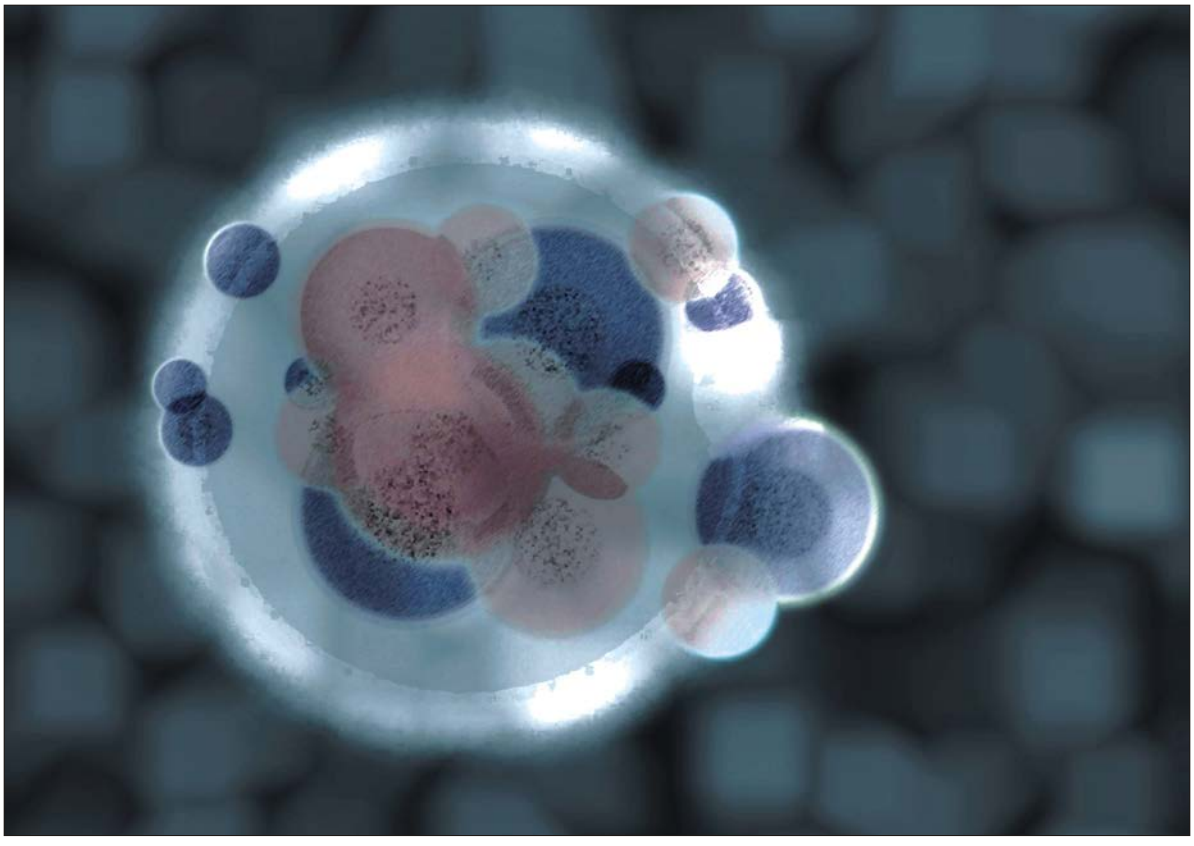

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# The Effects of Changes in Salinity on Gill Mitochondria-Rich Cells of Juvenile Yellowfin Seabream, *Acanthopagrus latus*

