J Comp Physiol B (2006) 176: 441–452
DOI 10.1007/s00360-006-0065-z

ORIGINAL PAPER

**Sergio Polakof · Francisco J. Arjona
Susana Sangiao-Alvarellos · María P. Martín del Río
Juan M. Mancera · José L. Soengas**

Food deprivation alters osmoregulatory and metabolic responses to salinity acclimation in gilthead sea bream *Sparus auratus*

Received: 15 September 2005 / Revised: 30 November 2005 / Accepted: 23 December 2005 / Published online: 24 January 2006
© Springer-Verlag 2006

Abstract The influence of acclimation to different environmental salinities (low salinity water, LSW; seawater, SW; and hyper saline water, HSW) and feeding conditions (fed and food deprived) for 14 days was assessed on osmoregulation and energy metabolism of several tissues of gilthead sea bream *Sparus auratus*. Fish were randomly assigned to one of six treatments: fed fish in LSW, SW, and HSW, and food-deprived fish in LSW, SW, and HSW. After 14 days, plasma, liver, gills, kidney and brain were taken for the assessment of plasma osmolality, plasma cortisol, metabolites and the activity of several enzymes involved in energy metabolism. Food deprivation abolished or attenuated the increase in gill Na^+,K^+-ATPase activity observed in LSW- and HSW-acclimated fish, respectively. In addition, a linear relationship between renal Na^+,K^+-ATPase activity and environmental salinity was observed after food deprivation, but values decreased with respect to fed fish. Food-deprived fish acclimated to extreme salinities increased production of glucose through hepatic gluconeogenesis, and the glucose produced was apparently exported to other tissues and served to sustain plasma glucose levels. Salinity acclimation to extreme salinities enhanced activity of osmoregulatory organs, which is probably sustained by higher glucose use in fed fish but by increased use of other fuels, such as lactate and amino acids in food-deprived fish.

Communicated by I.D. Hume

S. Polakof · S. Sangiao-Alvarellos · J. L. Soengas (✉)
Laboratorio de Fisioloxía Animal, Facultade de Ciencias do Mar, Edificio de Ciencias Experimentais, Universidade de Vigo, 36310 Vigo, Spain
E-mail: jsoengas@uvigo.es
Tel.: +34-986-812564
Fax: +34-986-812556

F. J. Arjona · M. P. Martín del Río · J. M. Mancera
Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain

Keywords Osmotic acclimation · Food deprivation · Gilthead sea bream · Energy metabolism

Abbreviations Ala-AT: Alanine aminotransferase (EC. 2.6.1.2) · Asp-AT: Aspartate aminotransferase (EC. 2.6.1.1) · ELISA: Indirect enzyme immunoassay · HK: Hexokinase (EC. 2.7.1.11) · FBPase: Fructose 1,6-bisphosphatase (EC. 3.1.3.11) · FW: Freshwater · G3PDH: Glyceraldehyde 3-phosphate dehydrogenase (EC. 1.1.1.8) · G6Pase: Glucose 6-phosphatase (EC. 3.1.3.9) · G6PDH: Glucose 6-phosphate dehydrogenase (EC. 1.1.1.49) · GDH: Glutamate dehydrogenase (EC. 1.4.1.2) · GK: Glucokinase (EC. 2.7.1.2) · GPase: Glycogen phosphorylase (EC. 2.4.1.1) · HOAD: 3-Hydroxiacil-CoA-dehydrogenase (EC. 1.1.1.35) · HSW: High salinity water · LDH-O: Lactate dehydrogenase-oxidase (EC. 1.1.1.27) · LSW: Low salinity water · PFK: 6-Phosphofructo 1-kinase (EC. 2.7.1.11) · PK: Pyruvate kinase (EC. 2.7.1.40) · SEI: Sucrose-EDTA-imidazole · SW: Seawater

Introduction

Salinity acclimation of euryhaline fish is known to induce changes in osmoregulatory parameters. Gilthead sea bream (*Sparus auratus*) is an euryhaline species capable of living in different environmental salinities (Chervinski 1984). This species showed a U-shaped relationship between gill Na^+,K^+-ATPase activity and environmental salinity and a linear relationship for renal Na^+,K^+-ATPase activity (Sangiao-Alvarellos et al. 2003, 2005c; Laiz-Carrión et al. 2005a, b). The acclimation to different environmental salinities requires a metabolic reorganization to meet the increased energy demands associated with exposure to the new environmental salinity. Accordingly, euryhaline fish, including gilthead sea bream, showed several metabolic changes to compensate for these salinity changes (Kelly et al. 1999; Nakano et al. 1997; Sangiao-Alvarellos et al. 2003, 2005c; Laiz-Carrión et al. 2005b).

The maintenance of energy homeostasis during food deprivation in fish is directly related to the capacity for mobilization of energy reserves such as lipids and hepatic glycogen, at least during the initial stages of fasting, and also depends on subsequent activation of hepatic gluconeogenesis and reduction in the rate of glucose utilization (Sheridan and Mommsen 1991; Navarro and Gutiérrez 1995). Information regarding metabolic changes in tissues other than liver during food deprivation is limited to some studies performed on brain (Soengas et al. 1996, 1998; Figueroa et al. 2000; Tripathi and Verma 2003), muscle (Collins and Anderson 1997; Kirchner et al. 2005), gills (Kirchner et al. 2005; Sangiao-Alvarellos et al. 2005b), kidney and intestine (Kirchner et al. 2005). Osmoregulatory parameters (like branchial and renal Na^+,K^+-ATPase pumps) are also influenced by food deprivation, suggesting that the nutritional state affects the capacity for salinity acclimation in euryhaline fish. However, few studies have addressed the effect of prior nutritional state on energy metabolism and the associated osmotic acclimation processes in fish (Jürss et al. 1986; Kültz and Jürss 1991; Vijayan et al. 1996). Our group has demonstrated the existence of osmoregulatory (Mancera et al. 1993a; Guzmán et al. 2004; Laiz-Carrión et al. 2005a, b), endocrine (Mancera et al. 1993b, 2002) and metabolic changes (Sangiao-Alvarellos et al. 2003, 2005a, c) during osmotic acclimation of *S. auratus*. However, there is no information available in this species regarding metabolic adjustments to salinity acclimation in combination with food deprivation. Therefore, we transferred gilthead sea bream to hypo- or hyper-osmotic environments and deprived them of food for 14 days to assess osmoregulatory and metabolic parameters and to evaluate whether food deprivation modifies the osmoregulatory and metabolic responses of fed fish to osmotic extremes.

Materials and methods

Fish

Sexually immature male gilthead seabream (*Sparus auratus* L., 400–450 g body mass) were provided by Planta de Cultivos Marinos (CASEM, Universidad de Cadiz, Puerto Real, Cádiz, Spain) and transferred to the laboratories at the Faculty of Marine Science (Puerto Real, Cádiz). Fish were acclimated to SW in 400 l aquaria for at least 2 weeks in flow-through tanks that provided a constant supply of fresh seawater (SW, 40 ppt salinity, 1,162 mOsm kg^{-1} H_2O) before the experiments. During the experiments, fish were kept under natural photoperiod (July 2004) and constant temperature (18°C). Fish were fed once daily at 1% body mass with commercial dry pellets (Dibaq-Diprotg SA, Segovia, Spain). The approximate food analysis was: 48% crude protein, 6% carbohydrate, 25% crude fat, and 11.5% ash; 20.2 MJ kg^{-1} of feed. Fish were fasted 24 h before sampling. The experiments described comply with the Guidelines of the European Union Council (86/609/EU) and of the University of Cádiz (Spain) for the use of laboratory animals.

Experimental design

Fish were randomly assigned to one of six treatments (2 tanks/treatment, $n=4$ per tank): fed fish acclimated to SW, LSW or HSW, and food-deprived fish acclimated to SW, LSW or HSW. Salinity in the group denoted as hyper-saline water (HSW, 55 ppt salinity, 1,354 mOsm kg^{-1} H_2O) was obtained by mixing full SW with natural marine salt (Unionsal, Cádiz, Spain), whereas salinity in the group denoted as low-salinity water (LSW, 6 ppt salinity, 160 mOsm kg^{-1} H_2O) was obtained by mixing full SW with dechlorinated tap water. The water salinity was checked daily and corrected when necessary. In addition, common water quality criteria (hardness, pH, concentrations of oxygen, carbon dioxide, hydrogen sulphide, nitrite, nitrate, ammonia, calcium, chlorine and suspended solids) were assessed daily (or every other day), with no major changes being observed.

Fish were fed once daily at 1% body mass whereas the fasted fish were deprived of food from the start of the experiment onwards. After 14 days, eight fish from each treatment (four fish from each tank) were removed by dip-net and tissue samples were taken as described below. The duration of food deprivation was specifically chosen because other studies in teleost fish, including *S. auratus*, showed significant changes in metabolic status after 2 weeks of fasting (Navarro and Gutiérrez 1995; Sangiao-Alvarellos et al. 2005b).

