) for the excretion of Na^+ and Cl^- (for a review, see Jurss and Bastrop, 1995). The higher gill Na^+/K^+-ATPase activity in the SW fish, however, argues against the inactivity of the sodium pump as a likely cause of the poor Cl^- regulation in food-deprived fish. Previous studies have shown that food-deprivation decreased both the size and numbers of chloride cells in SW-acclimated *Oreochromis mossambicus* (Kultz and Jurss, 1991). In our study, the plasma Cl^- concentration in the food-deprived fish in SW was higher than the plasma Na^+ concentration, which also suggests that food-deprivation may be modifying the transepithelial branchial Cl^- transport mechanism(s). Further work is necessary before this process can be clearly explained.

Hormonal changes

Plasma cortisol is considered to be an important hormone for SW acclimation because cortisol has been shown to increase both gill chloride cell proliferation and Na^+/K^+-ATPase activity in fish (for a review, see McCormick, 1995). In the present study, the absence of an increase in plasma cortisol concentration in the fed fish in SW is not surprising as previous studies have shown that plasma cortisol concentration is transient after SW entry, peaking at about 6 h, after which it drops to baseline levels (Assem and Hanke, 1981), perhaps due to the faster clearance of cortisol in SW (Redding *et al.* 1984). As our fish were in SW for 3 days, it is likely that plasma cortisol values had returned to the FW levels in these fish. The transient increase in plasma cortisol concentration is thought to assist in the SW acclimation process directly by enhancing the ion regulation process and/or indirectly by providing energy substrates for ion regulation (Assem and Hanke, 1981).

Plasma cortisol concentration was higher in the food-deprived fish and appeared to increase in SW (Fig. 2A), suggesting that these fish were subjected to an osmotic stress as shown by the higher plasma Cl^- concentration (Fig. 1B). The higher plasma cortisol concentration may be directly assisting in maintaining the gill Na^+/K^+-ATPase activity (McCormick, 1995) and/or indirectly assisting in the ion regulation process by mobilizing energy substrates for gill metabolism (Assem and Hanke, 1981; Vijayan *et al.* 1996*a*,*b*; see below).

The increase in plasma GH and decrease in $tPRL_{177}$ and $tPRL_{188}$ concentrations in SW are consistent with those reported in the literature for *O. mossambicus* (Yada *et al.* 1994) and further underscore the role of these hormones in ion regulation in fish (see Wendelaar Bonga, 1993; McCormick, 1995). The higher growth hormone response in the food-deprived fish in sea water, where there was evidence of impaired ion regulation, clearly supports the proposal that GH plays a role in SW adaptation in the tilapia (Yada *et al.* 1994).

Prolactin plays an important role in the ion regulation process in FW, but the role of this hormone in the acclimation process to SW is not clear. However, studies have shown that SW-acclimated fish injected with prolactin have high plasma Na^+ levels, indicating that prolactin attenuates Na^+ efflux in these animals (see McCormick, 1995). Thus, the decrease in plasma prolactin concentrations in SW (Fig. 3A,B) is probably required for the efficient regulation of plasma Na^+ concentration in SW. The significantly lower $tPRL_{188}$, but not $tPRL_{177}$, level in food-deprived compared with fed fish suggests that nutritional state may differentially modulate the plasma concentration of the two prolactins. Our results show that the plasma $tPRL_{188}$:$tPRL_{177}$ ratio in the fed fish increases in SW (Fig. 3C), and this is likely to be due to the higher release of $tPRL_{188}$ relative to $tPRL_{177}$ in SW. Interestingly, the abolition of this increased $tPRL_{188}$:$tPRL_{177}$ ratio in the food-deprived fish coincides with impaired Cl^- regulation in that group, raising the possibility that the ratio of the two prolactins may be related to the SW-acclimation process in tilapia. The changes in plasma prolactin concentration and $tPRL_{188}$:$tPRL_{177}$ ratio, however, support the contention that $tPRL_{188}$ and $tPRL_{177}$ are differentially regulated, as proposed earlier from studies either *in vivo* (Yada *et al.* 1994) or *in vitro* using pituitary preparations (Yoshikawa-Ebesu *et al.* 1995).

Changes in metabolite levels

The plasma metabolite concentrations (Fig. 4A–C; Table 1), liver glycogen content (Fig. 4D) and liver enzyme activities (Table 2) in the fed fish indicate that there was no excessive substrate mobilization for energy purposes in SW fish. The decreased hepatic GDH and HOAD activities in the fed fish in SW suggest a lower hepatic potential for amino acid and fatty acid catabolism, implying a lower energy demand. This hepatic metabolic organization is consistent with the observation that the metabolic rate of long-term SW-acclimated tilapia is lower than that of FW fish (Ron *et al.* 1995) and that, as a result, tilapia may have higher growth rates in SW than in FW (Kultz and Jurss, 1991; Kuwaye *et al.* 1993; Ron *et al.* 1995).

