OSMOREGULATORY RESPONSES TO ABRUPT SALINITY CHANGES IN THE EURYHALINE GILTHEAD SEA BREAM (SPARUS AURATA L.)

J. M. MANCERA,* t J. M. PEREZ-FIGARES* and P. FERNANDEZ-LLEBREZ†

*Departamentos de Biologia Celular; †Biolégia Animal, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain (Tel. 213-1956; Fax 213-2100)

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Abstract—1. Gilthead sea breams ( Sparus aurata L.) adapted to sea water (SW, 39% salinity) and brackish water (BW, 7%) were submitted to abrupt osmotic stress by transferring the specimens to 7% and 39%, respectively.
2. Plasma osmolality, Na+, Cl−, K+, Ca2+, cortisol and glucose were measured before and after the transfers.
3. The transfer from SW to BW led to transitory hypomineralization and hyperglycemia. In long-term adapted fish cortisol level increased, and osmolality slightly decreased.
4. Conversely, the transfer from BW to SW provoked transitory hypermineralization. In adapted fish, cortisol levels strongly decreased, and osmolality slightly increased.

INTRODUCTION

It is known that whereas stenohaline teleosts can face only small changes in the environmental salinity, euryhaline teleosts can live in a broad range of environmental salinities (see reviews by Holmes and Donaldson, 1969; Maetz, 1974). When an euryhaline teleost goes from hyperosmotic to hypoosmotic media it tends to lose ions and gain water; in the inverse transference it tends to lose water and gain ions. The physiological response is oriented to the maintenance of a stable internal milieu: ionic regulation and water balance. The organs involved in osmoregulation are: gills, gut, kidney and urinary bladder. The osmotic stress provokes changes in the plasma level of hormones such as prolactin, cortisol and adrenaline (Bern, 1975; Assem and Hanke, 1981; Redding and Schreck, 1983; Abo Hegab and Hanke, 1984; Hirano, 1986). Metabolic indicators such as glucose, transaminases, and lipids may also vary (Assem and Hanke, 1979; Woo and Wu, 1982; Roché et al., 1983; Roché et al., 1989).

The gilthead sea bream ( Sparus aurata L.), is an euryhaline and eurytermic teleost. That is to say, the fish are able to live in environments of different salinities and temperatures namely: coastal water (sea water, SW), estuaries (brackish, BW) and lagoons (hypersaline). Their presence in one or the other of these different habitats depends on the particular stage of the reproductive cycle. Gonadal growth and maturation takes place in estuaries and coastal waters but for the final maturation and egg-laying the animals must migrate to the open sea (Arias, 1976; Suau and López, 1976; Ben-Tuvia, 1979). Thus, under natural conditions Sparus aurata cope with a wide range of variations in environmental salinities.

The capability of young sea bream to adaptate to brackish water has been studied before (Chervinski, 1984). These studies revealed that juvenile specimens of gilthead sea bream were able to adapt and grow in brackish water, for 2 months at least under laboratory conditions. However, at present, there is no data about the change in plasma parameters during the adaptation.

The aim of the present investigation was to analyse the changes in osmoregulatory plasmatic parameters, as a result of abrupt osmotic stress due to transference from SW to BW or vice-versa. Osmolality, plasma levels of Na+, Cl−, K+, Ca2+, glucose and cortisol have been measured. The results will be discussed in relation to the osmoregulatory processes in this species.

MATERIALS AND METHODS

Specimens of gilthead sea bream ( Sparus aurata L.) (body wt approx. 100 g) were provided by an experimental fish culturing centre (El Toruíno, Pemares, El Puerto de Santa María, Cádiz, Spain); where the animals stayed during the experimental period (May–June, 1989). Two animal groups ( N = 120 each), were previously acclimatized for 3 months to sea water (SW, salinity ≈ 39%) and brackish water (BW, salinity ≈ 7%). The animals stayed in 1.500 l cylindric tanks with permanent water turnover (5 l/min) and supplied with oxygen. They were fed twice a day (9 a.m. and 6 p.m.) with Illex sp.

The fish were exposed to abrupt salinity changes

†To whom all correspondence should be addressed.
from SW to BW and from BW to SW (end of May, 1989); where they stayed for 1 month (June 1989), under natural environmental conditions of photoperiod and water temperature (18°–24°C). The animals were tested in their original media, SW or BW (0 hr), and 12 hr, 1, 2, 3, 4, 15 and 30 days after being transferred to BW or SW, respectively (N = 15 for each time). Before each sampling, the fish were fasted for 24 hr and after the transference fish were not fed for 4 days. There was no mortality in either groups during the experiments.