Sampling

Fish were deeply anaesthetized with 2-phenoxyethanol (0.1% v/v) and killed by decapitation. Blood was obtained in ammonium-heparin treated syringes by punction of the caudal veins. Plasma samples were obtained by centrifugation of blood (30 s at 13,000× *g*; Eppendorf 5415R), and divided into two aliquots. One aliquot was immediately frozen on liquid nitrogen for the assessment of plasma osmolality, cortisol and protein concentrations, whereas the other aliquot, for the assessment of plasma metabolites, was deproteinized immediately with 6% perchloric acid and neutralized with 1 mol l^{-1} potassium bicarbonate, frozen in liquid nitrogen and stored at −80°C until further assay. To assess Na^+,K^+-ATPase activity, 3–5 filaments from the second branchial arch (cut just above the septum with fine point scissors) and a small portion of posterior kidney was placed on 100 μl of ice-cold SEI buffer (150 mmol l^{-1} sucrose, 10 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole, pH 7.3) and frozen at −80°C. Brain, liver, the remaining kidney, and the remaining branchial arches were removed quickly from each fish, freeze-clamped in liquid nitrogen, and stored at −80°C until assay.

Analytical techniques

Plasma cortisol concentrations were measured by ELISA validated for gilthead sea bream (Tintos et al. 2005). Plasma glucose, lactate and triglycerides were measured using commercial microplate kits from Spinreact (Spain). Plasma protein was measured using bicinchoninic acid method with a BCA protein kit (Pierce, Rockford, USA) for microplates; bovine albumin served as standard. Plasma osmolality was measured with a vapour pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT, USA).

Gill and kidney Na^{+},K^{+}-ATPase activity was determined using the microassay procedure of McCormick (1993) adapted to *S. auratus* (Mancera et al. 2002).

Frozen liver, brain, kidney, and gill were finely minced on an ice-cooled Petri dish, vigorously mixed and divided into two aliquots to assess enzyme activities and metabolite levels. The frozen tissue used for the assessment of metabolite concentrations was homogenized by ultrasonic disruption with 7.5 vols of ice-cooled 0.6 N perchloric acid, neutralized (using 1 mol l^{-1} potassium bicarbonate), centrifuged (2 min at 13,000× *g*, Eppendorf 5415R), and the supernatant used to assay tissue metabolites. Tissue lactate and triglyceride levels were determined spectrophotometrically using a commercial kit (Spinreact, Spain). Tissue glycogen concentrations were assessed using the method of Keppler and Decker (1974). Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined with a commercial kit (Biomérieux, Spain). Tissue total α-amino acids were assessed colorimetrically using the nynhidrin method of Moore (1968) with modifications to adapt the assay to a microplate format. The aliquots of tissues used for the assessment of enzyme activities were homogenized by ultrasonic disruption with 10 vols of ice-cold stopping-buffer containing 50 mmol l^{-1} imidazole-HCl (pH 7.5), 15 mmol l^{-1} 2-mercaptoethanol, 100 mmol l^{-1} KF, 5 mmol l^{-1} EDTA, 5 mmol l^{-1} EGTA, and a protease inhibitor cocktail (Sigma, P-2714). The homogenate was centrifuged (2 min at 13,000× *g*, Eppendorf 5415R) and the supernatant used in enzyme assays. In those cases where non-cytosolic enzymes were assessed, appropriate centrifugations were carried out to obtain samples. Enzyme activities were determined using a Unicam UV-2 spectrophotometer (Thermo Unicam, Waltham, USA). Reaction rates of enzymes were determined by change in absorbance of NAD(P)H at 340 nm. The reactions were started by the addition of homogenates (0.05 ml) at a pre-established protein concentration, omitting the substrate in control cuvettes (final volume 1.35 ml), and allowing the reactions to proceed at 15°C for pre-established times (5–15 min). No changes were found in tissue protein concentrations in any of the groups studied. Therefore, enzyme activities are expressed per milligram of protein. Protein was assayed in triplicate in homogenates according to Bradford (1976) with bovine serum albumin (Sigma, USA) as standard. Enzymatic analyses were carried out at conditions meeting requeriments for optimal velocities. The specific conditions for enzymes were described previously (Sangiao-Alvarellos et al. 2003, 2004, 2005a, b, c).

Statistical analyses

The effect of acclimation to different salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) as well as its possible interaction in parameters assessed was analyzed using a two-way ANOVA with salinity and feeding conditions as main factors. When significant differences were obtained from the ANOVA, multiple comparisons were carried out using the Student–Newman–Keuls test. Significance level was set at $P < 0.05$.

Results

No mortality, health disturbances or any alterations in behaviour were observed in any group of fish throughout the 14 days of experiment. Fed fish in LSW, SW and HSW gained, respectively, an average (± SEM) of 3.5 ± 0.3, 4.7 ± 0.3 and 7.3 ± 0.4 g in mass, while food-deprived fish lost an average (± SEM) of 9.1 ± 0.5, 8.2 ± 0.4 and 11.7 ± 0.7 g in mass. The differences between fed and food-deprived fish were significant ($P < 0.05$) whereas the only significant difference observed among salinities within each treatment was in fed fish where there was an increased fish mass acclimated to HSW compared with those in SW and LSW. *P* values resulting from the two way ANOVA of all parameters assessed are displayed in Table 1.

Concentrations of osmoregulatory parameters are displayed in Fig. 1. Food deprivation abolished or attenuated the increase in gill Na^{+},K^{+}-ATPase activity observed in LSW- and HSW-acclimated fish, respectively. In addition, a linear relationship between renal Na^{+},K^{+}-ATPase activity and environmental salinity was observed both in fed and food-deprived fish (r^2 of 0.92 and 0.91 for fed and food-deprived fish, respectively), though values decreased in food-deprived with respect to fed fish. In gill Na^{+},K^{+}-ATPase activity the increase seen in fed fish acclimated to LSW was not observed in food-deprived fish.

No significant effects of salinity were observed for plasma cortisol (Fig. 2), glucose and lactate (Fig. 3a, b), and triglyceride and protein concentration (Fig. 4a, b). However, food deprivation increased plasma cortisol (Fig. 2) and acetoacetate concentrations (Fig. 4c), had no effect on plasma glucose (Fig. 3a) and decreased the remaining plasma parameters.

The changes in the concentrations of metabolites and enzyme activities elicited by salinity adaptation and feeding conditions in liver are shown in Table 2. Significant salinity effects were observed in glycogen, lactate

Table 1 *P* values from two-way ANOVA of parameters measured in plasma, liver, gills, kidney, and brain of gilthead seabream after acclimation for 14 days to different environmental salinities and/or food deprivation

Tissue	Parameter	Salinity	Feeding	Salinity × feeding
Plasma	Cortisol levels	NS	< 0.001	NS
	Glucose levels	NS	NS	0.05
	Lactate levels	NS	< 0.001	NS
	Protein levels	NS	< 0.001	NS
	Triglyceride levels	NS	< 0.001	NS
	Acetoacetate levels	NS	< 0.001	NS
	Osmolality	< 0.001	0.033	NS
Liver	Hepatosomatic index	NS	< 0.001	NS
	Glycogen levels	0.011	< 0.001	NS
	Glucose levels	NS	NS	NS
	Lactate levels	NS	< 0.001	< 0.001
	Triglyceride levels	0.020	< 0.001	NS
	Acetoacetate levels	NS	0.001	NS
	α-Amino acid levels	NS	< 0.001	NS
	HK activity	0.015	< 0.001	0.011
	GK activity	NS	NS	NS
	PFK activity (Optimal)	0.017	0.024	NS
	PFK activity (activity ratio)	NS	< 0.001	NS
	PFK activity (activation ratio)	NS	0.003	NS
	FBPase activity	0.046	0.030	0.022
	G6Pase activity	NS	0.004	0.016
	G6PDH activity	0.042	NS	0.002
	GDH activity	NS	< 0.001	NS
	Ala-AT activity	0.001	< 0.001	0.055
	Asp-AT activity	< 0.001	0.010	NS
	HOAD activity	0.040	< 0.001	NS
	G3PDH activity	NS	< 0.001	NS
Gills	Na^+,K^+-ATPase activity	< 0.001	< 0.001	0.047
	Glycogen levels	< 0.001	< 0.001	< 0.001
	Glucose levels	NS	< 0.001	NS
	Lactate levels	0.003	NS	NS
	α-Amino acid levels	< 0.001	< 0.001	< 0.001
	HK activity	< 0.001	< 0.001	0.009
	GK activity	< 0.001	0.033	0.002
	PK activity (Optimal)	0.001	0.001	NS
	PK activity (activity ratio)	< 0.001	< 0.001	< 0.001
	PK activity (activation ratio)	NS	NS	NS
	G6PDH activity	< 0.001	< 0.001	NS
	GDH activity	0.006	< 0.001	0.007
	HOAD activity	NS	0.003	NS
	LDH-O activity	0.096	< 0.001	NS
Kidney	Na^+,K^+-ATPase activity	< 0.001	< 0.001	NS
	Glycogen levels	0.002	NS	0.002
	Glucose levels	< 0.001	< 0.001	NS
	Lactate levels	0.012	NS	< 0.001
	Triglyceride levels	0.024	NS	NS
	α-amino acid levels	NS	< 0.001	0.002
	HK activity	< 0.001	NS	0.013
	GK activity	NS	< 0.001	NS
	PK activity (Optimal)	0.010	< 0.001	NS
	PK activity (activity ratio)	0.012	< 0.001	NS
	PK activity (activation ratio)	NS	< 0.001	0.004
	G6Pase activity	< 0.001	NS	< 0.001
	G6PDH activity	< 0.001	NS	NS
	GDH activity	NS	< 0.001	< 0.001
	HOAD activity	NS	NS	0.009
	LDH-O activity	0.035	NS	0.007
Brain	Glycogen levels	0.017	NS	0.034
	Glucose levels	0.023	0.017	NS
	Lactate levels	NS	NS	NS
	Triglyceride levels	NS	NS	NS
	Acetoacetate levels	NS	0.001	NS
	α-amino acid levels	NS	NS	NS
	HK activity	NS	0.044	0.034
	GK activity	NS	NS	NS
	PFK activity (Optimal)	0.002	< 0.001	0.005
	PFK activity (activity ratio)	0.002	< 0.001	NS
	PFK activity (activation ratio)	0.006	< 0.001	NS
	G6PDH activity	NS	0.028	NS
	GDH activity	NS	NS	NS
	HOAD activity	NS	NS	NS
	LDH-O activity	NS	NS	NS

