Growth performance and osmoregulation in the shi drum (Umbrina cirrosa) adapted to different environmental salinities

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A B S T R A C T

In order to investigate the ability of shi drum (Umbrina cirrosa) to be reared at diverse locations, growth and osmoregulatory performance were assessed at full-strength seawater (40 psu), nearly iso-osmotic water (10 psu) and low salinity water (4 psu). At the end of the 64-day experimental period, fish reared at 4 psu displayed shorter mean fork length, lower mean body weight, lower specific growth rate and higher food conversion efficiency than fish reared at 10 or 40 psu. The effect of salinity on growth performance was also reflected by changes in plasma triglycerides and cholesterol, with fish reared at 4 psu exhibiting the lowest mean concentrations, while there was no significant difference in mean plasma glucose concentrations among treatments. Plasma osmolality was lower at 4 psu from day 42 onwards, while there was no significant difference in mean plasma K- and Cl- concentrations. Plasma sodium and gill Na+/K+ -ATPase activity showed minimum values on day 42 at 4 psu, but at the end of the experiment there was no difference among groups. Pavement cells, mucus cells and chloride cells were identified by histology on the gill epithelium. In shi drum reared at full seawater, mucus cells contained a mixture of acid and neutral mucins, whereas in fish adapted to hypo-osmotic environment neutral mucins were mainly observed. There was a significant increase in chloride cell number over the course of the study in all fish, but there was no difference among the three experimental salinities. Finally, in fish reared at 40 psu salinity, chloride cells increased in size to a significantly larger extent than fish adapted to 4 psu, whereas at 10 psu after 42 they there was a significant reduction in chloride cell size. These results indicate that shi drum reared from full-strength seawater to iso-osmotic salinity do not face any osmoregulatory imbalance, while fish reared in hypo-osmotic water displayed osmoregulatory impairment and low growth performance.

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1. Introduction

Euryhaline fishes exhibit the ability to maintain relatively constant ionic composition of plasma, lymph and interstitial fluid, in a broad range of environmental salinities (Marshall and Grosell, 2006). This is achieved through morphological, cellular, physiological and endocrine adaptations (McCormick and Bradshaw, 2006; Sakamoto and McCormick, 2006; Shane et al., 2006). The strategy of ion exchange with the external medium is mediated mainly through the gastrointestinal epithelium, kidney and gills (Marshall and Grosell, 2006). In adult teleost fish, the gills are recognized as the main site for ion exchange (Wilson and Laurent, 2002) mediated by different transporsters, mainly Na+/K+ -ATPase (McCormick, 1995, 2001), and ion channels located in the chloride cells, which are also called mitochondria-rich cells (MRCs) or more generally ionocytes (Pisam and Rambourg, 1991; Hirose et al., 2003; Marshall and Grosell, 2006).

Adaptation of euryhaline fish to different salinities involves changes in ion transport mechanisms that generally induce changes in oxygen consumption, suggesting variations in the energetic demands for osmoregulation (Morgan and Iwama, 1991). Several scenarios have been suggested to describe changes in oxygen metabolism in fish following salinity challenge that, in some cases, can result in altered or enhanced growth (Boeuf and Payan, 2001). The relationship between osmoregulation and growth performance in marine fish has stimulated a large amount of research and, so far, it seems to be species-specific (Boeuf and Payan, 2001). Each fish species seems to have its own salinity optimum for growth, and osmoregulatory imbalance is often associated with whole animal-level stress changes, including decreased growth performance (De Silva and Perera, 1976; Boeuf and Payan, 2001; Imsland et al., 2001).

