# Cellular and Biochemical Characterization of Hyposmotic Adaptation in a Marine Teleost, Sparus sarba

Authors: Kelly, Scott P., and Woo, Norman Y. S.

Source: Zoological Science, 16(3) : 505-514

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.16.505

ZOOLOGICAL SCIENCE **16**: 505–514 (1999)


# Cellular and Biochemical Characterization of Hyposmotic Adaptation in a Marine Teleost, *Sparus sarba*

Scott P. Kelly† and Norman Y.S. Woo*

*Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, China*

**ABSTRACT** —Silver sea bream (*Sparus sarba*) were adapted to a hyposmotic environment of 6‰ for 5 or 21 day periods. Hyposmotic adaptation did not significantly alter serum $Na^+$, $Cl^-$ or muscle moisture content. After 5 days in 6‰, chloride cell (CC) apical and fractional areas increased with no alteration in CC numbers. Elevated CC apical and fractional area was coupled with an increase in CC numbers after 21 days in 6‰. In fish adapted to 6‰, an increase was found in the area of CC cytoplasm occupied by mitochondria. Branchial $Na^+$-$K^+$-ATPase decreased after 21 days in 6‰, resulting in an uncoupled "typical" correlative relationship between this enzyme and CC numbers. Kidney $Na^+$-$K^+$-ATPase activity elevated after 5 days in 6‰ but was not significantly elevated after 21 days. In gill and kidney tissue, alterations were found in the activity of key metabolic enzymes after 5 days acclimation to 6‰, with few differences occurring after 21 days. Serum cortisol levels were unaltered by low salinity acclimation suggesting that an increase in the number of CCs found in 6‰-adapted *S. sarba* did not occur as a result of hypercortisolemia. Based on the present evidence, it seems possible that alterations in the form and function of the branchial epithelium may play an important role in the ability of *S. sarba* to acclimate to low salinity conditions, with renal assistance occurring during short term exposure. The response of *S. sarba* to low salinity adaptation appears to differ from the generally accepted teleostean model of response and offers insight into the as yet undefined hyperosmoregulatory strategies of estuarine marine migrant fish.

## INTRODUCTION

Numerous studies have demonstrated the ability of "true" marine fish to adapt to low salinity environments (Woo and Wu, 1982; Wu and Woo, 1983; Mancera *et al.*, 1993; Provencher *et al.*, 1993; Munro *et al.*, 1994; Gaumet *et al.*, 1995; Woo and Chung, 1995; Woo and Kelly, 1995; Kelly *et al.*, 1999), however, few studies have addressed the cellular and biochemical adjustments associated with low salinity acclimation of marine fish. This is particularly evident in studies concerned with the teleostean branchial chloride cell (CC), where an increase in CC numbers is generally associated with the exposure of euryhaline or diadromid fish to elevated salinities (Utida *et al.*, 1971; Thomson and Sargent, 1977; Langdon and Thorpe, 1984; King and Hossler, 1991; Kültz and Jürss, 1993). The functional importance of elevated CC numbers is evident in the salmonid model of osmoregulatory response where increased CC numbers appear to be necessary for successful seawater adaptation (Ouchi, 1985; Madsen, 1990; Franklin, 1990). Recent reports, however, indicate that euryhaline fish adapted to high salinity environments may not always exhibit increased CC numbers when compared to fish held in freshwater conditions (Ciccotti *et al.*, 1994; Van Der Heijden *et al.*, 1997). Moreover, elevated CC numbers are implicated in increased branchial ion uptake in freshwater fish adapted to ion poor conditions (for review see Perry, 1997). Overall, current evidence suggests that the branchial CC is equally important for ionoregulation in both freshwater and seawater conditions.

Numerous fish species can be found in the nekton fauna of estuarine ecosystems (Day *et al.*, 1989) and included in this fauna are the non-estuarine dependent marine migrants, such as certain members of the Sparidae (commonly referred to as sea bream) (Blaber, 1997). A number of sparid species have been demonstrated capable of adapting to low salinity environments (Woo and Wu, 1982; Wu and Woo, 1983; Mancera *et al.*, 1993; Woo and Kelly, 1995; Kelly *et al.*, 1999) and as marine migrants in estuarine ecosystems, silver sea bream (*Sparus sarba*) can experience low salinity conditions as both juveniles and adults. The present study addressed key elements involved in the adaptation of *S. sarba* to a low salinity environment of 6‰. More specifically, the morphology and presence of the branchial CC were investigated along with the response of gill and kidney tissue in order to gain

* Corresponding author: Tel. (852) 2609 6148;
FAX. (852) 2603 5646.
E-mail: normanwoo@cuhk.edu.hk
† Present address: Department of Biology, MacMaster University, Hamilton, Ontario, Canada

further insight into the adaptive response of a marine teleost to low salinity exposure.

## MATERIALS AND METHODS

### Culture conditions

Silver sea bream (*S. sarba*) (52.3g ± 2.3g) were obtained from a local fish farm and held in recirculating seawater systems in The Marine Science Laboratory, The Chinese University of Hong Kong. Three days prior to starting the experiment, fish were taken from stock tanks and placed in 1000 l experimental tanks. The experimental tanks contained recirculating filtration systems and water temperature ranged between 22–24°C. All the tanks were located outdoors and received a natural photoperiod (11 hr L:13 hr D). Fish were fed *ad libitum* once daily with diets formulated according to Woo and Kelly (1995). Under these conditions fish were cultured for either (1) 21 days in seawater (33‰), (2) 21 days, the final 5 days of which were in a salinity of 6‰ or (3) 21 days in 6‰. The salinity of seawater in 6‰-treated groups (2) and (3) was gradually reduced (≈8‰/day) using aged tap water until a final experimental salinity of 6‰ was reached.

### Sampling

Fish were netted from the water and blood was taken via a syringe inserted into the caudal vessels. Fish were then killed by spinal transection. All blood sampling was completed within a period of 2 min. Blood was allowed to clot at room temperature and centrifuged (5, 000g for 5 min) to obtain serum. The body cavity was opened and the kidney removed. A standardized region of the musculature (full flank dorsal to the lateral line) was removed from the fish and all tissues and serum were quick frozen in liquid nitrogen. Tissues and serum were stored at –70°C until further analysis.