Salinity (LSW, SW, and HSW) and feeding conditions (fed and food deprived) are the main factors
NS not significant

and triglyceride levels, and HK, PFK (optimal), FBPase, G6PDH, Ala-AT, Asp-AT and HOAD activities. Significant effects of food deprivation were observed in hepatosomatic index, glycogen, lactate, triglyceride, acetoacetate, and amino acid concentrations as well as in HK, PFK (optimal activity, and activity and activation ratios), FBPase, G6Pase, GDH, Ala-AT, Asp-AT, HOAD, and G3PDH activities. FBPase, G6Pase, G6PDH, and Ala-AT activities presented a significant interaction between salinity and feeding conditions. Thus, FBPase and Ala-AT activities increased more in food-deprived fish acclimated to extreme salinities. G6Pase activity in fed fish was lower in fish acclimated to extreme salinities, whereas G6PDH activity was lower in food-deprived fish acclimated to HSW than in SW.

The analysis of metabolite concentrations and enzyme activities in gills are presented in Table 3. Significant salinity effects were noticed in glycogen, lactate, and amino acid concentrations as well as in HK, GK, PK (optimal activity and activity ratio), G6PDH, GDH, and LDH-O activities, while feeding conditions modified glycogen, glucose, and amino acid levels as well as HK, GK, PK (optimal activity, and activity ratio), G6PDH, GDH, HOAD, and LDH-O activities. A significant interaction (salinity × feeding conditions) was noticed in glycogen and amino acid concentrations as well as in HK, GK, PK (activity ratio), and GDH activities. Thus, glycogen and amino acid concentrations and HK activity in food-deprived fish were higher in HSW-acclimated fish. GK activity was higher in fed HSW-acclimated fish whereas PK (activity ratio) and GDH activities in food-deprived fish showed no differences due to salinity, in contrast to that observed in fed fish.

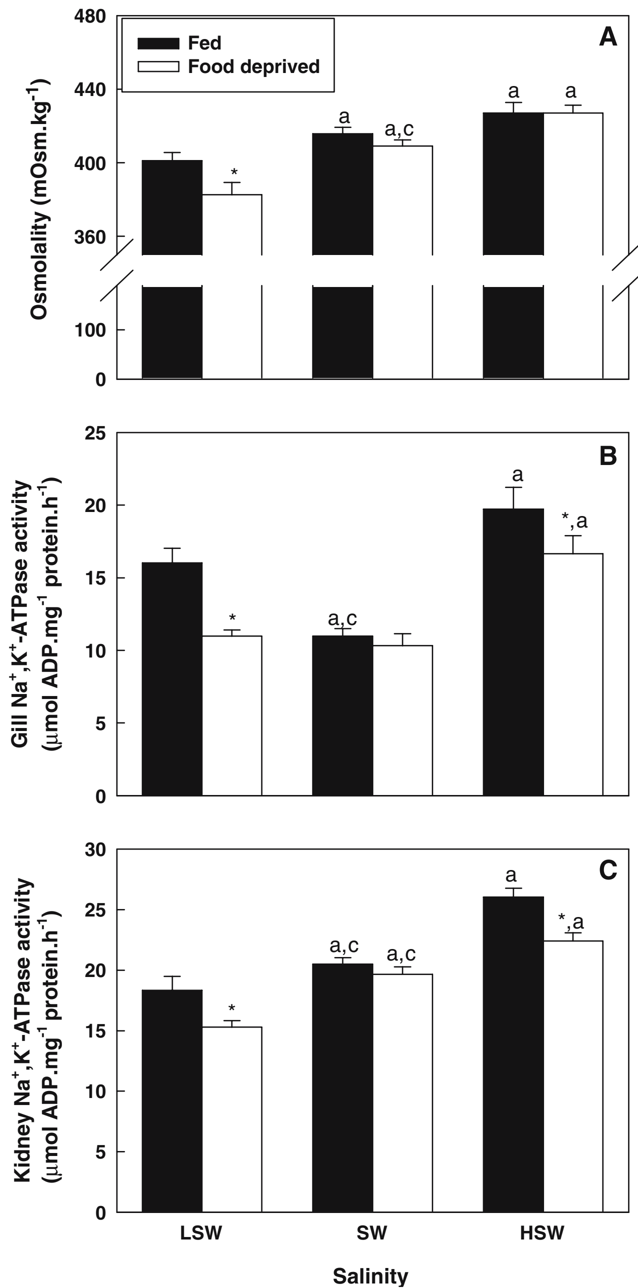

Fig. 1 Effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on plasma osmolality (**a**), gills (**b**) and kidney (**c**) Na^+,K^+-ATPase activities of gilthead sea bream. Data represent mean ± SEM of eight measurements. *Significantly different ($P < 0.05$) from fed group at the same salinity. a, b, c significantly different from LSW, SW, and HSW at the same feeding condition

Metabolic parameters assessed in kidney are displayed in Table 4. Significant salinity effects were apparent in glycogen, glucose, lactate and triglyceride concentrations as well as in HK, PK (optimal activity and activity ratio), G6Pase, G6PDH, and LDH-O activities. Food deprivation significantly affected glucose and amino acid concentrations as well as GK, PK (optimal activity and activity and activation ratios), and GDH activities. Glycogen, lactate, and amino acid concentrations as well as HK, PK (activation ratio), G6PDH, GDH, HOAD and LDH-O activities presented a significant interaction between salinity and feeding conditions. For glycogen, the higher concentrations observed in LSW-acclimated fed fish changed to higher levels in HSW-acclimated food-deprived fish. For lactate, the lower concentration in fed fish observed in LSW changed to higher levels in SW in food-deprived fish. For amino acids, the lower concentrations in SW-acclimated fed fish disappeared in food-deprived fish. The differences in HK, PK (activation ratio), G6PDH, GDH, HOAD and LDH-O activities observed in fed fish changed in food-deprived fish.

Metabolic parameters assessed in brain are presented in Table 5. Glucose concentration and PFK activity (optimal activity and activity and activation ratios) were affected by salinity acclimation. Feeding conditions modified glucose and acetoacetate concentrations, and HK, PFK (optimal activity, and activity and activation ratios) and G6PDH activities. A significant interaction (salinity × feeding conditions) was observed in glycogen concentration, and HK and PFK (optimal) activities. Thus, glycogen concentration that in fed fish were higher in SW-acclimated changed to lower levels in food-deprived LSW-acclimated fish. For HK and PFK (activation ratio) activities, the differences due to salinity in fed fish disappeared in food-deprived fish.