Some members of the family Sciaenidae (Nelson, 2006) display a great potential for aquaculture, and some have been reared in commercial facilities for some time. These include the red drum (Sciaenops ocellatus)
in the United States (Miranda and A.J., 1985; Thomas et al., 1995; Gardes et al., 2000), the mulloway (Argyrosomus japonicus) in Australia (Battaglene and Talbot, 1994; Fielder and Bardsley, 1999), and the maigre (Argyrosomus regius) in Europe (Pastor Garcia et al., 2002; Poli et al., 2003). Another Sciaenid species of interest in the eastern part of the Mediterranean Sea is the shi drum (Umbrina cirrosa) (Cardellini et al., 1999; Barbaro et al., 2002; Mylonas et al., 2004; Zaiss et al., 2006). Sciaenid fishes are considered as euryhaline species (Miran and A.J., 1985; Fielder and Bardsley, 1999; Doroudi et al., 2006), therefore they could be considered for aquaculture diversification in coastal lagoons with brackish water and areas near the mouth of large rivers, where the commonly employed marine species are not suitable. In the case of the shi drum, as well as some of the other sciaenids with aquaculture potential, there is a complete lack of knowledge on its adaptation potential and growth performance in different salinities.

The objective of the present work was to study the osmoregulatory capacity and the interaction between salinity adaptation and growth performance in shi drum, in order to provide scientific background for the development of optimized husbandry practices for this species. For this purpose, fish were reared for three months at different salinities representing full-strength seawater (40 psu), nearly iso-osmotic water (10 psu) and hypo-osmotic water (4 psu), and changes in gill histology, osmoregulation, growth and energetic metabolism related parameters were evaluated.

2. Material and methods

2.1. Animal husbandry and experimental design

Experiments were conducted using shi drum juveniles at the facilities of the Institute of Aquaculture, Hellenic Centre for Marine Research (Crete, Greece). Fish were produced in July 2004 from wild-caught, captive-reared broodstock, after hormonal induction of spawning with gonadotropin-releasing hormone agonist (GnRHa) implants (Mylonas et al., 2004). Fish with mean ± S.D. total length (TL) of 11.8 ± 0.8 cm and body weight (BW) of 22.8 ± 4.5 g were reared in three independent recirculating aquaculture systems (RAS) at different salinities (40,10 and 4 psu). Each RAS was fitted with mechanical and biological filters, and supported two replicated 500-l cylinder conical polyester tanks containing the experimental fish (n = 30). The recirculated water was maintained at 19 ± 1°C and >80% oxygen saturation throughout the course of the experiment (84 days), and new water exchange rate was 30% every 2–3 d. Fish were fed ad libitum with commercial feed (1st period EXCEL, SKRETING) by the use of a demand-feeder, and photoperiod was 24L:0D two months prior to and throughout the salinity manipulation. A constant light photoperiod was used due to mortalities from jumping out of the tanks during lights-on and off. After randomly allocating the fish in their tanks (n = 30) at 40 psu, two replicated groups were acclimated gradually to the lower experimental salinities over the course of 6 d. Total length and BW measurements were taken from all fish in each tank at 0, 14, 28, 42, 56, 70 and 84 days from the start of the experiment. Blood samples were taken initially (day 0) from ten fish from the stock population and then from five fish from each tank at 46 and 84 d. Blood was collected from the caudal vasculature with heparinized syringes, centrifuged at 5000 rpm for 15 min at 4°C, and the plasma was stored at −80°C until further analysis. Five fish from each treatment group were also sacrificed at 46 and 84 days, the second gill arch was excised for histological analysis and the third gill arch was quickly stored at −80°C for determination of Na+/K+-ATPase activity (see later for method). For all measurements, fish were first anesthetized in a solution of 0.4 mL·L⁻¹ of clove oil (Mylonas et al., 2005), dissolved first 1:10 v/v in 96% ETOH. Fish handling was carried out according to the European Union Directive (86/609/ECC) for the protection of animals used for experimental and other scientific purposes (EEC, 1986) and the “Guidelines for the treatment of animals in research and teaching” (Anonymous, 1998).

2.2. Biochemical analyses

Plasma metabolites were determined by enzymatic colorimetric procedures (glucose GOD/PAP; cholesterol PAP; triglycerides GPO/PAP) using commercial kits (Biosis, Greece). Osmosolality was measured using a cryoscopic osmometer (Gonotec, Osmomat 030). Plasma samples were diluted 1:600 with lithium nitrate 15 mmol L⁻¹ and electrolytes (K⁺, Na⁺) were determined by flame emission spectrophotometry. Chloride ions were determined by spectrophotometric method based on the reaction of CI⁻ with mercuric thiocyanate.