### Preparation of specimens for electron microscopy

The first branchial arch, on the right hand side of the branchial basket, was removed for scanning electron microscope (SEM) studies, fixed in 5% glutaraldehyde-0.1M phosphate buffer (pH 7.4) at 0–4°C for 2–4 hr. For transmission electron microscope (TEM) studies, the tissue was fixed in 2.5% glutaraldehyde-0.1M phosphate buffer (pH 7.4) at 0–4°C for 2 hr and then post-fixed in 1% osmium tetroxide-0.1 M phosphate buffer (pH 7.4) for 1 hr at room temperature.

Fixed gill filaments were washed twice in 0.1 M phosphate buffer (pH 7.4), cut into pieces ≈ 1 $mm^3$, and dehydrated in a graded ethanol series (50–100%). After dehydration tissues were embedded in spurr resin. Blocks were sectioned using glass knives and an ultramicrotome (Reichert-Jung). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Jeol JEM-1200EXII). Analysis of CC mitochondrial area was conducted using photomicrographs of CC cytoplasm taken at 15, 000×mag and computer assisted image analysis (Quantimet 500, Leica). All CCs selected for mitochondrial area analysis were exposed to the external environment at the apical surface.

### SEM studies and morphometric analysis

Fixed gill filaments were washed twice in 0.1 M phosphate buffer (pH 7.4) and dehydrated in a graded acetone series (50–100%). Critical point drying was achieved using two consecutive 10 min baths of tetramethylsilane (Sigma). After air drying, filaments were mounted on copper stubs using double sided non-conductive tape. Samples were given a fine coating of gold using a sputter coater (Edwards, model S150B). All observations were conducted using a scanning electron microscope (Jeol, JSM-5300). Videographs of afferent filament surfaces were taken parallel to the stub at the point of separation from the septum, near the base of the lamellae. Quantification of CC morphometrics was conducted using videograph images taken at 5000×mag and subsequent computer assisted image analysis (Quantimet 500, Leica). Calculation of CC apical and fractional exposure area was based on calculations outlined in Kelly *et al.* (1999).

### Isolation of gill epithelial cells

Fish were decapitated and the heads were immersed in a dish containing ice-cold $Ca^{2+}$ and $Mg^{2+}$-free Hanks balanced salt solution (HBSS) of the following composition (mM): NaCl: 137, KCl: 5.4, $Na_2HPO_4$: 3.4, $NaHCO_3$: 4.2, $KH_2PO_4$: 0.4, glucose: 5.6; pH 7.4. The pericardium was opened and the gills were perfused with 0.8% saline containing 1 mg/ml heparin (Sigma). After all the blood was perfused from the gills, the branchial basket was removed and individual gill arches were dissected and rinsed in $Ca^{2+}$ and $Mg^{2+}$-free HBSS. Epithelial tissue was scraped off from the underlying cartilage using a glass slide. The tissue was incubated in $Ca^{2+}$ and $Mg^{2+}$-free HBSS containing 5 mM EDTA and 0.02 mg/ml elastase (Sigma, Type I) for 15 min at room temperature, with frequent agitation. After incubation, the crude cell suspension was filtered through a graded series of gauze (120 μm, 80 μm and 45 μm) and gently passed through a syringe needle 10 times. Aliquots of this suspension were removed for viability studies and fluorescent staining while the rest of the cell suspension was washed twice with $Ca^{2+}$ and $Mg^{2+}$ free HBSS and quick frozen in liquid nitrogen. These samples were stored at –70°C until further analysis.

For cell viability determination, cells that were unstained after 10 min incubation in $Ca^{2+}$ and $Mg^{2+}$-free HBSS containing 0.2 mg/ml trypan blue were considered viable (Sharpe, 1988). CCs were selectively stained with the vital mitochondrial-specific fluorescent dye DASPMI [2-(*p*-dimethylaminostyryl)-1-ethylpyridiniumiodine] (Aldrich, Milwaukee). The cell isolates were incubated in $Ca^{2+}$ and $Mg^{2+}$-free HBSS containing 25 μM DASPMI for 15 min at 0–4°C in the dark. Cells were washed twice with $Ca^{2+}$ and $Mg^{2+}$-free HBSS and viewed under a microfluorophotometric microscope (Nikon, microphot-fx). The proportion of CCs in cell isolates was determined using a hemocytometer and by viewing the cell isolates with and without fluorescent illumination. A number (3–4) of viewing fields were counted for each fish and each site was chosen randomly before fluorescent excitation. The total number of cells counted in each field ranged from 200-300.

### Serum chemistry

Serum $Na^+$ was measured using atomic absorption spectrophotometry (Hitachi). Serum $Cl^-$ levels were determined using a chloride meter (Corning-eel 920). Cortisol was measured using a commercial enzyme immunoassay (ELISA) kit (IBL, Hamburg).

### Tissue composition and enzyme activities

Muscle moisture was determined after drying tissue overnight at 105°C. Kidney tissue was homogenized in ice-cold sucrose-EDTA-imidazole (SEI)/sucrose-EDTA-imidazole-deoxycholic acid (SEID) buffer according to McCormick (1993). Homogenates were centrifuged at 5,000g for 1 min and an aliquot taken for the analysis of $Na^+$-$K^+$-ATPase activity. Suspensions were re-centrifuged for a further 10 min at 12,000g and the supernatants were used for all other enzymes assays. Branchial cell pellets were resuspended in ice-cold SEI/SEID buffer as indicated above and sonicated using a sonicator (MSE Soniprep 150). The resulting suspensions were centrifuged either at 5,000g for 1 min for the determination of $Na^+$-$K^+$- ATPase activity or for 10 min at 12,000g for all other branchial enzymes.

All centrifugation steps were carried out at 0–4°C in a refrigerated centrifuge (Beckman model GS-15R) and, in the case of kidney tissue, homogenization was conducted using an Ultra-turrax homogenizer. The protein content of supernatants were determined according to Hartree (1972), using bovine serum albumin (Sigma) as a standard, and all enzyme activity is expressed as protein specific activity measured at 25°C.