Food-deprivation decreased the hepatic capacity for amino acid (AlaAT, AspAT, GDH) and fatty acid (HOAD) catabolism in FW (Table 2), and this may represent a general decrease in hepatic metabolism as shown before in fasted fish (Foster and Moon, 1991). The maintenance of plasma glucose concentration under these circumstances may have been achieved by increased glycogenolysis and/or gluconeogenesis as shown by lower liver glycogen and plasma lactate concentrations, and higher levels of plasma total amino acids in food-deprived compared with fed fish (Fig. 4B–D). In support of this argument, previous studies have shown that amino acids and lactate are preferred substrates for hepatocyte gluconeogenesis in fish (Suarez and Mommsen, 1987).

The appearance of a lower hepatic potential for protein catabolism in food-deprived fish in FW is certainly reduced in SW, implying an increased energy demand. The higher plasma free amino acid concentrations, especially of alanine and serine (important energy substrates in fish; French *et al.* 1981, 1983), with the concomitant increase in hepatic AlaAT and AspAT activities suggest peripheral proteolysis and amino acid catabolism in food-deprived fish in SW. A recent study has shown that cortisol mobilizes amino acids and increases amino

acid catabolism in *O. mossambicus* (Vijayan *et al.* 1996*b*). That observation coupled with a higher plasma cortisol concentration in food-deprived fish in sea water in the present study suggests a significant role for cortisol in the amino acid mobilization process (Fig. 2A; Tables 1, 2).

The C3 precursors arising from amino acid breakdown may be channelled to gluconeogenesis in fish (Vijayan *et al.* 1994) and may partly account for the higher glucose concentration in the food-deprived fish in SW (Fig. 4A), perhaps mediated by cortisol (Vijayan *et al.* 1996*b*). The elevated plasma glucose concentration of food-deprived fish in SW may also have been maintained by glycogenolysis, although the absence of a further decrease in hepatic glycogen content in SW could be due to the concurrent channelling of C3 precursors for glycogen repletion, as suggested by Pereira *et al.* (1995). This glucose may be utilized to fuel gill metabolism, including the energy required for ion regulation, since previous studies have shown glucose to be the preferred oxidative substrate for gill metabolism (Mommsen, 1984*a*,*b*; Perry and Walsh, 1989). The reduction of liver glycogen content in the food-deprived fish together with the elevation of PK and LDH activity, may imply a higher hepatic glycolytic potential in these animals in order to fuel liver metabolism (Vijayan *et al.* 1996*b*).

It is interesting that the plasma concentrations of several essential amino acid (methionine, phenylalanine, leucine, isoleucine and valine) increased in the SW fish regardless of the nutritional state of the animal (Table 1). The significance of this increase in SW is not known, although we can speculate that some of these amino acids may play a role in the SW acclimation process in fish. These essential amino acids are required for the synthesis of peptides and proteins and, therefore, their availability may regulate the synthesis of hormones that are important in the ion regulation process. In support of this argument, *in vitro* studies using tilapia pituitary gland have shown that both GH- and prolactin-secreting cells respond to variations in amino acid levels in the medium (Rodgers *et al.* 1992). Furthermore, mammalian studies have shown that branched-chain amino acids (isoleucine, leucine and valine) increase plasma GH levels (Stewart *et al.* 1984). Since levels of these three amino acids were higher in the SW fish (which had higher GH concentration), it is possible that a mechanism similar to that operating in mammals may regulate the secretion of GH in the tilapia.

In conclusion, our results support the possibility that food-deprivation affects the SW acclimation process by impairing Cl^- regulation in *O. mossambicus*. This effect does not appear to be due to the inactivation of gill Na^+/K^+-ATPase since the activity of this enzyme was higher in SW than in FW fish. Furthermore, the impairment was not due to an inability to release cortisol or GH as levels of both these hormones were also higher in the food-deprived fish. Furthermore, amino acid and glycogen mobilization and plasma glucose concentration were higher in food-deprived fish, clearly indicating that energy substrate availability was not a factor in the poor Cl^- regulation. We conclude either that the Na^+/K^+-ATPase activity was not functioning to its full capacity or that the Na^+/K^+-ATPase levels achieved were not sufficient to maintain ion balance. It is also possible that some other factor(s) independent of this ion transporter contributed significantly to the impaired Cl^- excretion. Kultz and Jurss (1991) have shown that food-deprivation decreases the size and number of chloride cells in tilapia. This, together with our finding that plasma Cl^- concentration is elevated relative to plasma Na^+ concentration in the food-deprived fish, suggests that nutritional state can modulate Cl^- transport in tilapia. We hypothesize that food-deprivation, directly or indirectly, affects transepithelial branchial Cl^- transport mechanism(s). The results also suggest that plasma prolactin and essential amino acids may play an important role in the SW acclimation process in tilapia. Future studies will examine the possible interaction(s) between nutritional states and hormones, especially GH and insulin-like growth factors, on osmoregulation in the tilapia.

The authors gratefully acknowledge the help of Benny Ron and others at the Hawaiian Institute of Marine Biology, Coconut Island, Hawaii. We extend our thanks to Shannon Balfry and Ellen Teng for help during the experiment and Cristina Pereira for her assistance with enzyme measurements. The study was supported by an NSERC Industrial Post-doctoral Fellowship to M.M.V. and operating grants from NSERC and NSERC/CBDN Centre of Excellence to G.K.I.

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