Gill Na⁺/K⁺-ATPase activity was determined according to Varsamos et al. (2004). Briefly, gill arches were homogenized with an IKA disperser (IKA®/Yellow™, USA), at 9,500 rpm for 5 sec, with 1 mL of buffer containing: 250 mM sucrose, 10 mM Heps, 5 mM MgCl₂, 0.1 mM Na₂EDTA, pH 7.4. The homogenate was centrifuged (4000 rpm, 15 min, 4°C) and the supernatant used for assays. Homogenate protein content was determined in triplicate as detailed by Bradford (1976), using BSA (Sigma, A-4503) as standard. The Na⁺/K⁺-ATPase activity was assessed as the difference of total ATP hydrolysis (in presence of Na⁺, K⁺, Mg²⁺ and ATP) and that in absence of K⁺ but in presence of an optimal concentration of the specific inhibitor ouabain (1 mg mL⁻¹). The amount of released phosphate was assessed by comparison with commercial reference standards (Cellmat, BioMérieux, France). The Na⁺/K⁺-ATPase specific activity was expressed in µmol Pi h⁻¹ mg⁻¹ protein. The total activity was calculated as the product of the specific activity and the total protein amount of the sample and expressed in µmol Pi h⁻¹.

2.3. Histological analyses

A section of the second gill arch was fixed in 4% formaldehyde: 1% gluteraldehyde buffered saline (Mcdowall and Trump, 1976) for at least 24 h. Before embedding in a methacrylate resin (Technovit 7100®), Heraeus Kulzer, Germany), gill samples were dehydrated through a series of increasing concentrations of ethanol (70–96%). The mounted gills were cut in 3-µm thick sections on a microtome (RM 2245, Leica, Germany), stained with Methylene Blue (Sigma, Germany)/Azur II (Sigma, Germany)/Basic Fuchsin (Polysciences, USA) (Bennett et al., 1976) and examined under a light microscope (BH-2, Olympus, Japan). In addition, four sections per gill sample were stained with the combined Alcian blue — periodic acid Schiff (AB-PAS) technique for neutral and acid mucins (Mowry, 1956) which stains mucus cells specifically and allows identification of different types of mucins (Sheppard, 1994). Chloride cells were located along the primary lamellae, in the area between the bases of the secondary lamellae (Fig. 1). They contained a large, but lighter staining nucleus than the pavement cells or red blood cells visible in the sections. As reported earlier (McCormick, 2001), chloride cells in fish reared in sea water contain a deep apical crypt and are larger than in freshwater fish, where an apical surface substitutes the apical crypt (Fig. 1). Chloride cell counts were made on histological sections of primary lamellae, where both the cartilaginous vestige of the gill septum was visible and the primary lamella was sectioned in the middle, along its whole longitudinal axis. Chloride cell counts were made along 1 mm length of three different primary lamellae (starting from the base of each primary lamella on the gill arch) and the average value was used to represent each fish sampled. Size estimation of the chloride cells was made using the average of the maximum (D) and minimum diameter (d). All chloride cells sectioned through the nucleus (n = 13–42), along the length of a primary filament containing three secondary lamellae were measured for three different areas and the average was used to represent each fish sampled. It was not possible to make reliable mucus cell counts, because different sections of the gill filament contained markedly different numbers.

2.4. Estimation of growth parameters and statistical analyses

Comparisons of TL and BW between groups reared at different salinities were made using regression analysis (Sokal and Rohlf, 1981). The
A general model used was of the form: \( Y = a_0 + a_1 \cdot t + a_2 \cdot D + a_3 \cdot t \cdot D \), where \( Y \) is the dependent variable, \( t \) the time, \( D \) a dummy variable with values 0 and 1 for each condition tested and \( a_i \) (i = 1, 2 or 3) constants. This method tests the hypothesis that the constants \( a_2 \) and \( a_3 \) are zero. Time series have the same slope when constant \( a_2 \) is zero, and the same initial value when \( a_3 \) is zero. When both constants are zero, time series describe similar dependent variables. Specific growth rate (SGR) was estimated as the slope of the above model. Food conversion ratio (FCR) was estimated as \( FCR = F / (B_f - B_i) \), where \( F \) = consumed food (kg), \( B_f \) = final biomass (kg), \( B_i \) = initial biomass (kg).