$Na^+$-$K^+$-ATPase (E.C. 3.6.1.3) was measured according to McCormick (1993). Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; G6P-DH) activity was measured according to Löhr and Waller

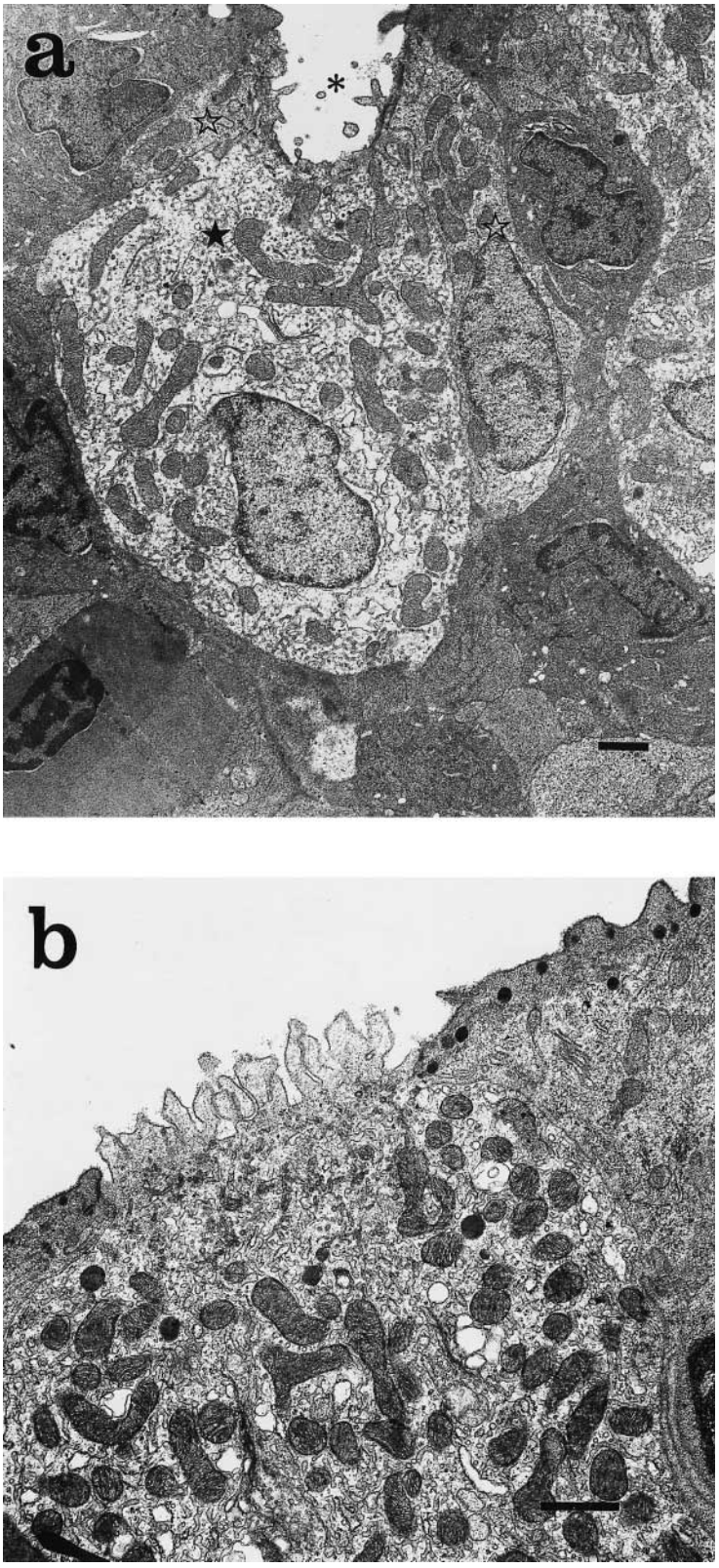


**Fig. 1.** Transmission electron micrographs of (**a**) a chloride cell (★) and an accessory cell (☆) from the gill of *Sparus sarba* held in seawater (33‰) and (**b**) the apical region of a chloride cell from *Sparus sarba* acclimated to 6‰ for 21 days. Note that the typical apical invagination of seawater (33‰) acclimated fish is absent in 6‰ acclimated fish and chloride cell openings appear almost planar with the respiratory surface in 6‰ acclimated fish. Scale bar = (**a**) 1 μm and (**b**) 2 μm.

(1974), lactate dehydrogenase (E.C. 1.6.4.3; LDH) according to Bergmeyer and Bernt (1974) and isocitrate dehydrogenase (E.C. 1.1.1.42; ICDH) according to Bernt and Bergmeyer (1974).

**Statistical analysis**

All data are expressed as mean values±standard error of the mean (SEM). To determine significance between groups, data were subjected to a one-way analysis of variance followed by a Student-Neuman-Keuls multiple comparison test (Sigmastat software, Jandel Scientific) to delineate significance.