Discussion

Effects of salinity

Osmoregulatory parameters of fed fish after acclimation to different environmental salinities displayed changes

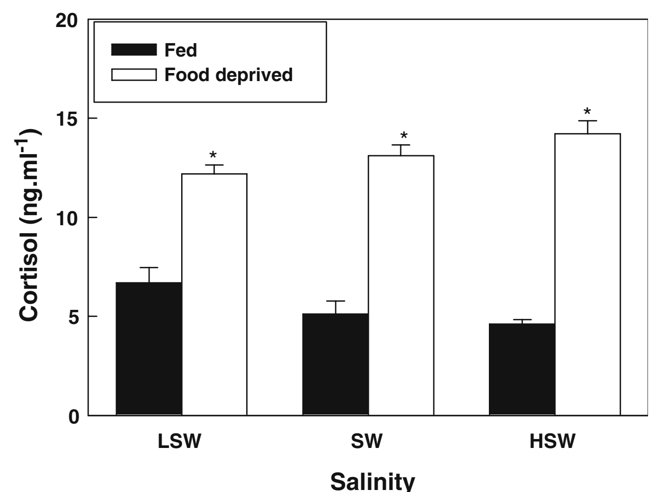

Fig. 2 Effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on plasma levels of cortisol in gilthead sea bream. Further details as in legend of Fig. 1

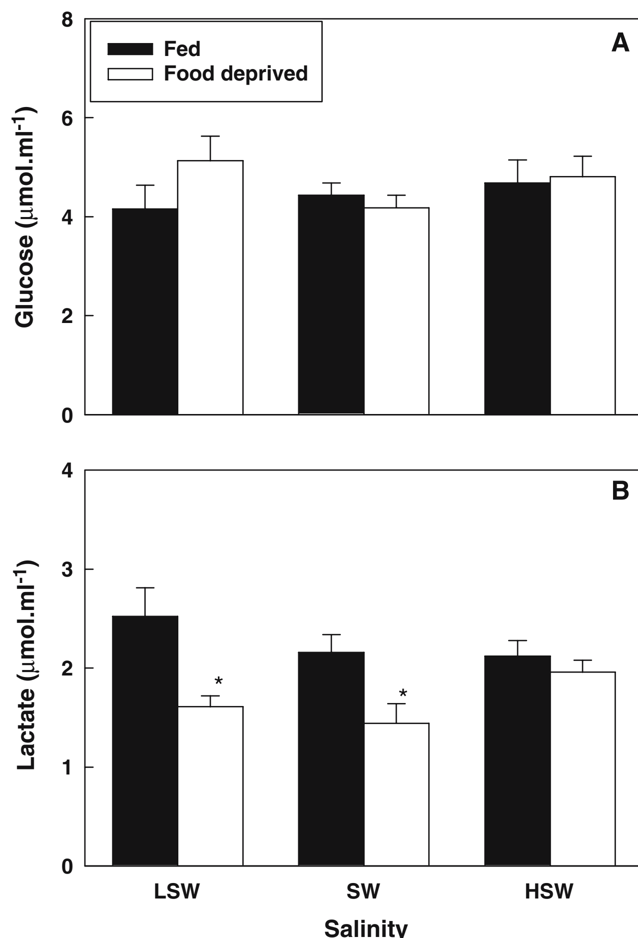

Fig. 3 Effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on plasma levels of glucose (**a**) and lactate (**b**) in gilthead sea bream. Further details as in legend of Fig. 1

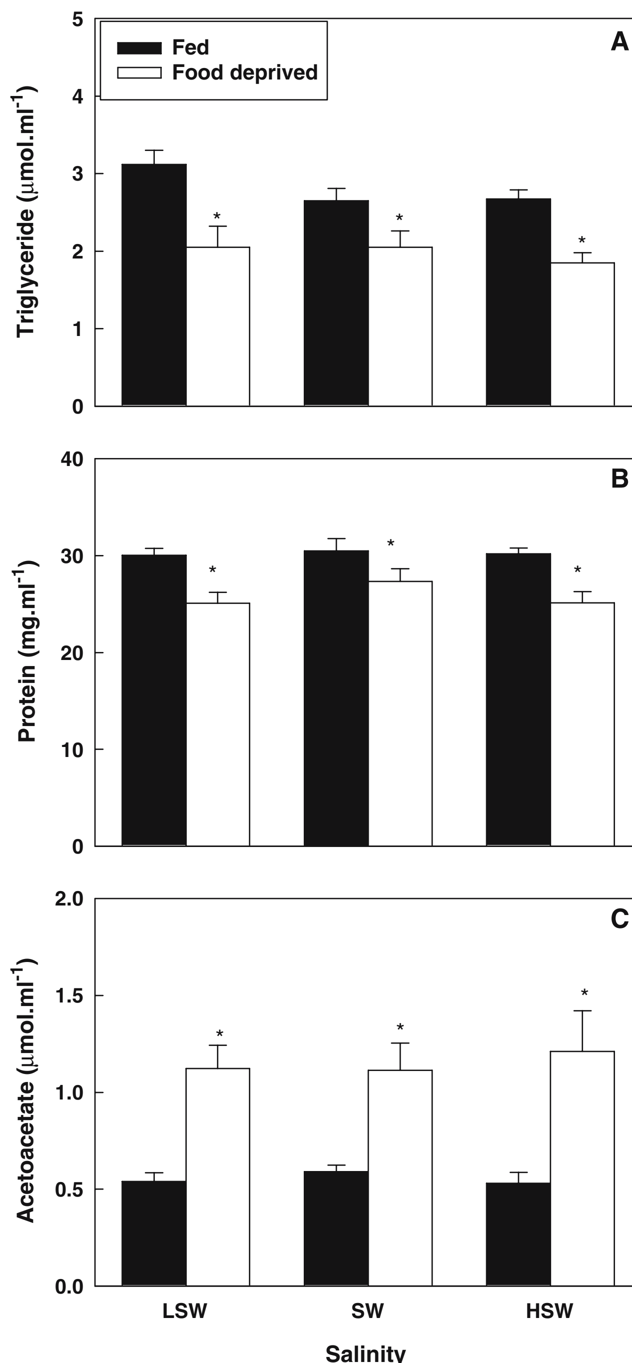

Fig. 4 Effects of acclimation to different environmental salinities (LSW, SW, and HSW) feeding conditions (fed and food deprived) for 14 days on plasma levels of triglyceride (**a**), protein (**b**) and acetoacetate (**c**) in gilthead sea bream. Further details as in legend of Fig. 1

similar to those previously observed in the same species (Laiz-Carrión et al. 2003, 2005a, b; Sangiao-Alvarellos et al. 2003, 2005a, c). Gill Na^{+},K^{+}-ATPase activity presented a U-shaped relationship with respect to environmental salinity, with fish adapted to extreme salinities (LSW and HSW) showing significantly higher gill Na^{+},K^{+}-ATPase activity than SW-adapted fish. In addition, kidney Na^{+},K^{+}-ATPase activity increased in parallel with salinity.

This relationship in kidney had been observed previously in the black sea bream (Kelly et al. 1999) and also in gilthead sea bream (Sangiao-Alvarellos et al. 2003), and it has been attributed to renal modification in urine production (drecreases) and/or ion transport (increases) due to hyperosmotic acclimation.

Cortisol concentrations did not show any changes after salinity acclimation for 14 days, in agreement with previous results in gilthead sea bream describing an initial increase in cortisol concentrations in the first days after transfer from SW to LSW and HSW (adaptative period) followed by a return after 14 days of transfer (regulatory period) to values of SW-acclimated fish (Laiz-Carrión et al. 2005a; Sangiao-Alvarellos et al. 2005c).

Changes described for many metabolic parameters assessed in the different tissues in fed fish acclimated to different osmotic conditions are similar to those previously reported in this species (Laiz-Carrión et al. 2002, 2003, 2005b; Sangiao-Alvarellos et al. 2003). The fol-

Table 2 Liver effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on hepatosomatic index, metabolites levels and enzyme activities in gilthead sea bream

