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# Acclimation of *S. aurata* to various salinities alters energy metabolism of osmoregulatory and nonosmoregulatory organs

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**Sangiao-Alvarellos, Susana, Raúl Laiz-Carrión, José M. Guzmán, María P. Martín del Río, Jesús M. Miguez, Juan M. Mancera, and José L. Soengas.** Acclimation of *S. aurata* to various salinities alters energy metabolism of osmoregulatory and nonosmoregulatory organs. *Am J Physiol Regul Integr Comp Physiol* 285: R897–R907, 2003. First published June 19, 2003; 10.1152/ajpregu.00161.2003.—The impact of different environmental salinities on the energy metabolism of gills, kidney, liver, and brain was assessed in gilthead sea bream (*Sparus aurata*) acclimated to brackish water [BW, 12 parts/thousand (ppt)], seawater (SW, 38 ppt) and hyper saline water (HSW, 55 ppt) for 14 days. Plasma osmolality and levels of sodium and chloride presented a clear direct relationship with environmental salinities. A general activation of energy metabolism was observed under different osmotic conditions. In liver, an enhancement of glycogenolytic and glycolytic potential was observed in fish acclimated to BW and HSW compared with those in SW. In plasma, an increased availability of glucose, lactate, and protein was observed in parallel with the increase in salinity. In gills, an increased $Na^+$-$K^+$-ATPase activity, a clear decrease in the capacity for use of exogenous glucose and the pentose phosphate pathway, as well as an increased glycolytic potential were observed in parallel with the increased salinity. In kidney, $Na^+$-$K^+$-ATPase activity and lactate levels increased in HSW, whereas the capacity for the use of exogenous glucose decreased in BW- and HSW-acclimated fish compared with SW-acclimated fish. In brain, fish acclimated to BW or HSW displayed an enhancement in their potential for glycogenolysis, use of exogenous glucose, and glycolysis compared with SW-acclimated fish. Also in brain, lactate and ATP levels decreased in parallel with the increase in salinity. The data are discussed in the context of energy expenditure associated with osmotic acclimation to different environmental salinities in fish euryhaline species.

Gilthead sea bream; *Sparus aurata*; osmoregulation; energy metabolism

ADAPTATION OF EURYHALINE FISH to different environmental salinities induces changes/activation of ion transport mechanisms. This adaptation is usually accompanied by changes in oxygen consumption, suggesting variations in the energetic demands for osmoregulation. Thus five patterns of metabolic response to altered environmental salinities have been suggested by Morgan and Iwama (37) in fish, including: *1*) no change in metabolic rate, *2*) metabolic rate is minimum in isotonic salinity and increased at lower and higher salinities, *3*) metabolic rate increases linearly with salinity, *4*) metabolic rates higher in freshwater (FW) that decrease in isotonic media [do not tolerate seawater (SW)], and *5*) rate highest in SW and decreasing in other salinities. These changes in oxygen consumption can lead to variations in whole body metabolism. The metabolic response of the fish to different osmotic conditions undoubtedly includes both stress and osmoregulation components, but the relative energetic demands of these processes cannot be discerned from whole animal oxygen consumption. Thus, not unexpectedly, alterations in intermediary metabolism related to osmoregulation are not fully understood in fish (41). In addition, the influence of environmental salinities on the growth rate in fish is also poorly understood (4).

Although the functions of osmoregulatory organs, such as the gills, kidney, and intestine, have been extensively investigated, less attention has been paid to metabolic aspects of osmoregulation in these organs (14, 16, 39).

The gills are probably the organs that consume most energy during osmoregulation since they must ensure isosmotic regulation of intracellular fluid and also anisosmotic regulation of extracellular fluid. Thus, on transfer of teleost fish to different salinities, $Na^+$ and $Cl^-$ transport across the gill epithelia switches from ion uptake from hypoosmotic water to $Na^+$ and $Cl^-$ excretion in hyperosmotic water. Those ion transport mechanisms involve cotransporters, ion conductive channels, and ion transport proteins driven by ATP (30, 63).

Kidney plays a minor role in osmoregulation compared with gills. However, several changes occur in this organ during osmotic adaptation, including changes in morphology, excretion of divalent ions, glomerular filtration rate, and urine production (2, 43).

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Most of these changes need energy in the form of ATP and may be associated with an altered energetic demand that would lead to changes in kidney intermediary metabolism (14, 54).

The energy requirement of the gills and kidney is thought to be maintained by oxidation of glucose and lactate obtained from the circulation (34, 36). Because the liver is the main site involved in glucose turnover in fish, it is likely that liver metabolism is enhanced during osmotic adaptation. However, very little is known about the reorganization of liver metabolism and the mobilization of substrates during acclimation to different osmotic conditions in fish (40, 58).

Brain energy metabolism in fish changes under stress conditions (49), and also after treatment with stress hormones such as catecholamines (45) and cortisol (19). In this way, a stressful situation such as adaptation to different osmotic conditions should produce effects similar to those of other stressors already evaluated in fish brain. Nevertheless, this possibility has received little attention to date (62).

Gilthead sea bream (*Sparus aurata*) is a euryhaline teleost capable of living in environments of different salinities (7, 26). The osmoregulatory system of this species has been studied previously by analyzing aspects related to adenohypophyseal and plasmatic parameters (26, 28, 29), including the role of different hormones in the adaptation to hyperosmotic and hypoosmotic environments (25, 27).

There are several studies addressing metabolic changes in euryhaline fish during acclimation to different osmotic conditions (40, 41, 50, 52, 58). However, those studies, except for a few cases (14), lack the integrative view of assessing osmoregulatory and metabolic changes simultaneously, and they are often focused only on one tissue (usually liver) during acclimation to only one different (lower or higher) environmental salinity. Therefore, the purpose of the present study was to assess in *S. aurata* the impact of acclimation to very different environmental salinities (lower and higher) on both osmoregulation and energy metabolism of different tissues simultaneously. Therefore, levels of several metabolites and the activities of key enzymes of the major pathways of energy metabolism (use of exogenous glucose, glycolysis, glycogenolysis, gluconeogenesis, and pentose phosphate) were measured in the gill, kidney, liver, and brain of *S. aurata* acclimated for 14 days to brackish water [BW, 12 parts/thousand (ppt)], SW (38 ppt), and hypersaline water (HSW, 55 ppt).