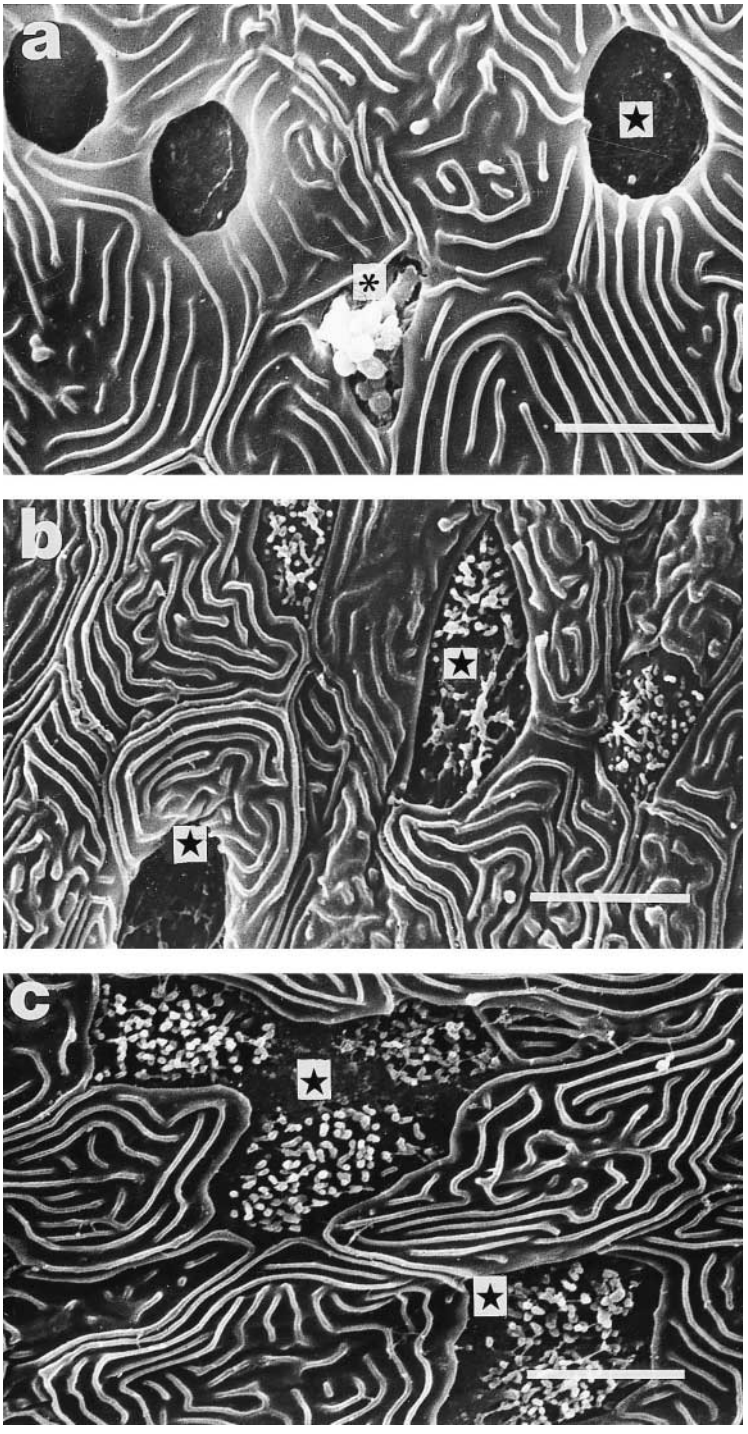


**Fig. 2.** Scanning electron micrographs of afferent gill filament surfaces from *Sparus sarba* adapted to (**a**) seawater (33‰), (**b**) 6‰ for 5 days, and (**c**) 6‰ for 21 days. Representative chloride cell apical areas are indicated by ★'s and the asterisk indicates a mucous cell. Fish adapted for 5 days are generally found to have two cell types. Fish adapted for 21 days most frequently display apical areas planar with the respiratory surface and numerous microvillous extensions. All scale bars = 5 μm

## RESULTS

### Chloride cell ultrastructure

The gill consisted of several branchial arches bearing filaments (or primary lamellae) from which radiated the secondary (or respiratory) lamellae. The CCs could only be observed on the primary epithelium and no CCs could be observed on the respiratory lamellae. Normally CCs could be easily seen at the base of the respiratory lamellae and were large and cuboidal in shape (Fig. 1a). The tubular system formed an extensive network throughout the cytoplasm of the CC and numerous mitochondria were distributed uniformly throughout the cytoplasm, except in the apical region. The apical region facing the external medium was depressed to form an apical cavity (Fig. 1a). Accessory cells were often found adjacent to the apical portion of the chloride cell (Fig. 1a).

Accessory cells were small and relatively rich in mitochondria. They were located in the superficial regions of the

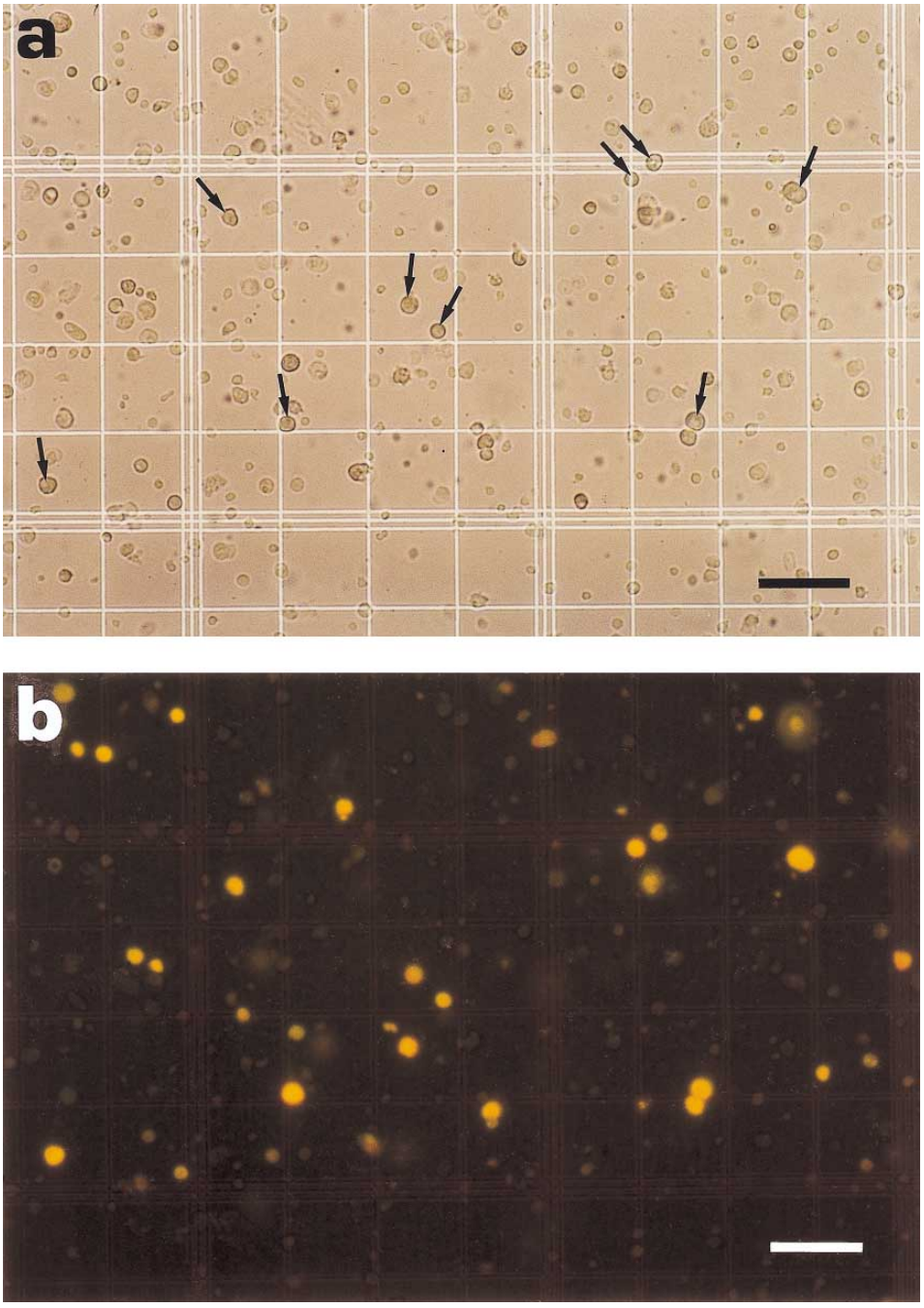


**Fig. 3.** Micrographs showing DASPMI-stained *Sparus sarba* gill cell isolates (**a**) without and (**b**) with fluorescent excitation. In micrograph (**a**) chloride cells are indicated by arrows and in micrograph (**b**) the same cells can be seen fluorescing. Scale bar = 50 μm.