Parameter	Feeding conditions	Salinity		
		LSW	SW	HSW
Hepatosomatic index	Fed	1.85 ± 0.12	1.48 ± 0.09	1.51 ± 0.16
	Food deprived	0.89 ± 0.05*	0.86 ± 0.05*	0.78 ± 0.03*
Metabolites				
Glycogen (μmol glycosyl units g^{-1} wet wt)	Fed	314 ± 20.9	738 ± 8.24[a,c]	436 ± 13.4
	Food deprived	44.1 ± 13.8*	177 ± 30.8*	13.4 ± 5.02*,[b]
Glucose (μmol g^{-1} wet wt)	Fed	30.3 ± 2.07	27.1 ± 0.95	28.9 ± 2.14
	Food deprived	29.4 ± 2.48	30.4 ± 1.33	32.7 ± 3.37
Lactate (μmol g^{-1} wet wt)	Fed	2.23 ± 0.30	3.46 ± 0.45	2.67 ± 1.38
	Food deprived	0.27 ± 0.03*	1.45 ± 0.73*	0.32 ± 0.06*
Triglyceride (μmol g^{-1} wet wt)	Fed	3.53 ± 0.23	3.92 ± 0.25	5.02 ± 0.54[a]
	Food deprived	5.32 ± 0.48*	5.22 ± 0.34*	5.72 ± 0.33
Acetoacetate (μmol g^{-1} wetwt)	Fed	5.49 ± 0.63	5.68 ± 0.26	4.93 ± 0.63
	Food deprived	3.98 ± 0.30	4.53 ± 0.38*	3.59 ± 0.38
Total α-amino acids (μmol g^{-1} wet wt)	Fed	69.4 ± 2.04	71.1 ± 3.09	78.1 ± 4.55
	Food deprived	65.1 ± 3.59	67.2 ± 3.79	63.4 ± 1.54*
Enzyme activities				
Carbohydrate metabolism				
HK activity (U mg^{-1} protein)	Fed	1.37 ± 0.18	0.90 ± 0.14[a,c]	0.23 ± 0.02[a]
	Food deprived	0.14 ± 0.02*	0.17 ± 0.02*	0.15 ± 0.02
GK activity (U mg^{-1} protein)	Fed	0.05 ± 0.001	0.11 ± 0.03	0.03 ± 0.001[b]
	Food deprived	0.06 ± 0.01	0.09 ± 0.03	0.06 ± 0.02
PFK activity				
Total activity (U mg^{-1} protein)	Fed	0.83 ± 0.05	1.08 ± 0.08	0.89 ± 0.07
	Food deprived	0.79 ± 0.08	0.90 ± 0.08	0.68 ± 0.06*
Activity ratio (%)	Fed	10.3 ± 1.03	8.19 ± 0.69	9.57 ± 0.69
	Food deprived	4.76 ± 1.13*	5.63 ± 1.01*	2.86 ± 0.77*
Fructose 2,6-P_2 activation ratio (%)	Fed	25.2 ± 2.79	31.7 ± 5.06	29.4 ± 2.79
	Food deprived	25.0 ± 3.75	15.1 ± 2.39*,[a]	12.8 ± 4.61*,[a]
FBPase activity (U mg^{-1} protein)	Fed	0.87 ± 0.07	0.88 ± 0.06	0.96 ± 0.07
	Food deprived	2.09 ± 0.06*	1.16 ± 0.07*,[a,c]	1.96 ± 0.09*
G6Pase activity (U mg^{-1} protein)	Fed	4.78 ± 0.47	10.9 ± 2.03[a]	6.38 ± 1.05
	Food deprived	5.21 ± 0.63	4.45 ± 0.90*	5.02 ± 0.47
G6PDH activity (U mg^{-1} protein)	Fed	2.23 ± 0.18	1.45 ± 0.16[a]	1.73 ± 0.12
	Food deprived	1.53 ± 0.22*	1.93 ± 0.15*	1.40 ± 0.18*,[b]
Amino acid metabolism				
Ala-AT activity (U mg^{-1} protein)	Fed	8.57 ± 0.48	9.36 ± 0.67	8.06 ± 0.58
	Food deprived	12.2 ± 0.91*	10.9 ± 0.71[a,c]	12.8 ± 0.74*
Asp-AT activity (U mg^{-1} protein)	Fed	5.57 ± 0.29	6.22 ± 0.60[a]	5.79 ± 0.21
	Food deprived	6.97 ± 0.48*	7.34 ± 0.51	7.05 ± 0.61
GDH activity (U mg^{-1} protein)	Fed	11.8 ± 0.52	11.9 ± 0.82	9.74 ± 0.53
	Food deprived	14.4 ± 0.70*	15.9 ± 0.57*	16.7 ± 0.77*
Lipid metabolism				
HOAD activity (U mg^{-1} protein)	Fed	0.70 ± 0.07	0.58 ± 0.08	0.74 ± 0.07
	Food deprived	1.37 ± 0.10*	1.09 ± 0.06*	1.19 ± 0.05*
G3PDH activity (U mg^{-1} protein)	Fed	1.92 ± 0.17	2.05 ± 0.19	2.17 ± 0.21
	Food deprived	1.06 ± 0.06*	1.27 ± 0.15*	0.98 ± 0.04*

Data represent mean ± SEM of eight measurements
*Significantly different ($P < 0.05$) from fed group at the same salinity
[a,b,c]Significantly different from LSW, SW, and HSW at the same feeding condition

lowing paragraphs are, therefore, devoted exclusively to metabolic parameters assessed for the first time in *S. auratus* in the present study.

In gills, minor changes were noticed for amino acid metabolism including decreased GDH activity in LSW- and increased amino acid concentrations in HSW-acclimated fish. This is in agreement with studies showing changes in amino acid metabolism in gills during osmotic acclimation in other fish species (Jürss et al. 1986; Jarvis and Ballantyne 2003).

In kidney, lactate metabolism seems to be altered in HSW-acclimated fish as judged by decreased lactate concentrations and increased LDH-O activity. This suggests an enhanced use of lactate to sustain the osmoregulatory work of this tissue in that salinity, in agreement with previous suggestions for this species (Sangiao-Alvarellos et al. 2003).

In liver, only minor changes were observed in parameters of lipid (triglyceride levels increased in HSW-acclimated fish) and amino acid (Asp-AT activity

Table 3 Gill effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on metabolites levels and enzyme activities in gilthead sea bream

Parameter	Feeding conditions	Salinity		
		LSW	SW	HSW
Metabolites				
Glycogen (μmol glycosyl units g^{-1} wet wt)	Fed	121.6 ± 17.7	80.9 ± 5.38	89.9 ± 9.18
	Food deprived	96.5 ± 5.44	74.4 ± 5.53	394 ± 35.3*[,a,b]
Glucose (μmol g^{-1} wet wt)	Fed	74.9 ± 6.77	67.1 ± 8.12	83.2 ± 12.1
	Food deprived	118 ± 17.4*	112 ± 15.9*	154 ± 12.4*
Lactate (μmol g^{-1} wet wt)	Fed	1.74 ± 0.17	1.53 ± 0.13	2.12 ± 0.15[b]
	Food deprived	1.85 ± 0.19	1.32 ± 0.12	2.07 ± 0.22
Total α-amino acids (μmol g^{-1} wet wt)	Fed	49.0 ± 1.44	49.5 ± 1.56	55.7 ± 2.09[a,b]
	Food deprived	49.4 ± 3.36	50.6 ± 2.03	85.8 ± 2.87*[,a,b]
Enzyme activities				
Carbohydrate metabolism				
HK activity (U mg^{-1}protein)	Fed	0.67 ± 0.05	0.71 ± 0.06	1.60 ± 0.09[a,b]
	Food deprived	0.65 ± 0.03	0.74 ± 0.07	2.31 ± 0.05*[,a,b]
GK activity (U mg^{-1} protein)	Fed	0.13 ± 0.03	0.24 ± 0.03	0.43 ± 0.04[a,b]
	Food deprived	0.22 ± 0.03	0.15 ± 0.02	0.22 ± 0.02*
PK activity				
Total activity (U mg^{-1} protein)	Fed	4.78 ± 0.32	6.52 ± 0.53[a,c]	4.62 ± 0.36
	Food deprived	3.41 ± 0.22*	4.59 ± 0.19*[,a]	4.59 ± 0.52
Activity ratio (%)	Fed	29.6 ± 2.88	14.4 ± 1.41[a,c]	38.0 ± 3.57[a]
	Food deprived	46.5 ± 3.07*	37.3 ± 1.42*[,a]	40.3 ± 2.17
Fructose 1,6-P_2 activation ratio (%)	Fed	86.3 ± 5.28	78.5 ± 5.37	94.5 ± 10.9
	Food deprived	87.9 ± 6.54	89.8 ± 5.33	88.3 ± 7.07
G6PDH activity (U mg^{-1} protein)	Fed	0.80 ± 0.06	1.05 ± 0.08	0.68 ± 0.04[a,b]
	Food deprived	0.64 ± 0.04	0.74 ± 0.07*	0.47 ± 0.03*[,a,b]
Amino acid metabolism				
GDH activity (U mg^{-1} protein)	Fed	2.37 ± 0.08	3.55 ± 0.19[a]	3.27 ± 0.05[a]
	Food deprived	3.85 ± 0.20*	3.83 ± 0.23	3.95 ± 0.21*
Lipid metabolism				
HOAD activity (U mg^{-1} protein)	Fed	0.25 ± 0.02	0.29 ± 0.02	0.23 ± 0.02
	Food deprived	0.17 ± 0.01*	0.21 ± 0.02*	0.21 ± 0.02
Lactate metabolism				
LDH-O activity (U mg^{-1} protein)	Fed	3.17 ± 0.08	3.22 ± 0.23	2.83 ± 0.15
	Food deprived	2.39 ± 0.13*	2.56 ± 0.18*	2.17 ± 0.23*

Further details as in legend of Table 2

decreased in LSW-acclimated fish) metabolism among salinities. In brain, none of the new parameters assessed displayed any changes across the different osmotic conditions assessed.

These results suggest that the higher energy consumption of liver and brain during osmotic acclimation is mainly based on carbohydrates, whereas an increased importance of amino acids and lactate was apparent in osmoregulatory tissues like gills and kidney, in agreement with the hypothesis raised in previous studies using *S. auratus* (Sangiao-Alvarellos et al. 2003, 2005c, Laiz-Carrión et al. 2005b).