## MATERIALS AND METHODS

*Fish.* Immature male gilthead sea bream (*S. aurata* L., 100–150 g body wt) were provided by Planta de Cultivos Marinos (CASEM, University of Cádiz, Puerto Real, Cádiz, Spain) and were transferred to the laboratory at the Faculty of Marine Science (Puerto Real, Cádiz). They were acclimated to SW in 400 liters of aquaria for at least 2 wk in an open system (38 ppt salinity). During the experiments (May 2002), fish were maintained under a natural photoperiod and constant temperature (18°C). Fish were fed daily with 1% body wt using commercial dry pellets (Dibaq-Diprotg, Segovia, Spain). 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 protocol.* Fish were randomly divided into three different groups (12 fish/group), kept in an open system of 400-liter tanks containing SW (38 ppt). After an initial acclimation period (30 days), salinity was progressively changed to BW (12 ppt) or HSW (55 ppt) in recirculating systems, whereas in the tank with the SW group the system was also recirculated to be comparable with the other groups. Salinity in the group denoted as BW was decreased gradually to 12 ppt over 2 h by mixing full SW with dechlorinated tap water. In the group denoted as HSW, salinity was increased gradually during 2 h up to 55 ppt by mixing full SW with natural marine salt water (Unionsal, Cádiz, Spain). Fish from three groups remained in their specific conditions for 2 wk during which the common water quality criteria (hardness, and the levels of oxygen, carbon dioxide, hydrogen sulfide, nitrite, nitrate, ammonia, calcium, chlorine, and suspended solids) were assessed, with no major changes being observed. The water salinity was checked every day and corrected when it was necessary. No mortality was observed during the experiments.

*Sampling.* Fish were anesthetized with 2-phenoxyethanol (1 ml/l water), weighed, and sampled. Blood was obtained in ammonium-heparinized syringes from the caudal peduncle. Plasma samples were obtained after centrifugation of blood (1 min at 10,000 *g*) and were immediately frozen on dry ice and stored at −80°C until further assay. To assess gill $Na^+$-$K^+$-ATPase activity (25), three to five filaments coming from the second branchial arch were cut just above the septum with fine-point scissors, placed in 100 μl of ice-cold SEI buffer (in mmol/l: 150 sucrose, 10 EDTA, and 50 imidazole, pH 7.3), and frozen at −80°C. For evaluating $Na^+$-$K^+$-ATPase activity in kidney, a small biopsy from the posterior portion of the kidney was placed in ice-cold SEI buffer and frozen at −80°C. Brain, liver, the remaining kidney, and the remaining branchial arches were removed in a few seconds from each fish, frozen on dry ice, and stored at −80°C until assay.

*Analytic techniques.* Plasma glucose and lactate were measured using commercial kits from Sigma (nos. 16–20UV and 735, respectively) adapted to microplates (12, 56). Plasma protein was measured using the bicinchoninic acid (BCA) method (48) with the BCA protein kit (Pierce, Rockford, IL) for microplates, with bovine albumin as the standard. Plasma triglyceride (TG) levels were determined enzymatically with a commercial kit from Sigma (no. 334-UV; see Ref. 6) in microplates. Those assays were run on a Bio Kinetics EL-340i Automated Microplate Reader (Bio-Tek Instruments, Winooski, VT) using DeltaSoft3 software for Macintosh (BioMetallics). Plasma osmolality was measured with a vapor pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT). Plasma $Na^+$ was measured using an atomic absorption spectrophotometer, and plasma $Cl^-$ levels were measured with the Chloride Sigma kit (no. 461-3).

Gill and kidney $Na^+$-$K^+$-ATPase activity was determined using the microassay method of McCormick (31) adapted to *S. aurata* (25). Tissue was homogenized in 125 μl of SEI buffer with 0.1% deoxycholic acid and then centrifuged at 2,000 *g* for 30 s. Duplicate 10-μl homogenate samples were added to 200-μl assay mixture with and without 0.5 mmol/l ouabain in 96-well microplates at 25°C and read at 340 nm for 10 min with intermittent mixing. Ouabain-sensitive ATPase activity was detected by enzymatic coupling of ATP dephosphorylation to NADH oxidation. The Pierce BCA Pro-

tein kit was used to assess protein in homogenates with bovine albumin as standard.

Brain, liver, gill, and kidney samples were minced on a chilled petri dish to very small pieces that, once formed, were mixed and divided into two homogeneous aliquots to assess enzyme activities and metabolite levels, respectively. The tissue used for the assessment of metabolite levels was homogenized immediately by ultrasonic disruption in cold (UP200H from Dr. Hielscher) with 7.5 vol ice-cooled 6% perchloric acid and neutralized (using 1 mol/l potassium bicarbonate). The homogenate was centrifuged (2 min at 13,000 *g*; Eppendorf 5415R), and the supernatant was used for assays. Tissue lactate and ATP levels were determined spectrophotometrically using commercial kits (Sigma Diagnostics). Tissue glycogen levels were assessed using the method of Keppler and Decker (17). Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined enzymatically using a commercial kit (Biomérieux).

The tissue used for the assessment of enzyme activities was homogenized by ultrasonic disruption in cold (UP200H from Dr. Hielscher) with 10 vol ice-cold stopping buffer containing (in mmol/l) 50 imidazole hydrochloride (pH 7.5), 2-mercaptoethanol, 50 NaF, 4 EDTA, 250 sucrose, and 0.5 PMSF (added as dry crystals immediately before homogenization). The homogenate was centrifuged (2 min at 13,000 *g*; Eppendorf 5415R), and the supernatant was used for assays.

Enzyme activities were determined using a Unicam UV6–220 spectrophotometer (Thermo Unicam, Waltham, MA). Reaction rates of enzymes were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm. The reactions were started by the addition of homogenates (0.05 ml) at a preestablished protein concentration, omitting the substrate in control cuvettes (final volume 1.35 ml) and allowing the reactions to proceed at 15°C for preestablished times. Homogenate protein was assayed in triplicate, as detailed by Bradford (5), using BSA (Sigma) as standard. Enzyme analyses were all carried out to achieve maximum rates in each tissue, as defined in preliminary tests. The specific conditions for enzyme assays were described previously (19), after adaptation of methods described for salmonids (51, 55), and were as follows.

Hexokinase (HK; EC 2.7.1.1) was assayed in gills, kidney, and brain with (in mmol/l) 50 imidazole hydrochloride (pH 8), 5 $MgCl_2$, 0.15 NADP, 1 ATP, excess glucose-6-phosphate dehydrogenase (G-6-PDH), and 5 glucose (omitted for control).

6-Phosphofructo 1-kinase (PFK; EC 2.7.1.11) was assessed in brain using (in mmol/l) 100 imidazole hydrochloride (pH 8.25), 5 $MgCl_2$, 50 KCl, 4 $SO_4(NH_4)_2$, 0.15 NADH, 1 ATP, excess aldolase, excess triose phosphate isomerase, and excess α-glycerol phosphate dehydrogenase. Activities were determined at low (0.1 mmol/l) and high (5 mmol/l) fructose 6-phosphate concentrations (omitted for controls). An activity ratio was calculated as the activity at low fructose 6-phosphate/high fructose 6-phosphate. Similarly, a fructose 2,6-bisphosphate activation ratio was determined using 5 μmol/l fructose 2,6-bisphosphate and 0.1 mmol/l fructose 6-phosphate concentrations.