To evaluate differences in FCR, plasma metabolites, electrolytes, osmolality, gill Na\(^{+}/K^{+}\)-ATPase activity and chloride cell density or size, data were subjected to 2-way Analysis of Variance (ANOVA) (time x salinity), followed by Duncan's New Multiple Range (DNMR) test on main effects, and mean comparisons for interactions. Statistical analysis was performed using linear statistics software (SuperAnova, Abacus Concepts or SigmaStat, Systat Software, Inc.) at a minimum significance level of \( P<0.05 \). All results are presented as mean ± SEM.

3. Results

3.1. Growth performance of shi drum in different salinities

Survival was unaffected by salinity and at the end of the 84-day experimental period it was greater than 97% in all salinities. Also, no differences in feeding behaviour or appetite were observed. However, salinity did have a statistically significant effect on growth (\( P<0.05 \)), with fish reared at 4 psu having significantly smaller TL and BW than the fish reared at 10 or 40 psu, whereas there were no differences between fish reared at 10 or 40 psu salinity (Fig. 2). The final mean BW of shi drum reared at the three salinities were 63.3 ± 3.2, 67.5 ± 2.1 and 44.5 ± 2.2 g for 40, 10 and 4 psu, respectively. As expected from the above measurements, SGR over the course of the study was also affected by environmental salinity, and in fish reared at 4 psu was 0.265 d\(^{-1}\), significantly lower (\( P<0.05 \)) than of fish adapted to 10 or 40 psu, which was the same at 0.513 d\(^{-1}\).

There was large variation in the FCR between the two replicates of the 4 psu treatment at different times, as indicated by the large SEM values (Table 1), which could not be explained by the experimental design. Nevertheless, the statistical analysis over the course of the study indicated the existence of a significant difference (\( P<0.05 \)) between the FCR of fish reared at 4 psu and the FCR of fish reared at 10 or 40 psu (Table 1). The differences were due to higher values of the 4 psu fish during the first 56 days, when FCR ranged between 1.36 ± 0.14 and 4.71 ± 2.2, compared to a range of 1.06 ± 0.05 and 1.78 ± 0.07 of fish reared at 10 or 40 psu salinity. During the period between 57 and 84 days after the onset of the experiment no significant difference in FCR was found among the three salinities.

3.2. Plasma metabolites, osmolality, electrolytes and gill Na\(^{+}/K^{+}\)-ATPase activity

There were no significant differences in mean plasma glucose concentration among fish reared at the different salinities and fish reared at 10 or 40 psu salinity.

Fig. 1. Microphotograph of primary lamella from shi drum reared at different salinities. (A) 40 psu. Note the larger size of chloride cells and the presence of a prominent apical crypt. (B) 4 psu. The chloride cells are smaller and an apical surface has substituted the apical crypt. pc = pavement cells, ac = apical crypt, as = apical surface, cc = chloride cells. Bar represents 25 µm.

Fig. 2. Changes in mean (± SEM) total length (A) and body weight (B) of shi drum (n=25–30) reared in duplicated tanks at different salinities (4, 10 and 40 psu) during a period of 84 days. Reduction of salinity to 4 psu had a statistically significant effect on growth over the whole course of the study (regression analysis, \( P<0.05 \)), as indicated by different letter superscripts next to the growth curves.