Effects of food deprivation

Osmoregulatory parameters in SW-acclimated *S. auratus* were not affected by food deprivation for 14 days, suggesting that this species can preserve osmoregulatory balance during fasting, at least during this time. In contrast, food deprivation increased plasma cortisol concentrations in SW-acclimated *S. auratus* in a way similar to that previously observed in the same (Sangiao-Alvarellos et al. 2005b) and other species (Moon et al. 1989; Vijayan et al. 1996; Jorgensen et al. 2002) under similar experimental protocols. This increase can be associated with the increased gluconeogenic potential already observed in the liver of food-deprived fish (Navarro and Gutiérrez 1995) that could be attributed to cortisol action (Mommsen et al. 1999).

Food deprivation resulted in changes in several parameters of hepatic energy metabolism, as in previous studies carried out in *S. auratus* and other teleosts. These included (1) increased glycogenolytic and gluconeogenic potentials (Foster and Moon 1991; Sheridan and Mommsen 1991; Bonamusa et al. 1992; Sangiao-Alvarellos et al. 2005b; Kirchner et al. 2005) that can be attributed, at least in part, to the increased plasma cortisol concentrations (Laiz-Carrión et al. 2002, 2003); (2) enhancement in the capacity of liver for exporting glucose (Caseras et al. 2002; Metón et al. 2004; Sangiao-Alvarellos et al. 2005b); (3) decreased concentrations of lactate in plasma (Soengas et al. 1996, 1998; Vijayan et al. 1996), probably related to an increased use as

Table 4 Kidney effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on metabolites levels and enzyme activities in gilthead sea bream

Parameter	Feeding conditions	Salinity		
		LSW	SW	HSW
Metabolites				
Glycogen (μmol glycosyl units g^{-1} wet wt)	Fed	6.57 ± 0.56	3.34 ± 0.71[a]	2.79 ± 0.88[a]
	Food deprived	5.01 ± 0.45	3.92 ± 0.58	6.13 ± 0.17*[,b]
Glucose (μmol g^{-1} wet wt)	Fed	9.29 ± 0.64	8.03 ± 0.52	11.9 ± 0.90[a,b]
	Food deprived	6.30 ± 0.51*	6.30 ± 0.62*	7.57 ± 0.53*
Lactate (μmol g^{-1} wet wt)	Fed	6.33 ± 0.40	6.08 ± 0.34	4.74 ± 0.24[a,b]
	Food deprived	4.91 ± 0.28*	7.17 ± 0.57[a,c]	1.95 ± 0.30*[,a]
Triglyceride (μmol g^{-1} wet wt)	Fed	1.21 ± 0.11	1.35 ± 0.15	1.89 ± 0.18
	Food deprived	1.17 ± 0.16	1.56 ± 0.16	1.35 ± 0.14*
Total α-amino acids (μmol g^{-1} wet wt)	Fed	60.2 ± 1.39	52.5 ± 2.03[a]	58.8 ± 1.62
	Food deprived	49.6 ± 2.19*	54.8 ± 1.34	49.5 ± 2.06*
Enzyme activities				
Carbohydrate metabolism				
HK activity (U mg^{-1} protein)	Fed	0.34 ± 0.04	0.33 ± 0.05	0.25 ± 0.04
	Food deprived	0.45 ± 0.03	0.28 ± 0.05[a]	0.17 ± 0.02[a]
GK activity (U mg^{-1} protein)	Fed	3.16 ± 0.32	4.48 ± 0.49	4.30 ± 0.41
	Food deprived	6.62 ± 0.22*	6.21 ± 0.59	4.93 ± 0.62
PK activity				
Total activity (U mg^{-1} protein)	Fed	7.42 ± 0.42	9.92 ± 0.68[a]	8.39 ± 0.52[a]
	Food deprived	5.06 ± 0.60*	7.24 ± 0.57*	7.78 ± 0.81[a]
Activity ratio (%)	Fed	38.3 ± 5.28	45.7 ± 4.45	32.5 ± 5.70
	Food deprived	67.8 ± 11.4*	101 ± 10.3*	54.5 ± 8.17[b]
Fructose 1,6-P_2 activation ratio (%)	Fed	50.1 ± 4.35	39.7 ± 2.66	41.2 ± 4.05
	Food deprived	47.2 ± 4.73	55.8 ± 3.50*	68.4 ± 4.33*[,a]
G6Pase activity (U mg^{-1} protein)	Fed	2.05 ± 0.20	1.22 ± 0.23	5.69 ± 0.48[a]
	Food deprived	1.01 ± 0.13*	6.99 ± 1.11*[,a,c]	2.78 ± 0.31*
G6PDH activity (U mg^{-1} protein)	Fed	1.09 ± 0.07	1.10 ± 0.06	1.53 ± 0.13[a,b]
	Food deprived	0.75 ± 0.08*	1.05 ± 0.08	1.54 ± 0.19[a,b]
Amino acid metabolism				
GDH activity (U mg^{-1} protein)	Fed	6.41 ± 0.37	6.32 ± 0.57	6.53 ± 0.72
	Food deprived	9.70 ± 0.21*	6.81 ± 0.43*[,a,c]	10.5 ± 0.42*
Lipid metabolism				
HOAD activity (U mg^{-1} protein)	Fed	0.49 ± 0.04	0.54 ± 0.03	0.38 ± 0.04[b]
	Food deprived	0.55 ± 0.05	0.44 ± 0.03*	0.57 ± 0.06*
Lactate metabolism				
LDH-O activity (U mg^{-1} protein)	Fed	1.84 ± 0.20	2.11 ± 0.19	3.20 ± 0.16[a,b]
	Food deprived	3.09 ± 0.18*	1.79 ± 0.18[a,c]	3.53 ± 0.22*

Further details as in legend of Table 2

substrate for hepatic gluconeogenesis; and (4) decreased concentrations of triglyceride and protein in plasma (Sheridan and Mommsen 1991).

Other parameters had not been assessed before in the liver of *S. auratus* during food deprivation. These include HK activity that decreased in food-deprived fish, in agreement with that observed in cod (Sundby et al. 1991) though not in salmonids (Sundby et al. 1991; Kirchner et al. 2005), which is not surprising considering that HK activity can be nutritionally regulated in fish liver including *S. auratus* (Panserat et al. 2000). HOAD activity increases in food-deprived fish, which converse to the response found by Vijayan et al. (1996) in tilapia. This finding together with the increase in liver triglyceride concentrations (and the decrease in plasma triglyceride concentrations) suggests an enhancement of lipid metabolism during food deprivation, which is similar to that addressed by several authors in other fish species (Sheridan and Mommsen 1991). The decreased concentrations of liver acetoacetate and the increased plasma concentration suggest an increase in ketone exporting capacity to the plasma, favouring an enhanced use of that fuel in other tissues. A decreased capacity for amino acid catabolism is suggested based on the decrease observed in Ala-AT activity and the lack of changes in GDH activity and amino acid concentrations. This decreased capacity agrees with the decreased Ala-AT activity reported in food-deprived tilapia (Vijayan et al. 1996). However, these data do not agree with the general concept that cortisol activates aminotransferases including Ala-AT and Asp-AT (Mommsen et al. 1999).

In gills and kidney, changes described for many parameters are comparable to those previously addressed in this species (Sangiao-Alvarellos et al. 2005b) including decreased capacity of gill pentose phosphate pathway and increased kidney glycolytic capacity. Other parameters assessed for the first time in this study in-

Table 5 Brain effects of acclimation to different environmental salinities (LSW, SW, and HSW) and feeding conditions (fed and food deprived) for 14 days on metabolites levels and enzyme activities in gilthead sea bream