Pyruvate kinase (PK; EC 2.7.1.40) was assessed in liver, gills, and kidney using (in mmol/l) 50 imidazole hydrochloride (pH 7.4), 10 $MgCl_2$, 100 KCl, 0.15 NADH, 0.5 ADP, and excess lactate dehydrogenase. Activities were determined at low (0.05 mmol/l for kidney and 0.1 mmol/l for liver and gills) and high (2.8 mmol/l) phospho*enol*pyruvate (PEP) concentrations (omitted for controls). An activity ratio was calculated as the activity at low PEP/high PEP. Similarly, a fructose 1,6-bisphosphate activation ratio was determined using 0.01 (brain), 0.1 (kidney), and 1 (liver) mmol/l FBPase and 0.1 mmol/l PEP concentrations.

Glycogen phosphorylase (GPase; EC 2.4.1.1) was assayed in liver, gills, kidney, and brain using 50 mmol/l phosphate buffer (pH 7.0), 27 mmol/l $MgSO_4$, 19.5 mmol/l EDTA, 0.5 mmol/l NADP, 50 mmol/l glucose 1,6-bisphosphate, 2.5 mmol/l AMP, excess phosphoglucomutase, excess G-6-PDH, and 5 mg/ml glycogen (omitted for control). GPase *a* activity was measured with 10 mmol/l caffeine present, and total GPase activities were estimated without caffeine. The ratio of GPase activities with and without caffeine multiplied by 100 represents the percentage of total GPase ($a + b$) in the active form (%GPase *a*).

G-6-PDH (EC 1.1.1.49) was determined in liver, gills, and brain using (in mmol/l) 78 imidazole hydrochloride (pH 7.7), 5 $MgCl_2$, 0.5 NADP, and 1 glucose 6-phosphate (omitted for control).

Fructose 1,6-bisphosphatase (FBPase; EC 3.1.3.11) was assessed in liver using (in mmol/l) 85 imidazole hydrochloride (pH 7.7), 0.5 NADP, 5 $MgCl_2$, excess phosphoglucose isomerase, excess G-6-PDH, and 0.05 FBPase (omitted for control).

*Statistics.* Data were statistically analyzed by a one-way ANOVA test in which treatment (12, 38, and 55 ppt) was the main factor and treated as a nominal independent variable. Logarithmic transformations of the data were made when necessary to fulfill the conditions of the ANOVA, but data are shown in their decimal values for clarity. Post hoc comparisons were made using a Tukey test, with the differences considered to be statistically significant at $P < 0.05$.

## RESULTS

The results obtained for osmoregulatory parameters are displayed on Table 1. Plasma osmolality increased in parallel with salinity, and significant differences ($P < 0.001$) were observed when comparing HSW- and BW-acclimated fish. Plasma $Na^+$ levels increased in

Table 1. *Osmolality, $Na^+$, and $Cl^-$ levels in plasma, and muscle water content in gilthead sea bream acclimated for 14 days to BW, SW, or HSW*

| Parameter | Groups | | |
|---|---|---|---|
| | BW acclimated | SW acclimated | HSW acclimated |
| Plasma osmolality, mosmol/kg $H_2O$ | 396 ± 3* | 401 ± 3*† | 410 ± 3† |
| Plasma sodium, mmol/l | 178 ± 2* | 182 ± 3* | 192 ± 4† |
| Plasma chloride, mmol/l | 145 ± 2* | 147 ± 1* | 156 ± 2† |
| Muscle water content, % | 75.4 ± 0.2* | 74.9 ± 0.5* | 74.5 ± 0.4* |

Values are means ± SE; $n$ = 10–12 fish/group. BW, brackish water (12 ppt); SW, seawater (38 ppt); HSW, hypersaline water (55 ppt). Different symbols indicate significant differences among groups by one-way ANOVA ($P < 0.05$).

parallel with salinity and were higher in HSW-acclimated fish compared with BW ($P = 0.013$)- and SW ($P = 0.023$)-acclimated fish. Plasma Cl− levels increased in parallel with increased salinity, with levels of fish acclimated to HSW being higher than those in BW ($P = 0.031$) or SW ($P = 0.021$). In contrast, muscle water content was not significantly different among the different salinities assessed. $Na^+$-$K^+$-ATPase activity (Fig. 1) displayed an increase in parallel with salinity in gills ($P < 0.001$), whereas in the kidney activity (Fig. 1) was higher in HSW- than in BW ($P = 0.025$)- and SW ($P = 0.039$)-acclimated fish.

The levels of plasma metabolites in fish acclimated to different salinities are displayed on Table 2. Plasma glucose levels increased in parallel with increased salinity ($P < 0.001$). Plasma lactate levels also increased in parallel with salinity, with levels being significantly higher in HSW-acclimated fish ($P = 0.031$) than those of BW-acclimated fish. Plasma protein levels also increased with salinity, and significant differences ($P < 0.001$) were observed when comparing HSW-acclimated fish with BW- or SW-acclimated fish. However, plasma levels of TG were not altered significantly by environmental salinity.

The metabolic profile of gills in fish acclimated to different environmental salinities is displayed on Table 3. ATP levels decreased significantly in parallel with the increase in salinity, with levels in HSW being lower ($P = 0.045$) than in BW. No significant differences were observed in the levels of glycogen and lactate, and GPase activity. A significant increase ($P < 0.001$) in activity in parallel with increased salinity

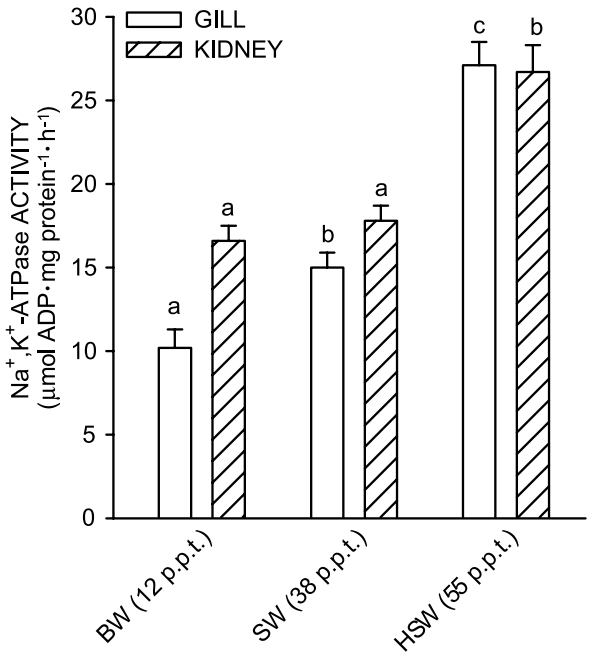


Fig. 1. $Na^+$-$K^+$-ATPase activity in gills and kidney of gilthead sea bream acclimated for 14 days to brackish water (BW; 12 p. p. t.), seawater (SW; 38 p. p. t.), or hypersaline water (HSW; 55 p. p. t.). Each value is the mean ± SE of $n = 10$–12 fish/group. Different letters indicate significant differences among groups for each tissue by one-way ANOVA ($P < 0.05$).