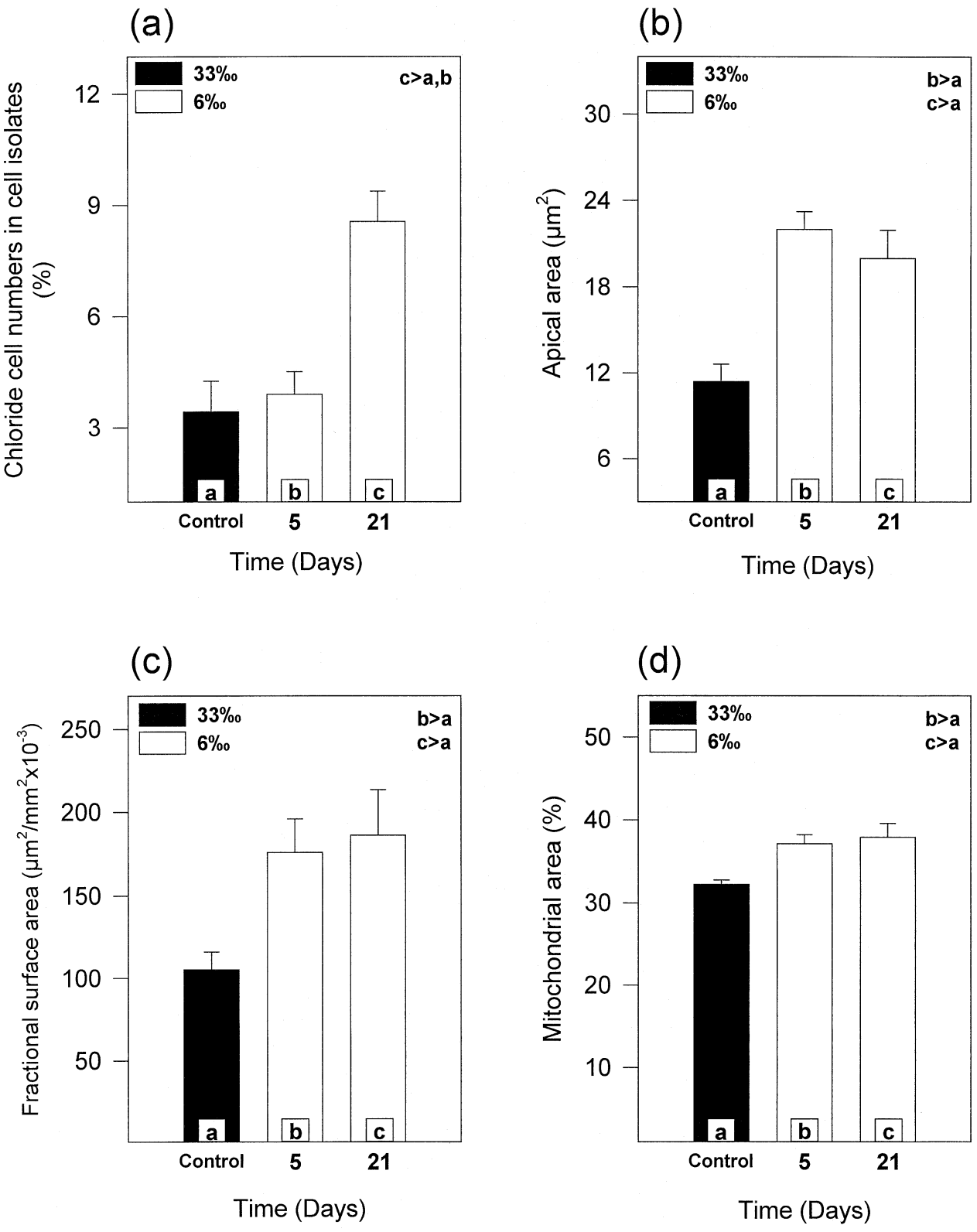


**Fig. 4.** The effect of hyposmotic (6‰) adaptation on chloride cell (**a**) numbers in gill cell isolates, (**b**) apical area, (**c**) fractional area and (**d**) mitochondrial area. Data area expressed as mean values±S.E.M. Significance ($P<0.05$) between groups a-c is denoted in the top right corner of each graph. n = 5.

primary epithelium and, thus, never reached the basal lamina. Accessory cells often sent apical interdigitations into the exposed regions of the chloride cell.

The large cuboidal shape of CCs was also seen in fish adapted to a hyposmotic environment. In contrast to seawater-adapted fish, the apical surface of 6‰-adapted fish extended numerous protrusions into the external medium (Fig. 1b). Furthermore, accessory cells were occasionally still present and bound to the apical portions of the chloride cells by shallow junctions.

**Chloride cell surface ultrastructure**

CC apical openings were most frequently observed on the afferent surface of the gill filament and in the interlamellar surfaces around the bases of the respiratory lamellae. Generally, no CCs were found on the efferent filament surface. In seawater fish, most CCs exhibited round or oval apical openings of varying depth (Fig. 2a). The branchial epithelium of fish adapted to 6‰ generally exhibited cells that were almost planar with the respiratory surface and possessed a dense covering of microvillous extensions (Fig. 2b, c). Although this apical form was most abundant, cells possessing a "seawater-type" apical form were occasionally present. In general, no difference could be seen between CC apical form of fish adapted to 6‰ for 5 or 21 days, however, in fish adapted to 6‰ for 5 days the presence of the "seawater-type" apical form was more common (Fig. 2b). In fish adapted to 6‰, neighboring CCs often appeared to converge apical surfaces. The appearance of an apical convergence manifested as large areas with distinct regions of microvillous concentration and cell peripheries that could be used to define individual cells (Fig. 2c).