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Table 1

<table>
<thead>
<tr>
<th>Time period (d)</th>
<th>10 psu</th>
<th>40 psu</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–14</td>
<td>3.39 ± 1.89 (^{b})</td>
<td>1.78 ± 0.05 (^{a})</td>
</tr>
<tr>
<td>15–28</td>
<td>3.32 ± 0.22 (^{a})</td>
<td>1.31 ± 0.08 (^{a})</td>
</tr>
<tr>
<td>29–42</td>
<td>1.36 ± 0.14</td>
<td>1.21 ± 0.14</td>
</tr>
<tr>
<td>43–56</td>
<td>4.71 ± 2.20 (^{b})</td>
<td>1.68 ± 0.26 (^{a})</td>
</tr>
<tr>
<td>57–70</td>
<td>1.28 ± 0.04</td>
<td>1.31 ± 0.21</td>
</tr>
<tr>
<td>70–84</td>
<td>1.54 ± 0.26</td>
<td>1.54 ± 0.40</td>
</tr>
</tbody>
</table>

Significant differences (P<0.05) at different sampling points and over the whole course of the study between the 4 psu and the 10 or 40 psu groups are indicated by different letter superscripts next to the mean values. Within each salinity treatment, there were no differences in FCR between different sampling times (P>0.19).

4. Discussion

It has been shown that several euryhaline teleosts display a two-stage adaptation pattern following salinity challenge, consisting of an adaptive period when changes in osmotic and metabolic parameters occur, and of a regulatory period which generally leads to a new steady state (Jensen et al., 1998; Sangiao-Alvarellos et al., 2005). Our data have clearly shown that shrimp juveniles are able to maintain constant plasma osmolality and electrolyte levels over a relatively broad range of environmental salinities and during a long period. However, it appears that rearing at very low salinities (4 psu) affects osmoregulation and growth. This body size, SCR and FCR were lower in shrimp exposed to 4 psu salinity compared to those reared at 10 or 40 psu salinity. The blood osmolality of teleosts is around 12 psu, i.e. approximately one third of that of sea water (~38 psu) and more than 50-fold higher than the salinity of freshwater (~0.2 psu). Consensus exists that marine fish achieve higher growth rates at lower salinity (Boeuf and Payan, 2001) and that each species has its own salinity optimum for growth, under a certain water temperature and ontogenetic phase, although existing data are sometimes contradictory. For instance, the best salinity conditions for growth in gilthead sea bream (Sparus aurata) have been reported at 12 psu (Laiz-Carrion et al., 2005b) or 28 psu (Klaoudatos and Conides, 1996), in grey mullet (Mugil cephalus) at 20 psu (De Silva and Perera, 1976), in turbot (Scophthalmus maximus) at 15 psu (Imsland et al., 2001) and in European sea bass (Dicentrarchus labrax) at 15 psu (Saillant et al., 2003) or 28–30 psu (Dendrinos and Thorpe, 1985; Conides and Glumuzina, 2006). The present results indicate that salinity affects FCR and growth in shrimp juveniles adapted to low salinity. The metabolic and energetic cost of osmoregulation should, at least partly, reflect the effect of salinity on growth, however other possibilities should be taken into account. This may include the effect of salinity on feeding behavior, appetite, or stimulation/inactivation of other metabolic and endocrine pathways (Boeuf and Payan, 2001; McCormick, 2001).

Table 2

<table>
<thead>
<tr>
<th>Glu (mmol l(^{-1}))</th>
<th>4 psu</th>
<th>10 psu</th>
<th>40 psu</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>7.8 ± 0.7(^{b})</td>
<td>3.5 ± 0.2(^{a})</td>
<td>3.6 ± 0.1(^{b})</td>
</tr>
<tr>
<td>0.04</td>
<td>3.3 ± 0.2(^{b})</td>
<td>3.1 ± 0.2(^{a})</td>
<td>2.6 ± 0.1(^{b})</td>
</tr>
<tr>
<td>0.08</td>
<td>3.3 ± 0.2(^{b})</td>
<td>4.0 ± 0.1(^{a})</td>
<td>3.2 ± 0.1(^{b})</td>
</tr>
<tr>
<td>0.10</td>
<td>3.3 ± 0.2(^{b})</td>
<td>7.1 ± 1.1(^{a})</td>
<td>10.4 ± 1.6(^{b})</td>
</tr>
</tbody>
</table>