Table 2. *Levels of glucose, lactate, triglyceride, and protein in plasma of gilthead sea bream acclimated for 14 days to BW, SW, or HSW*

| Parameter | Group | | |
|---|---|---|---|
| | BW acclimated | SW acclimated | HSW acclimated |
| Glucose levels | 3.89 ± 0.15* | 4.83 ± 0.12† | 5.77 ± 0.24‡ |
| Lactate levels | 1.85 ± 0.09* | 2.01 ± 0.11*† | 2.29 ± 0.12† |
| Triglyceride levels | 3.19 ± 0.09* | 3.24 ± 0.07* | 3.27 ± 0.12* |
| Protein levels | 32.6 ± 0.5* | 33.1 ± 0.7* | 36.5 ± 0.2† |

Values are means ± SE; $n = 10$–12 fish/group. Units are mmol/l. Different symbols indicate significant differences among groups by one-way ANOVA ($P < 0.05$).

was observed for HK activity. PK activity displayed no significant differences between groups when considering the optimal activity of the enzyme or the FBPase activation ratio; however, the activity ratio increased in parallel, with increased salinity being significantly higher in HSW ($P = 0.037$)- and SW ($P = 0.042$)-acclimated fish compared with BW-acclimated fish. Finally, with G-6-PDH activity, the response was the inverse, i.e., the highest activity was noticed in BW-acclimated fish compared with SW ($P = 0.01$)- or HSW ($P = 0.02$)-acclimated fish.

Kidney metabolic parameters are displayed on Table 4. The levels of glycogen and ATP in this tissue were very close to the limit of detection of the techniques used, so values obtained in only a few individuals were unreliable (data not shown). Thus the only metabolite displayed is lactate, the levels of which increased in parallel with salinity and which was significantly higher in HSW-acclimated fish than in BW ($P = 0.035$)- and SW ($P = 0.019$)-acclimated fish. HK activity decreased in BW- and HSW-acclimated fish compared with SW-acclimated fish ($P < 0.001$). However, no significant changes were observed when comparing fish acclimated to different salinities for GPase and PK activities.

The metabolic parameters assessed in liver are displayed on Table 5. Glycogen levels were significantly lower in BW ($P = 0.041$)- and HSW ($P = 0.025$)-acclimated fish than those in SW-acclimated fish. ATP levels decreased in parallel with the increase in salinity and were significantly higher ($P = 0.033$) in BW- than in HSW-acclimated fish. However, levels of lactate did not show any significant trend among salinities assessed. Changes observed in GPase activity, either total activity or the percentage of the enzyme in the active form, correlate with those of glycogen, since increased activity was observed in BW ($P = 0.031$ and $P < 0.001$ for total activity and %GPase *a*, respectively)- or HSW ($P = 0.024$ and $P < 0.001$ for total activity and %GPase *a*, respectively)-acclimated fish compared with SW-acclimated fish. PK activity was significantly higher in SW-acclimated fish compared with BW- or HSW-acclimated fish, either for the optimal activity of the enzyme ($P = 0.028$ and $P = 0.012$ for BW- and HSW-acclimated fish, respectively) or the activity ratio ($P = 0.03$ and $P = 0.031$ for BW- and HSW-acclimated fish, respectively) and FBPase activation ratio ($P = 0.036$ and $P = 0.021$ for BW- and

Table 3. *Levels of glycogen, lactate, ATP and activities of potential regulatory enzymes in gills of gilthead sea bream acclimated for 14 days to BW, SW, or HSW*

| Parameter | Group | | |
|---|---|---|---|
| | BW acclimated | SW acclimated | HSW acclimated |
| Glycogen levels, μmol glycosyl units/g wet wt | 0.20 ± 0.02* | 0.14 ± 0.03* | 0.17 ± 0.02* |
| Lactate levels, μmol/g wet wt | 2.72 ± 0.38* | 2.28 ± 0.11* | 3.20 ± 0.29* |
| ATP levels, μmol/g wet wt | 3.94 ± 0.17* | 3.57 ± 0.14*† | 3.21 ± 0.19† |
| HK activity, U/mg protein | 0.45 ± 0.02* | 0.55 ± 0.03† | 0.69 ± 0.04‡ |
| PK activity | | | |
| Optimal activity, U/mg protein | 2.99 ± 0.20* | 3.14 ± 0.15* | 3.24 ± 0.09* |
| Activity ratio | 0.49 ± 0.02* | 0.56 ± 0.01† | 0.58 ± 0.01† |
| Fructose 1,6-$P_2$ activation ratio | 0.92 ± 0.03* | 0.93 ± 0.02* | 0.93 ± 0.02* |
| GPase activity | | | |
| Total activity U/mg protein | 0.10 ± 0.01* | 0.09 ± 0.01* | 0.09 ± 0.01* |
| GPase *a*, % | 14.4 ± 4.22* | 6.22 ± 2.07* | 11.4 ± 2.56* |
| G6PDH activity, U/mg protein | 1.03 ± 0.06* | 0.84 ± 0.04† | 0.85 ± 0.05† |

Values are means ± SE; $n$ = 10–12 fish/group. Hexokinase (HK), pyruvate kinase (PK), glycogen phosphorylase (GPase), glucose 6-phosphate dehydrogenase (G6PDH) in gills of gilthead sea bream acclimated for 14 days to BW (12 p.p.t.), SW (38 p.p.t.), or HSW (55 p.p.t.). One unit of enzyme activity is defined for HK as that which, utilizes 1 μmol glucose/min, for PK as that which utilizes 1 μmol phospho*enol*pyruvate/min, for GPase as that which produces 1 μmol NADPH/min, and for G6PDH as that which utilizes 1 μmol glucose 6-phosphate/min. %GPase *a*, percentage of total glycogen phosphorylase ($a + b$) in the active form ($a$). The activity ratio of pyruvate kinase is defined as activity at low (0.1 mmol/l)/high (2.8 mmol/l) substrate (fructose 6-phosphate) concentration. Similarly, a fructose 1,6-bisphosphate (fructose 1,6-$P_2$) activation ratio was determined using 0.01 mmol/l fructose 1,6-bisphosphate concentrations and 0.1 mmol/l phospho*enol*pyruvate concentrations. Different symbols indicate significant differences among groups by one-way ANOVA ($P < 0.05$).