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**Abstract:** To determine the variation pattern of apical openings in mitochondrial-rich cells, short term and long term exposure to different salinities was studied. The results obtained suggest that morphological adaptations of mitochondria-rich cells reflect the animal's ionic and osmotic requirements. Yellowfin seabream, *Acanthopagrus latus*, are capable of tolerating direct exposure of salinities from 5 to 60‰ without showing mortalities. This species was also able to tolerate gradual decrease in salinity in the surrounding medium from seawater to freshwater through a period of 10 days and successfully adapt to freshwater without showing mortality. This is the shortest period reported in a true marine fish for adaptation to freshwater through an acceptable experimental duration. Three subtypes of mitochondria-rich cells were detected in photomicrographs created from scanning electron microscope; shallow basin, deep hole and wavy convex mitochondria-rich cells. After decrease in salinity to 20‰ the apical membrane of mitochondria-rich cells showed changes in morphology and on day 7 most of the mitochondria-rich cells were of the wavy convex or shallow basin subtypes. However, on day 21 they returned to their original state prior to changes in salinity. When transferred to hypoosmotic medium (5‰ and FW), rapid responses were exhibited in the apical membrane of mitochondria-rich cell which stabilized after 21 days and all subtypes of mitochondria-rich cells were observed in photomicrographs of gill filaments. Seabream transferred to 20‰ and hypoosmotic environments showed rapid decrease in mitochondria-rich cells, which was restored to normal levels as those in seawater within 7 days.

**Key words:** Osmoregulation, mitochondria-rich cell, ultrastructure, yellowfin seabream, *Acanthopagrus latus*

## INTRODUCTION

Many marine fish are capable of maintaining constant internal ionic concentrations relative to external or environmental ionic concentrations and maintain homeostasis in a wide salinity range (Fielder *et al*., 2007; Uchida *et al*., 1996). In teleost fish, osmoregulation and ionoregulation is the result of integrated activities of the gills, kidneys and intestine (Kaneko and Katoh, 2004) and fish exposed to changes in osmolality in water, regulate their body fluid osmolality by controlling ion concentration and water content by changing drinking rate and levels of different hormones as well as maintaining osmoregulatory surfaces (Fielder *et al*., 2007; Kelly and Woo, 1999). In adult teleost fish, gills are the most important osmoregulatory organs. Mitochondria Rich Cells (MRCs) present in branchial epithelia are involved in secretion and absorption and play a vital role in hydromineral regulation and adaptation to various osmotic environments (Pisam *et al*., 1993; Shikano and Fujio, 1998; Chang *et al*., 2001; Wilson and Laurent, 2002; Varsamos *et al*., 2002, 2005; Kaneko and Katoh, 2004; Giari *et al*., 2006).

Mitochondria Rich (MR) cells in teleosts are round or ovoid and are characteristically very different from normal epithelial cells. The MRCs are equipped with many mitochondria and an extensive intracytoplasmic membranous tubular system which is an expansion of their basolateral plasma membrane and is the site of expression for the active transport enzyme $Na^+/K^+$-ATPase showing their high ability in active transport of ions. The apical surface of MR cells is exposed to the

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ambient environment (Pisam *et al*., 1993; Helfman, *et al*., 1997; Shikano and Fujio, 1998; Hirose *et al*., 2003; Evans *et al*., 2005).

Transformations in the apical surface of MR cells occurs in response to various changes in the ambient environment such as acclimation to seawater, exposure to ion-poor water, cortisol and growth hormone treatments and exposure to high pH. Deep apical crypts are characteristic of seawater, however after acclimation to freshwater these crypts are shallow and are located at the same level or even protrusive. The apical membranes of MR cells were equipped with developed microvilli in freshwater (Hirose *et al*., 2003; Kaneko and Katoh, 2004). The phenotypic changes in apical surface of chloride cells have been suggested as a key mechanism in acid-base regulation and ion transfer. It has also been demonstrated that the uptake of diverse ions is significantly correlated to functional surfaces of MR cells on gill filament epithelium in some teleosts (Goss *et al*., 1998; Chang *et al*., 2001). The MR cells in seawater are responsible for the secretion of sodium ($Na^+$) and chloride ($Cl^-$) while in freshwater they regulate uptake of $Na^+$ and $Cl^-$ ions.

Hence, the conditions which arrests osmotic homeostasis leads to functional and morphological alterations in MR cells as a response to optimize ion transfer (Kelly and Woo, 1999; Chang *et al*., 2001; Moron *et al*., 2003). However, it is still not known whether MR cells alter their functions and morphology in response to environmental salinity change during transfer from seawater to freshwater or whether one type of cells degenerate and are replaced by newly differentiated cells of another type (Kaneko and Katoh, 2004).