Fish were anesthetized with tricaine (MS-22, Sigma E1626, St Louis, MO) (0.065 g/l water). The blood was extracted from the caudal artery using insulin heparinized syringes, as rapidly as possible, and collected into Eppendorff tubes. One ml of blood was obtained in each extraction. The blood was centrifuged for 20 min at 3000 r.p.m., and the plasma stored at -30°C.

Ten μl of each sample were used to measure the osmolality in a Fiske One-Ten Osmometer. The Na+, K+, Cl-, and Ca2+ levels were determined, in 200 μl of plasma, by means of selective electrodes in an Automated Stat/Routine Analyzer Systems (Beckman’s Synchron Astra, Palo Alto, CA). Glucose levels were measured in 100 μl of plasma in the same analyser. In the statistical analysis an ANOVA test (N = 15 for each time) as utilized.

The serum concentration of cortisol was measured, using a DPC Gambyt CR gamma γ counter with a computer RIA programme for cortisol of DPC. The radioactivity was quantified in 50 μl of each sample, using a Coat-A-Count Cortisol125I Kit (Diagnostic Products Corporation, Los Angeles, NV). The radioactivity was quantified using a DPC Gambyt CR gamma γ counter with a computer RIA programme for cortisol of DPC. The intra-assay precision (mean of variation coefficient of 12 duplicates) was 9.9%. The inter-assay precision (mean of variation coefficient of 12 duplicates) was 11–14%. The assay sensibility, smallest detected concentration, was 2 ng/ml. In the statistical analysis a Kruskal–Wallis test (N = 10 for each time) was utilized.

**RESULTS**

**SW to BW**

There was a strong decrease in the plasma osmolality during the first 12 hr after the transition from SW to BW. Then it gradually recovered until, 30 days after the transition to BW, the plasma osmolality reached a constant value slightly lower than that measured in SW fish (Table 1).

The plasma levels of Na+ and Cl− changed concomitantly with the osmolality, showing a decrease in the first 12 hr after the transference and a complete recovery at 24 hr. The Na+ and Cl− concentration did not differ from the values of SW fish during the remaining experimental time. The Na+/Cl− ratio had the same value in SW and in BW adapted animals; however a weak increase of this index occurred during the first days after the transference due to a faster recovering of the Na+ plasma values with respect to Cl− (Table 1). The plasma concentration of Ca2+ gradually decreased and it partially recovered after 4 days. However, in fishes long-term adapted to BW the Ca2+ levels remained lower than in SW adapted fish (Table 1).

The K+ levels behaved irregularly during the first 2 days after the transference, but on day 3 they recovered a stable value similar to that of SW adapted fishes (Table 1). The existence of a peak after the transference (12 hr) coincided with the presence of haemolysis in the extracted plasma at this time.

The plasmatic level of cortisol increased reaching a peak at 12 hr; then it recovered its original value at 24 hr. Later, as the time of permanency in BW proceeded, cortisol value increased again until, in the animals adapted to BW for 15 to 30 days, it reached a 3-fold value with respect to the animals adapted to SW (0 hr = 43.0 ± 14.2 ng/ml, 30 days = 140.6 ± 22.1 ng/ml; P < 0.001) (Fig. 1).

Plasma glucose showed a pattern of variation quite similar to cortisol with a strong increase at 12 hr and a posterior decrease at 24 hr. However the animals adapted for 30 days to BW showed plasma glucose values only slightly higher than those observed in SW adapted fishes (Fig. 1).

**BW to SW**

In the transference from BW to SW the osmolality increased and reached a peak at 12 hr, but 2 days later the original value of BW was totally recovered. However, 15 days after the transference, plasma osmolality was slightly higher in SW than in BW (Table 2).