HSW-acclimated fish, respectively). However, FBPase and G-6-PDH activities were not significantly altered by environmental salinity.

The results obtained for parameters assessed in brain are displayed on Table 6. Glycogen levels were significantly lower in BW ($P = 0.029$)- and HSW ($P = 0.022$)-acclimated fish with respect to SW-acclimated fish. Levels of lactate significantly increased in parallel with the increase in salinity ($P < 0.001$). ATP levels also increased in parallel with the increase in salinity, and those measured in HSW-acclimated fish were significantly higher than those measured in BW ($P = 0.005$)- and SW ($P = 0.015$)-acclimated fish. HK activity was significantly higher in BW ($P = 0.019$)- and HSW ($P = 0.005$)-acclimated fish compared with SW-acclimated fish. Changes observed in GPase activity match with those of glycogen levels, because the optimal activity of the enzyme increased in BW ($P = 0.028$)- and HSW ($P = 0.017$)-acclimated fish compared with the activity of SW-acclimated fish. A similar change in activity was also observed for PFK, which was higher in BW- and HSW-acclimated fish than in SW-acclimated fish when considering the optimal activity ($P = 0.027$ and $P = 0.036$ for BW- and HSW-acclimated fish, respectively) and the activity ratio of the enzyme ($P = 0.023$ and $P = 0.014$ for BW- and HSW-acclimated fish, respectively). Finally, G-6-PDH activity increased in parallel with salinity, although the differences were not statistically significant.

## DISCUSSION

Euryhaline fish present the capacity of adapting to different environmental salinities by activation of their

Table 4. *Levels of lactate and activities of potential regulatory enzymes in kidney of gilthead sea bream acclimated for 14 days to HSW*

| Parameter | Group | | |
|---|---|---|---|
| | BW acclimated | SW acclimated | HSW acclimated |
| Lactate levels, μmol/g wet wt | 1.25 ± 0.17* | 1.28 ± 0.21* | 1.99 ± 0.24† |
| HK activity, U/mg protein | 0.09 ± 0.01* | 0.19 ± 0.01† | 0.12 ± 0.02* |
| PK activity | | | |
| Optimal activity, U/mg protein | 3.14 ± 0.20* | 3.29 ± 0.24* | 3.27 ± 0.19* |
| Activity ratio | 0.13 ± 0.01* | 0.14 ± 0.01* | 0.14 ± 0.01* |
| Fructose 1,6-$P_2$ activation ratio | 0.34 ± 0.02* | 0.37 ± 0.01* | 0.35 ± 0.02* |
| GPase activity | | | |
| Total activity U/mg protein | 0.10 ± 0.02* | 0.10 ± 0.01* | 0.08 ± 0.01* |
| GPase *a*, % | 58.1 ± 7.74* | 53.5 ± 6.59* | 53.9 ± 3.56* |

Values are means ± SE; $n$ = 10–12 fish/groups. HK, PK, and GPase assayed in kidney of gilthead sea bream acclimated for 14 days to BW (12 p.p.t.), SW (38 p.p.t.), or HSW (55 p.p.t.). One unit of enzyme activity is defined for HK as that which utilizes 1 μmol glucose/min, for PK as that which utilizes 1 μmol phospho*enol*pyruvate/min, and for GPase as that which produces 1 μmol NADPH/min. The activity ratio of pyruvate kinase is defined as activity at low (0.05 mmol/l)/high (2.8 mmol/l) substrate (fructose 6-phosphate) concentration. Similarly, a fructose 1,6-bisphosphate activation ratio was determined using 0.1 mmol/l fructose 1,6-bisphosphate concentrations and 0.1 mmol/l phospho*enol*pyruvate concentrations. Different symbols indicate significant differences among groups by one-way ANOVA ($P < 0.05$).

Table 5. *Levels of glycogen, lactate, ATP, and activities of potential regulatory enzymes in liver of gilthead sea bream acclimated for 14 days to HSW*

| Parameter | Group | | |
|---|---|---|---|
| | BW acclimated | SW acclimated | HSW acclimated |
| Glycogen levels, μmol glycosyl units/g wet wt | 501.9 ± 42.1* | 627.8 ± 36.3† | 474.7 ± 41.5* |
| Lactate levels, μmol/g wet wt | 0.74 ± 0.11* | 0.79 ± 0.13* | 0.92 ± 0.19* |
| ATP levels, μmol/g wet wt | 1.56 ± 0.28* | 1.12 ± 0.18*† | 0.78 ± 0.11† |
| PK activity | | | |
| Optimal activity, U/mg protein | 0.42 ± 0.03* | 0.54 ± 0.03† | 0.42 ± 0.02* |
| Activity ratio | 0.28 ± 0.02* | 0.37 ± 0.03† | 0.29 ± 0.02* |
| Fructose 1,6-$P_2$ activation ratio | 0.20 ± 0.02* | 0.29 ± 0.02† | 0.21 ± 0.01* |
| GPase activity | | | |
| Total activity, U/mg protein | 0.07 ± 0.01*† | 0.04 ± 0.01* | 0.10 ± 0.02† |
| GPase *a*, % | 38.4 ± 2.82* | 8.30 ± 5.15† | 44.4 ± 4.31* |
| G6PDH activity, U/mg protein | 1.26 ± 0.03* | 1.24 ± 0.03* | 1.29 ± 0.08* |
| FBPase activity, U/mg protein | 0.70 ± 0.04* | 0.75 ± 0.04* | 0.66 ± 0.03* |

Values are means ± SE; $n$ = 10–12 fish/group. PK, GPase, G6PDH, and fructose 1,6-bisphosphatase (FBPase) in liver of gilthead sea bream acclimated for 14 days to BW (12 p.p.t.), SW (38 p.p.t.), or HSW (55 p.p.t.). One unit of enzyme activity is defined for PK as that which utilizes 1 μmol phospho*enol*pyruvate/min, for GPase as that which produces 1 μmol NADPH/min, for G6PDH as that which utilizes 1 μmol glucose 6-phosphate/min, and for FBPase as that which utilizes 1 μmol fructose 1,6-bisphosphate/min. The activity ratio of pyruvate kinase is defined as activity at low (0.1 mmol/l)/high (2.8 mmol/l) substrate (fructose 6-phosphate) concentration. Similarly, a fructose 1,6-bisphosphate activation ratio was determined using 1 mmol/l fructose 1,6-bisphosphate concentrations and 0.1 mmol/l phospho*enol*pyruvate concentrations. Different symbols indicate significant differences among groups by one-way ANOVA ($P < 0.05$).

osmoregulatory system (11, 23). The osmotic parameters in plasma of BW-, SW-, and HSW-acclimated *S. aurata* confirmed the good euryhalinity of this species and agree with previous data obtained by our group (19, 25, 26, 27).