Different small letter superscripts indicate significant differences (P<0.05) between different sampling times within salinity treatments, while capital letter superscripts indicate significant differences between different salinities within sampling time.
Table 3  
Mean (±SEM) plasma osmolality, sodium (Na⁺), potassium (K⁺) and chloride (Cl⁻) of shi drum (n=50–60) reared at different salinities, and sampled at different times after the onset of the experiment (0, 42, and 84 days)

<table>
<thead>
<tr>
<th>Salinity (psu)</th>
<th>0</th>
<th>42</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Osmolality (mOsm kg⁻¹)</strong></td>
<td>350±3*</td>
<td>338±2*</td>
<td>410±2*</td>
</tr>
<tr>
<td>Na⁺ (mmol l⁻¹)</td>
<td>196±8</td>
<td>181±5</td>
<td>179±5</td>
</tr>
<tr>
<td>K⁺ (mmol l⁻¹)</td>
<td>8.0±0.3*</td>
<td>6.0±0.2*</td>
<td>8.3±0.3*</td>
</tr>
<tr>
<td>Cl⁻ (mmol l⁻¹)</td>
<td>161±7*</td>
<td>145±1*</td>
<td>197±5*</td>
</tr>
</tbody>
</table>

Different small letter superscripts indicate significant differences (P<0.05) between different sampling times within salinity treatments, while capital letter superscripts indicate significant differences between different salinities within sampling time.

To our knowledge this is the first report on plasma cholesterol and triglyceride concentrations for shi drum, and the values reported are within the range measured in other marine species (Kavadias et al., 2003). Interestingly, both parameters seemed to reflect metabolic effects of long-term salinity adaptation in this species, whereas plasma glucose levels where unaffected by salinity treatments. Thus, the low growth performance of shi drum reared at 4 psu was concomitant with lower plasma cholesterol and triglyceride concentrations compared to those measured in fish reared at 10 or 40 psu, suggesting a negative effect of low salinity on energy metabolism. It has been already suggested that changes in plasma metabolites during the first days of salinity change are related to modifications in energy supplies in osmoregulatory tissues (Sangiao-Alvarelos et al., 2005). For example, in gillhead sea bream plasma triglycerides did not show significant changes following acclimation to different salinities (i.e., 6, 12, 38 psu) for 100 d, although growth and SGR were correlated to salinity (12–38–6 psu) (Laiz-Carrion et al., 2005b). However, in the same species, a 20 and 25% decrease in plasma glucose and triglyceride levels, respectively, was observed after adaptation to 6 psu salinity, which was then followed by full recovery within 7 d (Sangiao-Alvarelos et al., 2005). The low cholesterol levels recorded in shi drum adapted to 4 psu salinity could be explained partly by differences in feeding rate compared to the other experimental salinities. That kind of correlation between the feeding rate and plasma cholesterol levels has been reported in European sea bass (Lemaire et al., 1991) as well as in Eurasian perch (Perca fluviatilis) (Vellas et al., 1994). The absence of differences in glucose levels among the salinity treatments in the present experiments seems surprising, since the role of glucose as fuel for tissues during osmotic challenge or stress conditions, in general, is well known (Wendelaar Bonga, 1997).

However, in long term adaptation, such as in the present study, it seems that the involvement of this metabolite is less discriminating, and similar results have been obtained in gillhead sea bream (Laiz-Carrion et al., 2005b).

The results obtained in the present experiment indicate that shi drum reared at 40 or 10 psu salinity for 84 days did not face major osmoregulatory imbalance, based on the fact that there were no significant differences in plasma osmoregulatory parameters. On the contrary, fish reared at 4 psu displayed osmoregulatory difficulties, as indicated by the observed lower plasma Na⁺ after 42 d and osmolality after 42 and 84 d. Low Na⁺ levels have also been reported in the gillhead sea bream after 100 d rearing at 6 psu salinity (Laiz-Carrion et al., 2005b) and in European sea bass 24 h after transfer to salinities less than 20 psu (Vetturini et al., 1992). Difficulties in homeostasis in low salinities have also been reported in other euryhaline teleost species, such as the sheepshead minnow (Cyprinodon variegatus) (Nordlie, 1983) and flounder (Paralichthys orbignyanus) (Sampaio and Bianchini, 2002). Franklin et al. (1992) suggested that such osmoregulatory impairments indicate salinity stress, whereas the negative effect of stress on growth is well documented in teleosts (Wendelaar Bonga, 1997).