**Chloride cell numbers and exposure areas**

Trypan blue exclusion revealed that all gill cell isolates exhibited +90% viability. In gill cell isolates stained with DASPMI, fluorescing CCs were easily distinguished from other epithelial cells (Fig. 3). CC numbers in the branchial epithelium of silver sea bream did not significantly change after 5 days acclimation to 6‰ (Fig. 4a). After 21 days in 6‰ the

**Table 1.** Effect of hyposmotic adaptation on serum $Na^+$, $Cl^-$ and cortisol and muscle moisture content in silver sea bream (*Sparus sarba*)

| | 33‰ | 6‰/5 days | 6‰/21 days |
|---|---|---|---|
| $Na^+$ (mM) | 187.0±3.8 | 179.1±2.4 | 177.0±2.8 |
| $Cl^-$ (mM) | 149.0±2.1 | 150.6±1.3 | 152.3±1.7 |
| Cortisol (ng/ml) | 17.0±3.7 | 14.5±2.9 | 21.5±4.0 |
| Muscle moisture (%) | 75.8±0.5 | 77.0±0.3 | 77.1± 0.3 |

All data are expressed as mean values±S.E.M. (n = 7)

**Table 2.** Effect of hyposmotic acclimation on gill and kidney enzyme activities in silver sea bream (*Sparus sarba*)

| | 33‰ | 6‰/5 days | 6‰/21 days |
|---|---|---|---|
| Gills | | | |
| $Na^+$-$K^+$-ATPase (μmoles NADH/min/g) | 32.4±3.9 | 25.9±5.8 | 15.6±1.9* |
| LDH (μmoles NADH/h/mg) | 105.1±11.5 | 79.3±4.3 | 89.4±4.9 |
| G6P-DH (μmoles NADPH/h/mg) | 3.30±0.23 | 6.03±0.27* | 2.67±0.22 |
| ICDH (μmoles NADPH/h/mg) | 3.27±0.38 | 4.92±0.26* | 2.97±0.29 |
| Kidney | | | |
| $Na^+$-$K^+$-ATPase (μmoles NADH/min/g) | 115.1±10.5 | 173.5±10.4* | 157.9±21.4 |
| G6Pase (μmoles Pi/h/mg) | 6.8±1.1 | 11.3±1.5* | 4.8±1.5† |
| LDH (μmoles NADH/h/mg) | 30.0±1.2 | 29.2±2.7 | 43.8±5.3*† |
| G6P-DH (μmoles NADPH/h/mg) | 6.4±0.3 | 6.3±0.5 | 6.3±1.0 |
| ICDH (μmoles NADPH/h/mg) | 7.9±0.4 | 9.5±0.3* | 7.5±0.9† |

All data are expressed as mean values±S.E.M. (n = 7)
*: Significantly different from 33‰ value
†: Significantly different from 6‰/5 days

number of CCs significantly increased ($P<0.05$). CC apical area, fractional area and mitochondrial area all significantly ($P<0.05$) increased after a 5 day acclimation period in 6‰ (Fig. 4b, c, d). These elevations were sustained after 21 days in 6‰.

**Serum chemistry**

Serum $Na^+$, $Cl^-$ and cortisol levels were unaffected after both 5 and 21 days adaptation to 6‰ (Table 1). Hyposmotic acclimation resulted in a slight, but insignificant increase in muscle moisture content after 5 and 21 days adaptation (Table 1).

**Branchial enzyme activities**

Branchial $Na^+$-$K^+$-ATPase activity did not significantly alter after 5 days in 6‰ (Table 2), however, after 21 days in 6‰ the activity of this enzyme was significantly ($P<0.05$) lower than that found in the seawater-adapted control group. Branchial G6P-DH and ICDH activities were significantly elevated after 5 days adaptation to 6‰ ($P<0.05$) (Table 2). After 21 days in 6‰, no significant difference in G6P-DH or ICDH activities was found. Branchial LDH activity was unaffected by salinity after both 5 and 21 days adaptation (Table 2).

**Kidney enzyme activities**

Five days post-6‰ adaptation, the activity of kidney $Na^+$-$K^+$-ATPase was significantly elevated above seawater control values (Table 2). After 21 days in 6‰, kidney $Na^+$-$K^+$-ATPase activity still appeared to be slightly elevated, however this elevation was no longer statistically significant. After 5 days in 6‰, kidney G6Pase and ICDH activities were significantly ($P<0.05$) elevated (Table 2). No significant difference in the activities of G6Pase and ICDH was found between 33‰ control fish and fish adapted to 6‰ for 21 days (Table 2). After 21 days in 6‰, kidney LDH activity was significantly greater than that found in fish adapted to 6‰ for 5 days and 33‰ control fish (Table 2). The activity of kidney G6P-DH was unaltered by salinity.

## DISCUSSION

In *S. sarba* acclimated from seawater to a hyposmotic environment of 6‰, serum $Na^+$ and $Cl^-$ levels remained within tight limits. This observation, taken together with an absence of significant muscle hydration, demonstrated that *S. sarba* is capable of osmoregulating in low salinity environments. The branchial CC plays a key role in the ability of euryhaline fish to tolerate salinity change (for review see Jürss and Bastrop, 1995) and in *S. sarba* adapted to 6‰ for 21 days, an increase in branchial CC numbers indicated that this cell type may also play a role in the osmoregulatory homeostasis of low salinity acclimated sea bream. However, numerous studies conducted on diadromous and euryhaline fish species have demonstrated that an increase in branchial CC numbers is implicated in successful hyposmoregulatory strategies (Utida *et al.*, 1971; Thomson and Sargent, 1977; Langdon and Thorpe, 1984; King and Hossler, 1991; Kültz and Jürss, 1991; Kim *et al.*, 1993). In light of this, the response of *S. sarba* seemed to be analogous to that of diadromids adapted to ion poor conditions, where increased branchial CC numbers result in elevated ion uptake at the gill surface (for review see Perry, 1997). Additional support for this contention may come from the observed low salinity-induced elevation in CC apical and fractional exposure area, a phenomenon that in freshwater fish has also been demonstrated to correlate with increased ion uptake (Perry, 1997). Furthermore, the appearance of numerous apical structures in the CCs of 6‰-adapted fish would facilitate an increased surface area for ion transporting mechanisms, such as $Cl^-/HCO_3^-$ exchange, which may allow 6‰-adapted fish to compensate passive ion loss with active ion uptake. However, further investigation is needed to clarify whether passive ion losses across the branchial epithelium of 6‰-adapted sea bream are compensated by branchial ion uptake.