Gill $Na^+$-$K^+$-ATPase activity is related to the capacity of this osmoregulatory organ for extrusion of excess ions in the hyperosmotic environment (30, 32). In this way, the increase observed in the present study in gill $Na^+$-$K^+$-ATPase activity in parallel with the increase in environmental salinity agrees with the physiological role of this ion pump, and with previous data reported for this species (25).

In euryhaline fish, kidney $Na^+$-$K^+$-ATPase activity also presents changes in response to variation in environmental salinity (9, 16a,20, 57). In the black sea bream, *Mylio macrocephalus*, kidney $Na^+$-$K^+$-ATPase activity is lower in fish acclimated to BW (12 ppt) with respect to SW (33 ppt) or HSW (50 ppt; see Ref. 14). A similar trend was observed in the present study, since higher values of kidney $Na^+$-$K^+$-ATPase activity were observed in HSW-acclimated fish compared with those of BW- and SW-acclimated fish. This activation could be attributed to a reduction in urine production and/or to increases in ion transport in the kidney of individuals acclimated to a hyperosmotic environment.

Because the liver is the main site involved in glycogen/glucose turnover in fish, liver metabolism may be enhanced during osmotic adaptation to make glucose available to fuel other metabolic and osmoregulatory processes, specially in osmoregulatory tissues like gills

Table 6. *Levels of glycogen, lactate, ATP, and activities of potential regulatory enzymes in brain of gilthead sea bream acclimated for 14 days to HSW*

| Parameter | Group | | |
|---|---|---|---|
| | BW acclimated | SW acclimated | HSW acclimated |
| Glycogen levels, μmol glycosyl units/g wet wt | 0.06 ± 0.01* | 0.11 ± 0.01† | 0.07 ± 0.01* |
| Lactate levels, μmol/g wet wt | 5.77 ± 0.25* | 6.74 ± 0.24† | 8.82 ± 0.20‡ |
| ATP levels, μmol/g wet wt | 0.17 ± 0.02* | 0.20 ± 0.03* | 0.30 ± 0.03† |
| HK activity, U/mg protein | 0.40 ± 0.07* | 0.15 ± 0.05† | 0.52 ± 0.05* |
| PFK activity | | | |
| Optimal activity, U/mg protein | 3.60 ± 0.28* | 2.77 ± 0.22† | 3.90 ± 0.35* |
| Activity ratio | 0.13 ± 0.02* | 0.06 ± 0.01† | 0.11 ± 0.01* |
| Fructose 2,6-$P_2$ activation ratio | 0.22 ± 0.04* | 0.33 ± 0.06* | 0.33 ± 0.09* |
| GPase activity | | | |
| Total activity, U/mg protein | 0.25 ± 0.03* | 0.12 ± 0.02† | 0.31 ± 0.04* |
| GPase *a*, % | 59.2 ± 9.9* | 77.5 ± 22.3* | 49.4 ± 13.3* |
| G6PDH activity, U/mg protein | 0.11 ± 0.02* | 0.18 ± 0.07* | 0.20 ± 0.04* |

Values are means ± SE; $n$ = 10–12 fish/group. HK, 6-phosphofructo 1-kinase (PFK), GPase, and G6PDH in brain of gilthead sea bream acclimated for 14 days to BW (12 p.p.t.), SW (38 p.p.t.), or HSW (55 p.p.t.) One unit of enzyme activity is defined for HK as that which utilizes 1 μmol glucose/min, for PK as that which utilizes 1 μmol phospho*enol*pyruvate/min, for GPase as that which produces 1 μmol NADPH/min, and for G6PDH as that which utilizes 1 μmol glucose 6-phosphate/min. The activity ratio of 6-phosphofructo 1-kinase is defined as activity at low (0.1 mmol/l)/high (5 mmol/l) substrate (fructose 6-phosphate) concentration. Similarly, a fructose 1,6-bisphosphate activation ratio was determined using 5 μmol/l fructose 2,6-bisphosphate concentrations and 0.1 mmol/l fructose 6-phosphate concentrations. Different symbols indicate significant differences among groups by one-way ANOVA ($P < 0.05$).

and kidney (58). In the present study, liver glycogen levels decreased in BW- and HSW-acclimated fish compared with SW-acclimated fish, suggesting that acclimating *S. aurata* to salinities lower or higher than those of the natural environment induces an enhancement of the energy requirements of the liver through increased glycogenolysis. Increased liver glycogenolysis has been usually observed in fish in SW compared with those in FW or BW (10) either in euryhaline species such as tilapia (1, 58), black sea bream (14), and rainbow trout (50, 53) or in stenohaline species such as carp (8). In a similar way, several studies have addressed decreased liver glycogen levels when fish are transferred from SW to HSW (14, 40). The decrease observed in glycogen levels in the present study agrees well with changes observed in GPase activity, which showed an enhancement in BW- and HSW-acclimated fish compared with SW-acclimated fish. This enhancement was observed in both the total activity of the enzyme and the percentage of the enzyme in the active form. Similar changes in GPase activity had been observed previously in fish transferred from FW to SW, such as in rainbow trout (50) or in tilapia (40). In contrast, in fish acclimated to HSW, Nakano et al. (40) failed to address any significant difference in GPase activity compared with fish in SW. The mobilization of liver glycogen would provide glycosyl units ready to be used to fuel endogenous pathways such as glycolysis or to be exported to other tissues.

The enhancement of glycogenolysis in livers of BW- or HSW-acclimated *S. aurata* is accompanied by changes in liver ATP levels that decreased in parallel with increased salinity. In no comparable study has this parameter been assessed. When the activity of the glycolytic enzyme PK is considered, changes displayed were of a similar trend to those of glycogen levels, i.e., increased activity in BW- and HSW-acclimated fish compared with those in SW, suggesting that under those salinity conditions the highest energy requirements of the liver were taking place. In other studies, changes in the glycolytic capacity of the liver were evaluated when fish were transferred from FW to SW and HSW, displaying an enhancement in that capacity (14, 40, 50, 58). The drop in ATP levels in parallel with the increased salinity may suggest a lower energy requirement in livers of BW- compared with those of HSW-acclimated fish, i.e., that the production of ATP raised from glycosyl units exceeds ATP consumption in BW- compared with SW- and HSW-acclimated fish. Thus the liver of BW-acclimated fish may have a lower capacity to export glucose to plasma, which would agree with the finding of decreased liver glucose 6-phosphatase activity already reported in BW-acclimated fish compared with SW- and HSW-acclimated fish (64). Hence, the portion of glucose obtained from liver mobilization and therefore capable of being used in other tissues is probably higher in SW- and HSW- than in BW-acclimated fish. This lower energy requirement in BW-acclimated fish would agree with the fact that gilthead sea bream is known to grow better at 12 ppt than at 38 ppt salinity (R. Láiz-Carrión, M. P. Martín del Río, and J. M. Mancera, unpublished observation).