The plasma concentration of Na+ and Cl− changed in the same way. After an initial increase in

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**Table 1. Osmolality, Na+, Cl−, K+, and Ca2+ levels and Na+−Cl− ratio during abrupt transference from SW (39% salinity) to BW (7% salinity)**

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</thead>
<tbody>
<tr>
<td>Time (hr/day)</td>
<td>0 hr</td>
<td>12 hr</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>15</td>
<td>30</td>
<td></td>
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<tr>
<td>Osmolality</td>
<td>396.4 ± 9.3</td>
<td>339.7 ± 8.1</td>
<td>347.6 ± 8.1</td>
<td>362.1 ± 8.4</td>
<td>361.6 ± 9.4</td>
<td>368.2 ± 7.3</td>
<td>380.9 ± 8.7</td>
<td>382.2 ± 8.6</td>
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<tr>
<td>Na+</td>
<td>181.7 ± 5.7</td>
<td>175.8 ± 5.3</td>
<td>183.5 ± 5.4</td>
<td>180.0 ± 6.2</td>
<td>178.0 ± 5.5</td>
<td>181.8 ± 2.8</td>
<td>180.9 ± 3.0</td>
<td>180.1 ± 3.8</td>
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<tr>
<td>K+</td>
<td>152.6 ± 2.9</td>
<td>145.4 ± 5.2</td>
<td>151.8 ± 6.6</td>
<td>148.0 ± 6.2</td>
<td>149.3 ± 6.9</td>
<td>152.6 ± 5.1</td>
<td>153.0 ± 4.2</td>
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<tr>
<td>Na+/Cl−</td>
<td>1.16</td>
<td>1.20</td>
<td>1.20</td>
<td>1.23</td>
<td>1.18</td>
<td>1.19</td>
<td>1.18</td>
<td>1.16</td>
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<td>Ca2+</td>
<td>3.93 ± 0.19</td>
<td>4.28 ± 0.19</td>
<td>3.77 ± 0.23</td>
<td>4.16 ± 0.22</td>
<td>3.96 ± 0.24</td>
<td>4.04 ± 0.21</td>
<td>3.94 ± 0.29</td>
<td>3.95 ± 0.22</td>
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</table>

Values are the mean ± SD (N = 15). Osmolality in mOsm/kg and ions in mM/l. *P < 0.001; †P < 0.01; ‡P < 0.05 with respect to the values observed in SW (ANOVA test).
Osmoregulation in *Sparus*

**Fig. 1.** Changes in cortisol and glucose levels after an abrupt transference from SW (39%) to BW (7%). The points are the mean ± SD (N = 15 for glucose, ANOVA test; N = 10 for cortisol, Kruskal-Wallis test). *P < 0.001; **P < 0.01 with respect to the values observed in SW.

The first period of the experiment (12–24 hr), they tended to recover their original values. In the fish adapted to SW for 15 and 30 days, the values of Na⁺ and Cl⁻ were higher than those found in BW adapted fishes (Table 2). The Na⁺–Cl⁻ ratio decreased in the first 12 hr as a consequence of the higher increase of the plasma Cl⁻ values with respect to Na⁺ values.

Table 2. Osmolality, Na⁺, Cl⁻, K⁺, and Ca²⁺ levels and Na⁺–Cl⁻ ratio during abrupt transference from BW (7% salinity) to SW (39% salinity)

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>7 39 39 39 39 39 39 39</th>
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<tbody>
<tr>
<td>Time (hour/day)</td>
<td>0 hr 12 hr 1 2 3 4 15 30</td>
</tr>
<tr>
<td>Osmolality</td>
<td>376.0 ± 6.5 394.4 ± 7.5* 380.1 ± 7.9 374.9 ± 7.7 381.0 ± 4.6 379.8 ± 7.0 386.8 ± 7.5 390.3 ± 6.34*</td>
</tr>
<tr>
<td>Na⁺</td>
<td>177.7 ± 7.8 184.0 ± 7.2 184.4 ± 6.7 177.9 ± 8.7 177.7 ± 4.0 176.4 ± 3.8 189.9 ± 7.7* 189.1 ± 7.7*</td>
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<tr>
<td>Cl⁻</td>
<td>157.1 ± 6.0 169.3 ± 5.5* 168.1 ± 6.21 162.9 ± 2.2 163.3 ± 5.0 162.0 ± 3.2 174.0 ± 6.3* 175.9 ± 5.0*</td>
</tr>
<tr>
<td>Na⁺–Cl⁻</td>
<td>1.13 1.09 1.10 1.10 1.09 1.09 1.09 1.09</td>
</tr>
<tr>
<td>K⁺</td>
<td>4.03 ± 0.21 4.08 ± 0.25 4.22 ± 0.29 4.00 ± 0.25 3.86 ± 0.36 3.73 ± 0.20 4.05 ± 0.22 4.04 ± 0.22</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>3.13 ± 0.24 3.06 ± 0.23 2.96 ± 0.21 2.60 ± 0.18* 2.63 ± 0.18* 2.64 ± 0.14* 2.96 ± 0.22 2.19 ± 0.19</td>
</tr>
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</table>