Parameter	Feeding conditions	Salinity		
		LSW	SW	HSW
Metabolites				
Glycogen (μmol glycosyl units g^{-1} wet wt)	Fed	0.90 ± 0.11	2.79 ± 0.28[a,c]	1.15 ± 0.28
	Food deprived	1.16 ± 0.15	2.62 ± 0.17	2.41 ± 0.40*[,a,b]
Glucose (μmol g^{-1} wet wt)	Fed	0.41 ± 0.04	0.30 ± 0.02[a]	0.28 ± 0.02[a]
	Food deprived	0.24 ± 0.01*	0.26 ± 0.02	0.28 ± 0.02
Lactate (μmol g^{-1} wet wt)	Fed	10.8 ± 0.62	10.4 ± 0.74	10.7 ± 0.90
	Food deprived	12.8 ± 1.06	10.7 ± 0.76	12.6 ± 0.78
Triglyceride (μmol g^{-1} wet wt)	Fed	7.76 ± 1.25	6.48 ± 0.96	4.50 ± 0.50
	Food deprived	7.66 ± 0.98	4.70 ± 0.75	7.72 ± 0.70*
Acetoacetate (μmol g^{-1} wet wt)	Fed	5.49 ± 0.54	3.36 ± 0.24	3.78 ± 0.37
	Food deprived	3.68 ± 0.21*	4.62 ± 0.27*	5.23 ± 0.37*
Total α-amino acids (μmol g^{-1} wet wt)	Fed	58.8 ± 1.50	59.0 ± 1.80	54.0 ± 2.00
	Food deprived	56.9 ± 1.41	56.8 ± 3.42	56.5 ± 1.29
Enzyme activities				
Carbohydrate metabolism				
HK activity (U mg^{-1} protein)	Fed	1.98 ± 0.14	1.35 ± 0.09[a,c]	2.04 ± 0.18
	Food deprived	1.87 ± 0.06	2.24 ± 0.07*	2.12 ± 0.20
GK activity (U mg^{-1} protein)	Fed	0.47 ± 0.03	0.38 ± 0.06	0.44 ± 0.08
	Food deprived	0.48 ± 0.05	0.45 ± 0.05	0.45 ± 0.09
PFK activity				
Total activity (U mg^{-1} protein)	Fed	7.04 ± 0.53	7.34 ± 0.22	4.13 ± 0.42[a,b]
	Food deprived	4.57 ± 0.43*	4.88 ± 0.47*	4.59 ± 0.48
Activity ratio (%)	Fed	9.16 ± 0.84	6.24 ± 0.68	9.22 ± 1.20
	Food deprived	11.9 ± 0.89*	8.98 ± 0.95*	12.1 ± 0.70
Fructose 2,6-P_2 activation ratio (%)	Fed	27.1 ± 1.12	24.4 ± 2.75	30.2 ± 2.71
	Food deprived	41.0 ± 2.65*	30.3 ± 3.47	44.3 ± 3.65*[,b]
G6PDH activity (U mg^{-1} protein)	Fed	0.51 ± 0.05	0.54 ± 0.03	0.46 ± 0.04
	Food deprived	0.47 ± 0.05	0.43 ± 0.03*	0.37 ± 0.03
Amino acid metabolism				
GDH activity (U mg^{-1} protein)	Fed	0.78 ± 0.05	0.91 ± 0.09	0.81 ± 0.09
	Food deprived	0.79 ± 0.07	0.84 ± 0.04	0.89 ± 0.13
Lipid metabolism				
HOAD activity (U mg^{-1} protein)	Fed	0.20 ± 0.03	0.26 ± 0.06	0.26 ± 0.06
	Food deprived	0.27 ± 0.08	0.35 ± 0.05	0.32 ± 0.08
Lactate metabolism				
LDH-O activity (U mg^{-1} protein)	Fed	2.97 ± 0.26	2.72 ± 0.28	3.04 ± 0.24
	Food deprived	2.93 ± 0.14	2.70 ± 0.29	2.84 ± 0.22

Further details as in legend of Table 2

clude a decreased capacity of oxidation of fatty acids and lactate in gills, and decreased glucose concentrations and increased capacity for exporting glucose in kidney. Considering that in another study (Sangiao-Alvarellos et al. 2005b) food deprivation elicited in this species an increased FBPase activity (not measured in the present experiment due to technical reasons), this suggests increased gluconeogenesis in kidney of food-deprived fish to sustain plasma glucose concentrations.

In brain, the more important changes observed after 14 days of food deprivation (increase in glycolytic potential, use of exogenous glucose, and decreased capacity of the pentose phosphate pathway) agree in general with those previously addressed in the same species (Sangiao-Alvarellos et al. 2005b) and in salmonids (Soengas et al. 1996, 1998; Figueroa et al. 2000). There were minor changes in parameters of amino acid, lipid and lactate metabolism assessed for the first time in the present study, suggesting that the requirements of *S. auratus* brain for those metabolites are limited during food deprivation.

When taken together, the metabolic parameters assessed for the first time in food-deprived gilthead sea bream suggest an increased importance of lipid metabolism and ketone production in liver, with few changes in amino acid and lactate metabolism in liver, gills and kidney.

Interaction between salinity and feeding conditions

Food deprivation produces a significant interaction with salinity in gill Na^+,K^+-ATPase activity, abolishing the increase observed in LSW-acclimated fish and reducing the increase observed in HSW-acclimated fish. These results support the hypothesis of a negative influence of a poor nutritional state on acclimation capacity in fish (Jürss et al. 1986; Kültz and Jürss 1991; Vijayan et al. 1996).

In liver, several differences in parameters observed in fed fish due to salinity disappear in food-deprived fish.

The increase in Ala-AT activity in food-deprived fish in extreme salinities (LSW and HSW) compared with that in fed fish this, suggests that during food deprivation (i.e. lower glucose stores) the liver relies more on amino acids during acclimation to extreme salinities. However, in the only comparable study, Vijayan et al. (1996) showed that Ala-AT in tilapia did not show any significant interaction between food deprivation and salinity. Moreover, the increase in G6Pase activity in food-deprived fish compared with fed fish at extreme salinities also suggests that the increased capacity of liver for synthesizing glucose (through gluconeogenesis from amino acids) and exporting glucose is enhanced under acclimation to extreme salinities.

In gills, food deprivation in HSW-acclimated fish increased stores of glycogen and the use of exogenous glucose compared with other salinities. Because the capacity of liver for exporting glucose is also enhanced in food-deprived fish acclimated to extreme salinities, we hypothesize that at least part of the glucose increasingly being used in gills of these fish is of hepatic origin. The glycolytic enzyme PK also showed an increased activity ratio at extreme salinities in food-deprived fish supporting enhanced energy consumption. There were also changes in amino acid metabolism that resulted in a higher capacity for oxidation of amino acids in food-deprived LSW- and HSW-acclimated fish. It seems that the cumulative effects of food deprivation and salinity increase the energy demand of the gills to correct the accompanying hydromineral balance (Barton and Iwama 1991), resulting in increased use of amino acids and glucose in food-deprived fish acclimated to extreme salinities.

In kidney, food deprivation increased stores of glycogen in HSW-acclimated fish and lower amounts of lactate in LSW- and HSW-acclimated fish compared with fed fish. This suggests greater use of lactate as fuel under extreme salinities. This use is so important in HSW-acclimated fish that part of the C3 molecules are used through gluconeogenesis to synthesize glycogen. This is reinforced by increased LDH activity in food-deprived fish transferred to extreme salinities when compared with fed fish. This increased use of lactate in these fish is supported by the decreased capacity of exporting glucose in food-deprived fish transferred to the same salinities as well as by changes in PK activity. Since amino acid concentrations decreased in food-deprived compared with fed fish only in extreme salinities, an increased use of amino acids as fuel under those circumstances may also take place. This is also supported by an increase in GDH activity under the same conditions.

Finally, only marginal changes elicited by food deprivation in response to salinity were observed in the brain.

General considerations

The results of the present study demonstrate that osmotic acclimation and food deprivation alone elicit important osmoregulatory and metabolic changes in gilthead sea bream. Osmoregulatory results confirm those previously reported for *S. auratus* adapted to different environmental salinities (Sangiao-Alvarellos et al. 2003, 2005c, Laiz-Carrion et al. 2005a, b), and during food deprivation (Sangiao-Alvarellos et al. 2005b). As for energy metabolism, most of the changes in carbohydrate metabolism agree with previous studies (Sangiao-Alvarellos et al. 2005b), whereas for amino acid, lactate, and lipid metabolism results provide new evidence regarding metabolic adjustments to cope with food deprivation and osmotic conditions in liver, gills, kidney and brain. Furthermore, results indicate that food deprivation alters the response induced by salinity conditions compared with fed controls, resulting in interactions seen in other models while handling stress (Vijayan and Moon 1992; Reubush and Heath 1996), toxicant exposure (Jorgensen et al. 2002), or high stocking density (Sangiao-Alvarellos et al. 2005b). These interactions show increased production of glucose in liver through gluconeogenesis in food-deprived fish acclimated to extreme salinities (LSW and HSW), to be exported to other tissues and to sustain plasma glucose concentrations. The increased osmoregulatory work due to salinity acclimation to these extreme salinities, which in fed fish is sustained by increased glucose use, is apparently sustained in osmoregulatory tissues of food-deprived fish by increased use of other fuels such as lactate and amino acids.

Acknowledgements This study was partly supported by grants VEM2003-20062 (Ministerio de Ciencia y Tecnología and FEDER, Spain), and PGIDT04PXIC31208PN and PGIDIT05PXIC31202PN (Xunta de Galicia, Spain) to J.L. Soengas, and grant BFU2004-04439-CO2-01B (Ministerio de Ciencia y Tecnología and FEDER, Spain) to J.M. Mancera. The authors wish to thank Planta de Cultivos Marinos (CASEM, Universidad de Cádiz, Puerto Real, Cádiz, Spain) for providing them with experimental fish.