Several studies have focused on the possible existence of CC sub-types in the gills of fish (for review see Jürss and Bastrop, 1995). In the present experiment, changes in the ultrastucture of *S. sarba* CCs (particularly apical morphology) were similar to those found in the α-cell sub-type of seawater- or freshwater-acclimated tilapia (*Oreochromis niloticus*) (Pisam *et al.*, 1995) and guppy (*Lebistes reticulatus*) (Pisam *et al.*, 1987). However, clear distinction between α- and β-CC types is not possible in the present study due to the lack of definitive staining techniques required for CC sub-type identification. In previous studies, no evidence was found to indicate the presence of a β-CC type in the branchial epithelium of low salinity (5‰) acclimated marine fish, despite apical ultrastrutctural alterations that, again, suggest an alteration in the physiological role of the existing putative α-type CC (Pisam *et al.*, 1990).

A low salinity induced reduction in the activity of branchial $Na^+$-$K^+$-ATPase seemed paradoxical in *S. sarba* as elevated CC numbers in teleost fish are normally associated with an increase in $Na^+$-$K^+$-ATPase activity (Utida *et al.*, 1971; Thomson and Sargent, 1977; Perry and Walsh, 1989; Kültz and Jürss, 1993). However, several reports indicate that a parallel relationship between CC numbers and $Na^+$-$K^+$-ATPase activity may not always exist. This is certainly the case in tilapia (*O. mossambicus*) where both Van Der Heijden *et al.* (1997) and Kültz and Jürss (1991) reported no difference in the activity of branchial $Na^+$-$K^+$-ATPase between freshwater and seawater adapted fish, despite the former study reporting a greater number of CCs in the branchial epithelium of freshwater-adapted fish and the latter study reporting a greater number of CCs in seawater-adapted fish.

Although the relationship between CC numbers and $Na^+$-$K^+$-ATPase activity in *S. sarba* appeared to differ from that of the generally reported response, the activities of key metabolic enzymes in the branchial epithelium did not significantly differ from that found in 33‰-adapted fish, suggesting that a downregulation in the active metabolism of the gill tissue did not occur. Indeed, transient elevations in the activity of branchial G6P-DH and ICDH in 5-day acclimated fish suggested a

process of tissue reorganization consistent with an intermediate phase of acclimation prior to the appearance of increased CC numbers. This phenomenon was in line with an increase in CC mitochondrial area, found in the CCs of 5 day-adapted fish. However, a sustained increase in mitochondrial area was confusing considering that $Na^+$-$K^+$-ATPase activity was lower and other metabolic enzymes were unchanged. This may relate to possible artifacts in the selection of CCs for measurement as all the CCs used were exposed to the external environment at the apical surface. The cell isolation, and fluorescent staining, procedures did not distinguish between exposed and unexposed CCs and as such, the exposed cells may have been metabolically more active than those not exposed. Furthermore, a lower mitochondrial area in seawater-adapted fish may facilitate a greater cytosolic area for the proliferation of the tubular system, the location of $Na^+$-$K^+$-ATPase (De Renzis and Bornancin, 1984). Regardless of explanation, the measurement of CC mitochondrial area, despite its successful use in other studies (King *et al.*, 1989), was not a good morphological correlate for gill metabolism in 6‰-adapted *S. sarba*.

After a 5 day adaptation period to 6‰, the kidney of *S. sarba* also appeared to be in a state of functional and metabolic reorganization as elevated $Na^+$-$K^+$-ATPase activity was coupled with increased G6Pase and ICDH activities. The elevation in kidney $Na^+$-$K^+$-ATPase activity appeared to be sustained for the duration of the experiment, however, no significant difference could be found between the activity found in seawater fish and fish acclimated to 6‰ for 21 days. Despite this, elevated kidney LDH activity in fish adapted to 6‰ for 21 days seemed to indicate greater metabolic activity in the tissue.

In the present study, salinity had no effect on circulating cortisol levels, an observation that is in agreement with earlier studies on red sea bream (Woo and Fung, 1981). This would suggest that cortisol, which has been reported to cause an elevation in branchial CC numbers of some species of fish (Madsen, 1990), is probably not responsible for the elevated CC numbers in *S. sarba*. Indeed, previous studies have shown that daily injections of cortisol over a period of 7 days did not result in altered CC numbers in *S. sarba* (Kelly, 1997). This observation, taken together with the numerous studies that demonstrate the significance of cortisol in the control of teleostean CC differentiation (for review see McCormick, 1995) suggests that the importance of cortisol in sea bream osmoregulation requires further attention, an aspect that is being actively pursued in our laboratory at the present time.

In conclusion, the marine teleost *S. sarba* was able to adapt to a hyposmotic environment of 6‰ and maintain serum $Na^+$ and $Cl^-$ levels and muscle moisture content within tight limits. Morphological evidence suggested that the branchial CC may play a key role in the events associated with hyposmotic acclimation of *S. sarba*. In low salinity conditions, an increase in the number of branchial CCs and decrease in branchial $Na^+$-$K^+$-ATPase activity indicated a difference in the response of this fish species when compared to the general response of euryhaline fish. Elevated activities of key metabolic enzymes in gill and kidney tissue are transitionally evident during low salinity acclimation. After a 21-day acclimation period, most metabolic enzyme activity had returned to pre-acclimation levels. Unaltered cortisol levels indicated that an increase in branchial CC numbers under low salinity conditions did not originate from a hypercortisolemic response.

## ACKNOWLEDGMENT

This study was supported by an Earmarked Research Grant (CUHK 353/95M) awarded to NYSW by the Research Grants Council of Hong Kong.