It is well recognized that gills are the organs that consume most energy during adaptation of teleosts in different salinities, since they must maintain differential regulation of intracellular and extracellular fluids. Moreover, in case of transition from hypoosmotic to hyperosmotic conditions (or vice versa), Na⁺ and Cl⁻ fluxes across the gill epithelium must switch from ion uptake to ion excretion (and vice versa). The Na⁺/K⁺-ATPase plays a pivotal role in these processes and, thus, its activity could be related to some extent to the energetic cost of osmoregulation (McCormick, 1995). In our experiments the lowest activity for this enzyme was recorded in shi drum juveniles reared at low salinity after 42 d, and could suggest, at first sight, a lower energy cost for osmoregulation at that salinity. Our results are in contrast with the U-shaped variations in gill Na⁺/K⁺-ATPase activity reported for euryhaline fish, with high levels in freshwater or very low salinity and seawater, and lower levels in fish adapted to brackish water (10–12 psu) (Jensen et al., 1998; Boeuf and Payan, 2001; Varsamos et al., 2002). However, a direct relationship between salinity and Na⁺/K⁺-ATPase activity has been shown in some teleosts (McCormick, 1995, 2001; Marshall, 2002). Nevertheless, at the end of the present experiments there was no significant difference in Na⁺/K⁺-ATPase activity among the experimental groups, indicating that fish reached a steady state within 84 d after the challenge. These observations, together with the data mentioned above, suggest that it is rather unlikely that the low enzyme activity observed in the gills after 42 d at low salinity corresponds to low osmoregulatory cost. Rather, a transient alteration in cellular metabolism and in the function of membrane exchangers/transporters could be involved, due to salinity-induced stress. Hence, unlike other euryhaline teleosts, it appears that the adaptation period following low salinity acclimation in shi drum juveniles is much longer and displays a different pattern. Moreover, such an adaptation period is probably associated with a rapid growth.

![Fig. 3](image-url)  
Changes in mean (±SEM) gill Na⁺/K⁺-ATPase specific activity in shi drum (n=10) reared at different salinities during a period of 84 days. Different small letter superscripts indicate significant differences (P<0.05) between different salinities within a sampling date. There were no significant differences between sampling times.
other transporters, such as H+-ATPase (Lin and Randall, 1995) or Ca+-ATPase (Marshall, 2002), could be involved in low salinity adaptation of shi drum. In terms of the absolute values of mean ATPase activity in shi drum in the present study in comparison with other fishes, it was found that it was lower than in saltwater adapted Japanese eel (Anguilla japonica) (Utida et al., 1971), chum salmon (O. keta) (Uchida et al., 1996), silver perch (Bidyanus bidyanus) and golden perch (Macquaria ambigua) (Alam and Frankel, 2006), and was an order of magnitude lower than larval European sea bass (Varsamos et al., 2004).

In fish, the gill epithelium consists mainly of three types of cells: namely the pavements cells (representing 90–95% of the total epithelial area), the chloride cells and the mucus cells (Shephard, 1994; Wilson and Laurent, 2002). In shi drum reared at full seawater, most mucus cells of the gills stained purple indicating a mixture of acid and neutral mucins. Similar results were reported in other species, such as the gilthead sea bream, Senegal sole (Solea senegalensis) and Siberian sturgeon (Acipenser baeri) (Sarasquete et al., 2001). On the contrary, most mucus cells of shi drum exposed to hypo-osmotic environment stained reddish, indicating that they consisted mainly of neutral mucins, a result similar to that documented for species maintained in freshwater, such as the rainbow trout (Oncorhynchus mykiss) (Ferguson et al., 1992) and Atlantic salmon (Salmo salar) (Roberts and Powell, 2003). Our findings suggest that changes in the mucin production of gill mucus cells, could be part of the salinity adaptation process in the shi drum.