Members of the Sparidae (commonly referred to seabream) are marine fish that have been shown to tolerate a wide salinity range. Despite the physiologically euryhaline characteristics of this group of fish, they are considered to be true marine teleosts as they do not appear to require a fresh water environment for any part of their life cycle. In their natural setting, seabream experience salinity fluctuations during estuarine entry and by definition are categorized as marine migrants in such ecosystems (Kelly *et al*., 1999a, b).

The yellowfin seabream, *Acanthopagrus latus* (sub-family: Sparinae) a protandrous hermaphroditic sparid fish (Hesp *et al*., 2004) is considered to be one of the most commercially and ecologically important marine fish due to its consumer preference (Abou-Seedo *et al*., 2003; Xia *et al*., 2006; Jiang *et al*., 2008). This species usually inhabits warm and coastal waters (Xia *et al*., 2006) throughout the Indo-Pacific (Hesp *et al*., 2004; Jean *et al*., 2000).

There is a few information on the effects of different salinities on osmoregulatory organs of marine fish and very few studies have been conducted to investigate the effects of transfer from seawater to different salinities on morphological changes of apical membrane in MR cells. The present research was conducted with the aim to study ultrastructural responses of branchial chloride cells in yellowfin seabream (*Acanthopagrus latus*) using SEM (scanning electron microscope) and to study the effects of transferring fish from seawater in the Moosa creek (42‰), located in the north of the Persian Gulf to lower hyper and hypoosmotic (1, 5, 20‰) and higher (60‰) salinities on density and morphology of apical membranes of branchial MR cells.

## MATERIALS AND METHODS

**Fish and acclimation period:** Yellowfin seabream, *Acanthopagrus latus* (Houttuyn, 1782) required for this study were obtained from the Imam Khomeini Mariculture Research Station located in the North of the Persian Gulf in Southwest Iran (Khouzestan Province, Imam Khomeini Port) in March 2008. All the fish were one year of age, produced in 2007 at the same research station and were maintained in 6000 L indoor tanks containing filtered seawater treated with UV. About 600 fish specimens assigned for the present research were transferred to the research lab adjacent to the Imam Khomeini Mariculture Research Station (Khouzestan Province). The fish were randomly distributed in 15, 300 L tanks (40 fish per tank). Each of the acclimation tanks were filled with seawater. To prevent pollution and possible disease outbreaks in fish which might affect the histological and physiological effects of salinity in this stage and during the experimental stage, in addition to filtration and UV treatment, seawater was subjected to chlorination and dechlorination in 1500 L reservoirs. To accomplish this reservoir tanks were treated with chlorine solution at a concentration of 20 ppm 24 h prior to transferring seawater to experimental tanks and then sodium thiosulphate (10 ppm) was used as a dechlorinating agent just before transfer (Fielder *et al*., 2007). This gives the fish two weeks to acclimatize to the experimental conditions in the 300 L tanks filled with seawater (42‰, 1162 mOs mol $kg^{-1}$ $H_2O$) before exposing them to salinity variations and thus reduce potentially confounding effects of handling stress (such as increased cortisol levels) on osmoregulation (Biswas *et al*., 2006). All tanks were provided with aeration at a rate of 1000 mL $min^{-1}$ so that least degree of physical stress is caused (Fielder *et al*., 2007). Aeration was temporarily cut off during feeding and resumed again.

Salinity (to the nearest 0.1‰), pH (to the nearest 0.01 value) and dissolved oxygen concentrations (to the nearest 0.1 mg $L^{-1}$) were monitored daily using a digital conductivity meter (Hach company model Sension 5), portable digital pH meter (SUNTEX model TSI) and a digital oxygen meter (DO 5510), respectively.