References

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 129:3–26

Bonamusa L, García de Frutos P, Fernández F, Baanante IV (1992) Nutritional effects on key glycolytic–gluconeogenic enzyme activities and metabolite levels in the liver of the teleost fish *Sparus aurata*. Mol Marine Biol Biotechnol 1:113–125

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254

Caseras A, Metón I, Vives C, Egea M, Fernández F, Baanante IV (2002) Nutritional regulation of glucose-6-phosphatase gene expression in liver of the gilthead sea bream (*Sparus aurata*). Br J Nutr 88:607–614

Chervinski J (1984) Salinity tolerance of young gilthead sea bream *Sparus aurata*. Bamidgeh 36:121–124

Collins AL, Anderson TA (1997) The influence of changes in food availability on the activation of key degradative and metabolic enzymes in the liver and epaxial muscle of the golden Perch. J Fish Biol 50:1158–1165

Figueroa RI, Rodríguez-Sabarís R, Aldegunde M, Soengas JL (2000) Effects of food deprivation on 24 h-changes in brain and liver carbohydrate and ketone body metabolism of rainbow trout. J Fish Biol 57:631–646

Foster GD, Moon TW (1991) Hypometabolism with fasting in the yellow perch: a study of enzymes, hepatocyte metabolism, and tissue size. Physiol Zool 64:259–275

Guzmán JM, Sangiao-Alvarellos S, Laiz-Carrión R, Míguez JM, Martín del Rio MP, Soengas JL, Mancera JM (2004) Osmoregulatory action of 17β-estradiol in the gilthead sea bream *Sparus auratus*. J Exp Zool 301A:828–836

Jarvis PL, Ballantyne JS (2003) Metabolic responses to salinity acclimation in juvenile shortnose sturgeon *Acipenser brevirostrum*. Aquaculture 219:891–909

Jorgensen EH, Vijayan MM, Aluru N, Maule AG (2002) Fasting modifies Aroclor 1254 impact on plasma cortisol, glucose and lactate responses to handling disturbance in Arctic charr. Comp Biochem Physiol 132C:235–245

Jürss K, Bittorf T, Vökler T (1986) Influence of salinity and food deprivation on growth, RNA/DNA ratio and certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). Comp Biochem Physiol 83B:425–433

Kelly SP, Chow INK, Woo NYS (1999) Haloplasticity of black seabream (*Mylio macrocephalus*): hypersaline to freshwater acclimation. J Exp Zool 283:226–241

Keppler D, Decker K (1974) Glycogen. determination with amyloglucosidase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Acdemic, New York, pp 1127–1131

Kirchner S, Seixas P, Kaushik S, Panserat S (2005) Effects of low protein intake on extra-hepatic gluconeogenic enzyme expression and peripheral glucose phosphorylation in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol 140B:333–340

Kültz D, Jürss K (1991) Acclimation of chloride cells and Na/K-ATPase to energy deficiency in tilapia (*Oreochromis mossambicus*). Zool Jb Physiol 95:39–50

Laiz-Carrión R, Sangiao-Alvarellos S, Guzmán JM, Martín del Rio MP, Soengas JL, Mancera JM (2002) Energy metabolism in fish tissues related to osmoregulation and cortisol action. Fish Physiol Biochem 27:179–188

Laiz-Carrión R, Martín del Río MP, Míguez JM, Mancera JM, Soengas JL (2003) Influence of cortisol on osmoregulation and energy metabolism in gilthead sea bream *Sparus aurata*. J Exp Zool 298A:105–118

Laiz-Carrión R, Guerreiro PM, Fuentes J, Canario AVM, Martín del Rio MP, Mancera JM (2005a) Branchial osmoregulatory response to salinity in the gilthead sea bream, *Sparus auratus*. J Exp Zool 303A:563–576

Laiz-Carrión R, Sangiao-Alvarellos S, Guzmán JM, Martín del Rio MP, Soengas JL, Mancera JM (2005b) Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions: implications on osmoregulation and energy metabolism. Aquaculture 250:849–861

Mancera JM, Pérez-Fígares JM, Fernández-Llebrez P (1993a) Osmoregulatory responses to abrupt salinity changes in the euryhaline gilthead sea bream (*Sparus aurata*). Comp Biochem Physiol 106A:245–250

Mancera JM, Fernández-Llebrez P, Grondona JM, Pérez-Fígares JM (1993b) Influence of environmental salinity on prolactin and corticotropic cells in the euryhaline gilthead sea bream (*Sparus aurata* L.). Gen Comp Endocrinol 90:220–231

Mancera JM, Laiz-Carrión R, Martín del Río MP (2002) Osmoregulatory action of PRL, GH and cortisol in the gilthead sea bream (*Sparus aurata* L.). Gen Comp Endocrinol 129:95–103

McCormick SD (1993) Methods for nonlethal gill biopsy and measurement of Na^{+},K^{+}-ATPase activity. Can J Fish Aquat Sci 50:656–658

Metón I, Caseras A, Fernández F, Baanante IV (2004) Molecular cloning of hepatic glucose-6-phosphatase catalytic subunit from gilthead sea bream (*Sparus aurata*): response of its mRNA levels and glucokinase expression to refeeding and diet composition. Comp Biochem Physiol 138B:145–153

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268

Moon TW, Foster GD, Plisetskaya EM (1989) Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. Can J Zool 67:2189–2193

Moore S (1968). Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the nynhidrin reaction. J Biol Chem 1242:6281–6283

Nakano K, Tagawa M, Takemura A, Hirano T (1997) Effects of ambient salinities on carbohydrate metabolism in two species of tilapia: *Oreochromis mossambicus* and *O. niloticus*. Fish Sci 63:338–343

Navarro I, Gutiérrez J (1995) Fasting and starvation. In: Hochachka PW, Mommsen TP (eds) Metabolic biochemistry, biochemistry and molecular biology of fishes, vol 4. Elsevier, Amsterdam, pp 393–434

Panserat S, Médale F, Blin C, Brèque J, Vachot C, Plagnes-Juan E, Gomes E, Krishnamoorthy R, Kaushik S (2000) Hepatic glucokinase is induced by dietary carbohydrates in rainbow trout, gilhead seabream, and common carp. Am J Physiol 278:R1164–R1170

Reubush KJ, Heath AG (1996) Metabolic responses to acute handling by fingerling inland and anadromous striped bass. J Fish Biol 49:830–841

Sangiao-Alvarellos S, Laiz-Carrión R, Guzmán JM, Martín del Rio MP, Míguez JM, Mancera JM, Soengas JL (2003) Acclimation of *S. aurata* to various salinities alters energy metabolism of osmoregulatory and nonosmoregulatory organs. Am J Physiol 285:R897–R907

Sangiao-Alvarellos S, Lapido M, Míguez JM, Soengas JL (2004) Effects of central administration of arginine vasotocin on monoaminergic neurotransmitters and energy metabolism of rainbow trout brain. J Fish Biol 64:1313–1329

Sangiao-Alvarellos S, Guzmán JM, Laiz-Carrión R, Martín del Río MP, Míguez JM, Mancera JM, Soengas JL (2005a). Actions of 17β-estradiol on carbohydrate metabolism in liver, gills and brain of gilthead sea bream *Sparus auratus* during acclimation to different salinities. Marine Biol 146:607–617

Sangiao-Alvarellos S, Guzmán JM, Laiz-Carrión R, Míguez JM, Martín del Río MP, Mancera JM, Soengas JL (2005b) Interactive effects of high stocking density and food deprivation on carbohydrate metabolism in several tissues of gilthead sea bream *Sparus auratus*. J Exp Zool 303A:761–775

Sangiao-Alvarellos S, Arjona FJ, Martín del Río MP, Míguez JM, Mancera JM, Soengas JL (2005c) Time course of osmoregulatory and metabolic changes during osmotic acclimation in *Sparus auratus*. J Exp Biol 208:4291–4304

Sheridan MA, Mommsen TP (1991) Effects of nutritional state on in vivo lipid and carbohydrate metabolism of Coho Salmon, *Oncorhynchus kisutch*. Gen Comp Endocrinol 81:473–483

Soengas JL, Strong EF, Fuentes J, Veira JAR, Andrés MD (1996) Food deprivation and refeeding in Atlantic salmon, *Salmo salar*: effects on brain and liver carbohydrate and ketone bodies metabolism. Fish Physiol Biochem 15:491–511

Soengas JL, Strong EF, Andrés MD (1998) Glucose, lactate, and β-hydroxybutyrate utilization by rainbow trout brain: changes during food deprivation. Physiol Zool 71:285–293

Sundby A, Hemre GI, Borrebaek B, Christophersen B, Blom AK (1991) Insulin and glucagon family peptides in relation to activities of hepatic hexokinase and other enzymes in fed and starved atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). Comp Biochem Physiol 100B:467–470

Tintos A, Míguez JM, Mancera JM, Soengas JL (2005) Development of a microtitre plate indirect ELISA for measuring cortisol in teleost fish, and evaluation of stress responses in rainbow trout and gilthead sea bream. J Fish Biol (In press)

Tripathi G, Verma P (2003) Starvation-reduced impairment of metabolism in a freshwater catfish. Zeitschrift fur Naturforschung C 58:446–451

Vijayan MM, Moon TW (1992) Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). Can J Fish Aquat Sci 49:2260–2266

Vijayan MM, Morgan JD, Sakamoto T, Grau EG, Iwama GK (1996) Food-deprivation affects seawater acclimation in tilapia: hormonal and metabolic changes. J Exp Biol 199:2467–2475