## REFERENCES

Bergmeyer HU, Bernt E (1974) Lactate dehydrogenase. In "Methods of Enzymatic Analysis Vol II" Ed by HU Bergmeyer, Academic Press, New York, pp 574–579

Bernt E, Bergmeyer HU (1974) Isocitrate dehydrogenase. In "Methods of Enzymatic Analysis Vol II" Ed by HU Bergmeyer, Academic Press, New York, pp 624–627

Blaber SJM (1997) Fish and fisheries of tropical estuaries. Chapman and Hall, London

Ciccotti E, Marino G, Pucci P, Cataldi E, Cataudella S (1994) Acclimation trial of *Mugil cephalus* juveniles to freshwater: morphological and biochemical aspects. Env Biol Fish 43: 163–170

Day JW Jr, Hall CAS, Kemp WM, Yáñez-Arancibia A (1989) Estuarine ecology. Wiley, New York

De Renzis G, Bornancin M (1984) Ion transport and gill ATPases. In "Fish Physiology Vol XB" Ed by WS Hoar, DJ Randall, Academic Press, Orlando, pp 65–104

Franklin CE (1990) Surface ultrastructure changes in the gills of sockeye salmon (Teleostei: *Oncorhynchus nerka*) during seawater transfer: comparison of successful and unsuccessful seawater adaptation. J Morphol 206: 13–23

Gaumet F, Boeuf G, Severe A, Le Roux A, Mayer-Gostan N (1995) Effects of salinity on the ionic balance and growth of juvenile turbot. J Fish Biol 47: 865–876

Hartree EF (1972) Determination of protein: A modification of the Lowry method that gives a linear photometric response. Anal Biochem 48: 422–427

Jürss K, Bastrop R (1995) The function of mitochondria-rich cells (chloride cells) in teleost gills. Rev Fish Biol 5: 235–255

Kelly SP (1997) Strategies of hyposmotic adaptation in silver sea bream (*Sparus sarba*). PhD Thesis, The Chinese University of Hong Kong

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

King JAC, Hossler FE (1991) The gill arch of the striped bass (*Morone saxatilis*). IV. Alterations in the ultrastructure of chloride cell apical crypts and chloride efflux following exposure to seawater. J Morphol 209: 165–176

King JAC, Abel DC, DiBona DR (1989) Effects of salinity on chloride cells in the euryhaline cyprinodontid fish *Rivulus marmoratus*. Cell Tissue Res 257: 367–377

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

Kültz D, Jürss K (1993) Biochemical characterization of isolated branchial mitochondria-rich cells of *Oreochromis mossambicus* acclimated to fresh water or hyperhaline sea water. J Comp Physiol 163B: 406–412

Langdon JS, Thorpe JE (1984) Response of the gill $Na^+$-$K^+$ ATPase

activity, succinic dehydrogenase activity and chloride cells to saltwater adaptation in Atlantic salmon, *Salmo salar* L., parr and smolt. J Fish Biol 24: 323–331

Löhr GW, Waller HD (1974) Glucose-6-phosphate dehydrogenase. In "Methods of Enzymatic Analysis Vol II" Ed by HU Bergmeyer, Academic Press, New York, pp 636–643

Madsen SS (1990). The role of cortisol and growth hormone in seawater adaptation and development of hypoosmoregulatory mechanisms in sea trout parr (*Salmo trutta trutta*). Gen Comp Endocrinol 79: 1–11

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

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

McCormick SD (1995). Hormonal control of gill $Na^+$-$K^+$-ATPase and chloride cell function. In "Fish Physiology. Vol. XIV" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 285–315

Munro J, Audet C, Besner M, Dutil JD (1994) Physiological response of American plaice (*Hippoglossoides platessoides*) exposed to low salinity. Can J Fish Aquat Sci 51: 2448–2456

Ouchi K (1985) Effect of cortisol on the tolerance of masu salmon (*Oncorhynchus masou*) parr adapted to different salinities. Bull Natl Res Inst Aquaculture 7: 21–27

Perry SF (1997) The chloride cell: Structure and function in the gills of freshwater fishes. Ann Rev Physiol 59: 325–347

Perry SF, Walsh PJ (1989) Metabolism of isolated fish gill cells: Contribution of epithelial chloride cells. J Exp Biol 144: 507–520

Pisam M, Caroff A, Rambourg A (1987) Two types of chloride cells in the epithelium of a freshwater-adapted euryhaline fish: *Lebistes reticulatus*; their modifications during adaptation to saltwater. Am J Anat 179: 40–50

Pisam M, Boeuf G, Prunet P, Rambourg A (1990) Ultrastructural features of mitochondria-rich cells in stenohaline freshwater and seawater fishes. Am J Anat 187: 21–31

Pisam M, Le Moal C, Auperin B, Prunet P, Rambourg A (1995) Apical structures of "mitochondria-rich" $\alpha$ and $\beta$ cells in euryhaline fish gill: Their behaviour in various living conditions. Anat Rec 241: 13–24

Provencher L, Munro J, Dutil JD (1993) Osmotic performance and survival of Atlantic cod (*Gadus morhua*) at low salinities. Aquaculture 116: 219–231

Sharpe, PT (1988) Methods of cell separation. Elsevier, Amsterdam

Thomson AJ, Sargent JR (1977) Changes in the levels of chloride cells and ($Na^+$+$K^+$)-dependent ATPase in the gills of yellow and silver eels adapting to seawater. J Exp Zool 200: 33–40

Utida S, Kamiya M, Shirai N (1971) Relationship between the activity of $Na^+$-$K^+$-activated adenosinetriphosphatase and the number of chloride cells in eel gills with special reference to sea-water adaptation. Comp Biochem Physiol 38A: 443–447

Van Der Heijden AJH, Verbost PM, Eygensteyn J, Li J, Wendelaar Bonga SE, Flik G (1997) Mitochondria-rich cells in gills of tilapia (*Oreochromis mossambicus*) adapted to fresh water or sea water: quantification by confocal laser scanning microscopy. J Exp Biol 200: 55–64

Woo NYS, Fung ACY (1981) Studies on the biology of the red sea bream, *Chrysophrys major*-II. Salinity adaptation. Comp Biochem Physiol 69A: 237–242

Woo NYS, Wu RSS (1982) Metabolic and osmoregulatory response to reduced salinities in the red grouper, *Epinephelus akaara* (Temminck and Schlegel), and the black sea bream, *Mylio macrocephalus* (Basilewsky). J Exp Mar Biol Ecol 65: 139–161

Woo NYS, Kelly SP (1995) Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. Aquaculture 135: 229–238

Woo NYS, Chung KC (1995) Tolerance of *Pomacanthus imperator* to hypoosmotic salinities: changes in body composition and hepatic enzyme activities. J Fish Biol 47: 70–81

Wu RSS, Woo NYS (1983) Tolerance of hypo-osmotic salinities in thirteen species of adult marine fish: Implications for estuarine fish culture. Aquaculture 32: 175–181

(Received July 21, 1998 / Accepted March 15, 1999)