Gill chloride cells are recognized as the main site for ion exchange, mediated by different transporters and ion channels (Lin and Randall, 1995; Marshall, 2002; Marshall and Singer, 2002; McCormick and Bradshaw, 2006). Changes in the number and/or size of these cells have been related to salinity acclimation in several teleost species throughout post-embryonic development (Karnaky et al., 1976a; Pisam and Rambourg, 1991; Zydlewski and McCormick, 2001; Varsamos et al., 2002; Hiroi and McCormick, 2007). In the present study there was a

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**Fig. 4.** Histological sections of gill filaments (consisting of primary filament and secondary lamellae) showing structural changes in the epithelium of shi drum reared at different salinities. A: Gill filament of shi drum at the beginning of the experiment (reared at 38 psu). B: Gill filament of shi drum control fish reared 40 psu for 84 days. C: Gill filament of shi drum reared at 4 psu for 84 days. Abbreviations: cc = chloride cell, mc = mucus cell, pc = pavement cell, pl = primary filament, sl = secondary lamella. Bars represent 50 µm.

**Fig. 5.** Mean (±SEM) number of chloride cells (mm⁻¹ of gill filament) (A) and cell diameter ([max diameter, D± min diameter, d]/2) of chloride cells (µm) in shi drum (n=5) reared at different salinities (4, 10 and 40 psu) during a period of 84 days. The results of the two-way ANOVA are indicated on the graphs. Different capital letter superscripts indicate significant differences (P<0.001) between sampling times, regardless of salinity; whereas lower letter superscripts indicate significant differences among salinity treatments, within the specific sampling time. n/a=not available.
significant increase in chloride cell numbers related to age, but there was no significant difference among shi drum reared at the three different salinities. These results are in accordance with studies in Adriatic sturgeon (A. naccarii) (Catrali et al., 1995; McKenzie et al., 1999) or sea trout (Salmo trutta) (Brown, 1992) acclimated to saltwater, but are contrary to what has been reported in other fishes (Mcormick et al., 2003; Catrali et al., 2004). More intuitive, however, were the observed increases in chloride cell size in shi drum adapted to 40 psu compared to 4 psu, as reported also in the guppy (Poecilia reticulata) (Shikano and Fujio, 1999), Mozambique tilapia (Oreochromis mossambicus) (Uchida et al., 2000), killifish (Fundulus heteroclitus) (Karnaky et al., 1976b) and salmonids (Uchida et al., 1996; Hiroi and McCormick, 2007). Such increases in chloride cell size are attributed to the considerable enlargement of the basolateral tubular system, which is related to an increased activity of Na+/K+-ATPase (Karnaky et al., 1976a,b; Laiz-Carrion et al., 2005a). Furthermore, the presence of a distinct apical crypt in shi drum reared in 40 psu in the present study has been described as a distinctive feature of chloride cells of marine teleosts, or as a structural change that occurs in this cell when a euryhaline species passes from fresh water to saltwater (Hosler et al., 1979a,b; Fossett et al., 1983). As expected, shi drum reared in 4 psu water showed no apical crypt and had smaller chloride cells. On the contrary, shi drum reared at 10 psu for 42 days had significantly smaller chloride cells compared to both fish reared at 4 and 40 psu, similar to what has been reported in gillhead sea bream (Laiz-Carrion et al., 2005a). It has been speculated, that this could be due to a lower need for ion pumps required in fish reared at isoosmotic media (Laiz-Carrion et al., 2005a). Overall, in view of some of the discrepancies observed in the results of the present study, further physiological, ultrastructural and molecular studies are necessary to study more thoroughly the role of chloride cells in shi drum adaptation to different salinities.

In conclusion, the present study indicates that shi drum acclimated to full seawater or nearly isoosmotic water do not phase any osmoregulatory imbalance, while fish reared at 4 psu displayed osmoregulatory difficulties and low growth performance. The ability of shi drum to utilize environments of lower salinity, up the isoosmotic point, opens new possibilities in the development of shi drum aquaculture in coastal lagoons and estuaries, thus further contributing to the sustainable diversification of the aquaculture industry.

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