A hypothetical increase in the glucose-exporting capacity of the liver as a result of glycogen mobilization has not been assessed but is in good agreement with the absence of changes in the use of glucose through other pathways, including the pentose phosphate pathway for which no changes were noticed in liver G-6-PDH activity. The absence of changes in the activity of this enzyme is in agreement with data obtained during SW acclimation in tilapia (59) but not with other studies carried out in other species during SW acclimation (13, 14, 40) or BW acclimation (64). The capacity of liver to synthesize glucose from other fuels via gluconeogenesis does not appear to be modified by the osmotic challenge, based on the absence of any changes in FBPase activity, in contrast with the increase observed in BW- compared with SW-acclimated red sea bream (65).

Plasma glucose levels increased in parallel with the increase in salinity in the present study. The source for this increased level could be, at least in HSW, the mobilization of liver glycogen. Increased plasma glucose levels had been previously observed during acclimation from FW or BW to SW in several species of fish such as sea bass (44), rainbow trout (50), cutthroat trout (38), tilapia (39, 41), or carp (8), suggesting a mobilization of glucose to peripheral tissues to satisfy the increased energetic demand observed in osmoregulatory organs during SW acclimation. When euryhaline fish usually living in SW are acclimated to BW or FW, both decreases, such as in red sea bream (66), or increases, such as in tilapia (1), silver sea bream (16), and gilthead sea bream (26), have been reported in plasma glucose levels. Several reasons may explain the absence of such an increase in this study when comparing SW- and BW-acclimated fish, including *1*) a fast glucose turnover in BW, *2*) a different source of fuel to be used under the different energy requirements of both osmotic conditions, or *3*) BW may be less stressful than HSW.

Another interesting fuel reported to be used in tissues like gills and kidney for their energy requirements is lactate (34, 36). The increase of plasma lactate levels observed in parallel with increased salinity suggests that this metabolite becomes more important in hyperosmotic conditions, presumably related to its metabolic use in osmoregulatory organs. This increase in plasma lactate levels is comparable with the decrease observed in SW-acclimated red sea bream when transferred to diluted SW (66), whereas increased plasma lactate levels were also reported in tilapia after acclimation to SW (58). As for the source of this lactate, no significant changes were observed in liver lactate levels in fish acclimated to different salinities, suggesting that the source of increased plasma levels of lactate must be of extrahepatic nature.

The other metabolites assessed in plasma of fish acclimated to different salinities were TG and protein. TG levels did not change in any group of fish, suggesting that the enhanced production of TG already reported in plasma of Atlantic salmon during smoltifica-

tion (42) do not appear to occur during osmotic acclimation, at least in *S. aurata*. However, considering that TG levels are known to decrease in liver and muscle of Atlantic salmon during SW acclimation (47), a possible metabolic role for TG cannot be excluded. As for plasma protein levels, these increased in parallel with the increase in salinity. This is in contrast with other studies reporting either no changes (66) or decreases (15) in plasma protein levels in parallel with increased salinity. The possible importance of increased plasma protein as a fuel for tissues during osmotic acclimation has not been addressed yet but may be related to a metabolic reallocation of energy resources in hyperosmotic environments once carbohydrate stores have been mobilized.

The gill constitutes the organ with probably the most active ion transport system in the fish. Whereas transport systems and associated ATPases have been described in ample detail (30, 63), biochemical aspects of the mechanisms supplying the required ATP have been only slightly studied to date (34). Our results showed that gill $Na^+$-$K^+$-ATPase activity increased in parallel with the increase in environmental salinity (12 ppt $<$ 38 ppt $<$ 55 ppt), in agreement with the accepted osmoregulatory role for this ion pump (30, 32).

Fish gills are highly oxidative tissues, even in FW, and its oxygen requirement increases even further when fish are transferred to SW (58). The energy requirement of the gills is thought to be maintained by oxidation of glucose and lactate obtained from the circulation (34). Although gill HK activity is usually low in teleosts, it is apparently active enough to pace the $CO_2$ release values observed in that tissue (34). In the present study, gill HK activity increased linearly, with salinity being lowest in BW- and highest in HSW-acclimated fish. Changes in the activity of this enzyme during osmotic acclimation had only been observed during acclimation of rainbow trout to SW displaying an enhanced activity (52). In this study, an important range of salinity (BW, SW, and HSW) was assessed, suggesting that an increased use of exogenous glucose occurs in gills in parallel with the increase in salinity. A simultaneous enhancement of the glycolytic capacity of gill tissue in SW- and HSW-acclimated fish is suggested based on changes observed in PK activity. This increased glycolytic capacity agrees with data obtained in black sea bream transferred from BW to SW and HSW (14) and rainbow trout transferred from FW to SW (52). Moreover, the decrease of ATP levels in gills along with salinity coincides with a similar decrease in gill ATP levels observed previously during acclimation of rainbow trout to SW (21, 44) and further suggests an enhancement of energy demand. The fall in ATP levels in gills should be correlated with $Na^+$-$K^+$-ATPase activity. Because both parameters were only slightly correlated, other ATP-consuming pumps like $H^+$-ATPase and $Ca^{2+}$-ATPase (30) could be involved in the acclimation of gilthead sea bream to different salinities.

The suspected increased energy demand of the gills appears to be fueled at least by one exogenous fuel such as glucose, since levels of endogenous glycogen and lactate did not change in gills. Considering the low amount of glycogen accumulated in gill tissue, it is not surprising to see no changes in the glycogenolytic potential, since a raised metabolic demand in gills would not be sufficiently covered by glycogen mobilization. Because gill tissue is able to oxidize lactate at rates comparable to those of glucose (34), and considering the raise observed in plasma lactate levels in parallel with increased salinity, an enhancement of the use of exogenous lactate through lactate dehydrogenase working in the oxidative direction cannot be excluded. Finally, more evidence for an increased energy demand in gills in high salinities comes from G-6-PDH activity, which showed a clear decrease in parallel with increased salinity. This result is in partial agreement with the decrease already described by Kültz and Jürss (18) in tilapia transferred from FW to SW. The subsequent lower capacity of the pentose phosphate pathway appears to be logical considering that this is an energy-consuming process that competes with glycolysis for glucose.

Altogether, the data obtained in gills lend support for an increased energy demand in parallel with the increased environmental salinity, as suggested by increased $Na^+$-$K^+$-ATPase activity, decreased ATP levels, and increased glycolytic capacity. This increase in demand appears to be fueled by an enhanced use of exogenous glucose, although the possibility of lactate being also used cannot be excluded. However, in *S. aurata* gill, $Na^+$-$K^+$-ATPase activity shows a "U-shaped" salinity dependency, with higher values in low (5 ppt) and high (55 ppt) salinities with respect to SW (38 ppt) and BW (15 ppt; P. Guerreiro, R. Láiz-Carrión, J. Fuentes, and J. M. Mancera, unpublished observations). In this way, it will be interesting to study how gill energy metabolism changes in *S. aurata* acclimated to a very low environmental salinity (5 ppt).