Values are mean ± SD (N = 15). Osmolality in mOsm/kg and ions in mM/l. *P < 0.001; †P < 0.01; ‡P < 0.05 with respect to the values observed in BW (ANOVA test).
Fig. 2. Changes in cortisol and glucose levels after an abrupt transference from BW (7%) to SW (39%). The points are the mean ± SD (N = 15 for glucose, ANOVA test; N = 10 for cortisol, Kruskal-Wallis test). *P < 0.001; **P < 0.01 with respect to the values observed in BW.

Cortisol and glucose

It has been reported that euryhaline teleosts submitted to any kind of abrupt salinity changes suffers a transitory increase in the cortisolemia; but when the animals are adapted to the new environments the original cortisol levels are fully recovered (Anguilla anguilla: Leloup-Hatey, 1974; Chrysophrys major: Ishioka, 1980; Sarotherodon mossambicus: Assem and Hanke, 1981; Oncorhynchus kisutch: Redding and Schreck, 1983; Fundulus heteroclitus: Jacob and Taylor, 1983; Cyprinus carpio: Abo Hegab and Hanke, 1984).

Cortisol has been reported to be an important hormone for the adaptation to SW of many euryhaline teleosts by its ability to stimulate the differentiation of the chloride cells and to increase the activity of their Na⁺-K⁺-ATPase (Henderson et al., 1970; Johnson, 1973; Sandor and Mehdi, 1980; Foskett et al., 1983). After the transference from BW to SW, during the adaptative phase, the cortisol level rise in Sparus aurata. However, during the regulatory phase, the cortisol values dramatically decreased, thus indicating that in long term SW adapted fish high cortisol levels are not required for osmoregulation.

After the transference from SW to BW cortisol values of Sparus aurata reach a peak at 12 hr and recover the initial values at 24 hr. From this time a gradual increase in the cortisol concentration occurs until, in animals adapted long-term to BW, a high and stable value is reached. The hormonal peak at 12 hr (adaptative period) coincides with the lowest plasma osmolality and Na⁺ and Cl⁻ levels. Since hypoosmolality and hypomineralization are stressing circumstances, the transitory higher corticolemia at 12 hr can be regarded as a response of the animal to
stress. This reaction is accompanied by a hyperglycemic peak characteristic of this circumstance (Assem and Hanke, 1979; Donaldson, 1981; Abo Hegab and Hanke, 1984).

The maintenance of high and stable amounts of plasma cortisol (3-fold) in Sparus aurata long-term adapted to BW with respect to SW adapted suggests additional functions for cortisol different from that derived of stress. In the sea bassDicentrarchus labrax adapted to hyposmotic (5% salinity) media high (4-fold) cortisolemia was also reported (Roche et al., 1989).

The high amounts of plasma cortisol may be due to an increased release and/or a decreased catabolism. Firstly, in Sparus aurata adapted to BW we have indirect evidence suggesting an activation of the inter-renal tissue. In a semiquantitative immunocytochemical investigation of the ACTH cells of the adenohypophysis of Sparus aurata we have observed an increased release of ACTH in BW fish thus leading to an activation of the release of cortisol by the inter-renal tissue (Mancera et al., 1993). On the other hand, cortisol catabolism has been reported to increase in Anguilla anguilla and Salmo salar placed in sea water (Leloup-Hatey, 1974; Nichols and Weisbart, 1985) and to decrease in Oncorhynchus nerka adapted to freshwater (Donaldson and Dye, 1970). In Sparus aurata the rise in cortisol in hyposmotic environment could also be due to a decreased catabolism.

In other teleost species it has been shown that cortisol plays an osmoregulatory role in hyposmotic media by acting, synergistically with the prolactin, increasing the ionic intake in the digestive tract and the kidney (Henderson et al., 1970; Johnson, 1973; Sandor and Mehdi, 1980; Foskett et al., 1983). The high cortisolemia of Sparus aurata in hyposmotic environment suggest also an osmoregulatory function for this hormone in this media.

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REFERENCES