Glucose appears to be an important exogenous substrate for the kidney in teleosts based on *1*) the high activities of PK and G-6-PDH measured in that tissue (36) and *2*) its rates of glucose use, which are similar to those observed in tissues with important rates such as brain and gills (3). The osmoregulatory assessment of kidney under the present experimental conditions showed that $Na^+$-$K^+$-ATPase activity was higher in HSW-acclimated than in BW- and SW-acclimated fish. In addition, a sharp increase in kidney lactate levels in parallel with the increase in salinity was also observed in HSW-acclimated fish compared with BW- and SW-acclimated fish, suggesting an increased importance for this metabolite in HSW-acclimated fish. In Atlantic salmon, the acclimation to SW induced a decrease in citrate synthase and cytochrome oxidase activities that lend McCormick et al. (33) to suggest a decreased activity of this tissue in higher salinities because of the fact that, after SW adaptation, the teleost kidney produces smaller volumes of a more concentrated urine than is produced in FW. The absence of changes in kidney $Na^+$-$K^+$-ATPase activity when comparing BW- and SW-acclimated fish also suggests that a lower energy demand occurs in fish acclimated to BW and

SW compared with those in HSW. When addressing kidney HK activity in the present experiment, a sharp decrease was noticed in BW- and HSW-acclimated fish compared with SW-acclimated fish, suggesting that the necessity of exogenous glucose was lower in those salinities different from usual. In BW-acclimated fish, a reduction in activity of the kidney could be expected because the osmotic and ionic gradients between the fish body and the environment are minimum. However, in HSW-acclimated fish, an increased excretion of ions by the kidney could be necessary; thus, our metabolic results would lend support for an increased use of another fuel instead of glucose to support the increased osmoregulatory work of the kidney in HSW. Considering the low amount of endogenous glycogen, this metabolite does not seem important for fueling purposes in the kidney, and another fuel, namely lactate, whose levels increased in plasma and kidney simultaneously, could be an ideal candidate for being increasingly used in HSW-acclimated fish. Despite not measuring how ATP levels changed in kidney of HSW-acclimated fish, the increased necessity of ATP to fuel ATPase activity in HSW-acclimated fish should be sufficiently covered by a hypothetical increased oxidation of lactate. The absence of changes in the remaining parameters assessed may also lend support to the lower activation of kidney metabolism during osmotic acclimation and coincides with similar results obtained by Kelly et al. (14) in kidneys of black sea bream in which no changes in parameters related to glycolysis, pentose phosphate shunt, and glucose export capacities in fish acclimated to SW and HSW were observed.

Only one previous study (62) addressed metabolic changes in fish brain associated with osmotic adaptation, describing changes in ATP levels and creatine kinase activity in tilapia during the first hours of transfer from FW to SW. The results obtained in the present experiments demonstrate that acclimation of *S. aurata* to salinities either lower or higher than normal produces a mobilization of brain glycogen levels, which constitutes the major energy store of fish brain (49). This mobilization can be perfectly attributable to changes observed in the activity of GPase. The role of this mobilization is not known but could be related to a stress effect of salinity in brain metabolism that leads this tissue to activate processes involved indirectly in the osmoregulatory work. In fact, stress hormones such as cortisol (19) and catecholamines (45) produce changes in brain energy metabolism similar to those described in the present study. Furthermore, the important increase observed in HK activity in fish acclimated to extreme salinities compared with those acclimated to SW further reflects that the necessity of glucose, the main fuel of brain energy metabolism in teleosts (51), is increased under the stress conditions imposed by the acclimation to extreme salinity environments. These changes are reflected in an increased energy demand based on the high glycolytic potential observed in BW- and HSW-acclimated fish, as suggested by changes displayed by PFK activity. This increased glycolysis may be related to the increase described by Weng et al. (62) in both $Na^+$-$K^+$-ATPase and creatine kinase activities in brain of tilapia transferred from FW to SW. Another interesting finding was the increase in brain ATP and lactate levels in parallel with salinity, suggesting that the increased use of carbohydrates is higher than the energy demand of the brain, producing an accumulation of both lactate and ATP as a result. This accumulation also probably reflects that the energy demand of the brain decreases at lower salinities, since BW appears to be of a less stressor capacity than HSW.

The connection between changes in the osmotic condition of the fish and the different metabolic responses of the different tissues described is not known. We can only hypothesize that those hormones in which levels change dramatically during acclimation to different osmotic conditions in euryhaline fish (32) and also in *S. aurata* (25) such as prolactin, cortisol, or growth hormone may be involved in the process. For all those hormones, several metabolic effects have been demonstrated in liver of euryhaline fish that are similar, at least in part, to those described in the present study (22, 35, 46). In gills and kidney, prolactin, cortisol, and growth hormone are inductors of osmoregulatory changes, whereas in gills IGF-I is the local mediator of $Na^+$-$K^+$-ATPase activity (32). Therefore, those hormones become the main candidates for a regulatory role in the energy metabolism of both osmoregulatory tissues during osmotic adaptation.

As for the possible regulation of brain metabolism, considering that *1*) cortisol levels increase in plasma during osmotic acclimation in euryhaline fish (32, 61), *2*) we have recently demonstrated that cortisol produces in *S. aurata* a clear enhancement of brain glycolytic capacity (19), and *3*) SW-acclimated specimens of *S. aurata* also showed higher values of cortisol than BW-acclimated fish (P. Rotllant and J. M. Mancera, unpublished), a possible role for cortisol as a mediator of the metabolic changes described in brain cannot be discarded. Another possible regulator of the metabolic changes described in brain may be the neurohypophysial hormone arginine vasotocin (AVT), since *1*) AVT levels change during osmotic adaptation (60), and *2*) AVT induces changes in energy metabolism of fish brain either directly or indirectly through changes in brain monoaminergic neurotransmitters (S. Sangiao-Alvarellos, M. Lapido, J. M. Miguel, and J. L. Soengas, unpublished observation), which are known to alter brain energy metabolism in mammals (24) and fish (45).

In summary, after 14 days of acclimation to different environmental salinities, *S. aurata* displayed a tissue-specific reorganization of energy metabolism in liver, gills, kidney, and brain that can be summarized by increased energy expenditure, a reallocation of resources, and a depletion of carbohydrate reserves. It is of particular importance to remark that brain and liver, as judged by the number of statistically significant changes and amplitude of these, are even more metabolically responsive to a change in ambient salin-

ity than the established osmoregulatory organs kidney and gills.

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## DISCLOSURES

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