Fish were fed formulated diets in the form of pellets (protein 58%; fat 13%; carbohydrate 10% fibre 5%; ash 7%) at 2% body weight per day, two times a day at 9:00 to 9:30 h and 14:30-15:00 h. Pellets were cut into 4-7 mm pieces and distributed in the tanks. Uneaten pellets were siphoned out from the bottom of the tanks 1/2 h after feeding to prevent water pollution (Imsland *et al*., 2003). Ammonia levels in the water are reduced by a 50% water change every day (Altinok *et al*., 1998). A black shade cloth lid was used 1 m over the experimental tanks to reduce the effect of sunlight (Fielder *et al*., 2007).

**Experimental period:** After acclimation period, adaptation of fish under study to different salinities was examined. To do this the fish were subject to sudden changes in salinities of 5, 20 and 60‰ and to gradual changes in salinity for adaptation to FW (~1‰). First the tank water was drained so that only 10% of water remained. The control tanks were then filled with seawater, while the experimental tanks were filled immediately with water of required salinity (5, 20 or 60‰) (Kalujnaia *et al*., 2006). For adaptation to FW, tanks were first filled with 35‰ water on the first day and then the salinity was gradually decreased by 5‰ every day until the salinity reached 5‰ after which salinity was decreased by 1‰ every day until the salinity in the tanks reached 1‰. The ionic composition of the water in each experimental condition is shown in Table 1. Three replicates were used for each experimental group and for the control group (total of 15 tanks).

Different salinities were obtained by diluting filtered and UV treated seawater with filtered tap water or by adding natural sea salt to filtered and UV treated seawater. All water salinities were chlorinated and then dechlorinated following standard methods of chlorination as mentioned above.

The experiment was run for 21 days. Fish were starved for 24 h before samples were collected (Stefanson *et al*., 1998). Water exchange in each tank was carried out every day in the evening after feeding (Altinok *et al*., 1998). Salinity, pH and DO were checked daily throughout the experimental period and salinity in the tanks was corrected if required.

**Sampling and analysis:** Fish samples were collected from three tanks at the end of the acclimation period and immediately before exposing them to changes in salinity. This was considered as day zero ($t_0$). Fish samples were also collected from control tanks and from study groups with 5, 20 and 60‰ salinities at the end of day 1 and 7 and from all 15 tanks at the end of the experimental period (day 21). One fish was taken with a hand-held net from each tank (n = 3 for each experimental group) at each sampling and anesthetized with 2-phenoxyethanol (Sigma-Aldrich) (1 ml $L^{-1}$ water) in a bucket (Laiz-Carrion *et al*., 2005). Each sample was then taken out of the bucket, dried with a dry towel and weighed to the closest 0.01 g. The length of each fish was also measured to the nearest 1 mm and recorded (Table 2).

Table 1: Ionic composition of water at different salinities used in the experiments[1]

| Salinity (‰) | $Na^+$ (mM)[2] | $Cl^-$ (mM) | $Ca^{++}$ (mg/100 mL) | $K^+$ (mM)[2] |
|---|---|---|---|---|
| 60 | 370.83±2.88 | 1059.00±13.23 | 18.75±0.67 | 10.600±0.329 |
| 42 | 293.50±1.41 | 611.83±2.98 | 19.27±1.50 | 11.150±0.279 |
| 20 | 194.17±1.14 | 284.17±0.95 | 15.93±0.02 | 5.750±0.131 |
| 5 | 85.50±2.55 | 80.57±0.86 | 10.55±0.07 | 1.383±0.070 |
| 1 | 25.00±1.15 | 4.45±0.39 | 6.57±0.14 | 0.267±0.021 |

[1]All data are expressed as Mean±SEM (n = 6), [2]Determined using flame photometer (GENWAY model PFP7, England), [3]Determined via colorimetry (Technicon RA1000 Analyzer using Shern enzyme and MAN kits for $Cl^-$ and $Ca^{++}$, respectively)

Table 2: Weight and size of the sampled fish in different sampling times*

| Sampling time (day) | Weight (g) | Fork length (cm) |
|---|---|---|
| 0 (n = 3) | 32.10±1.92 | 11.70±0.25 |
| 1 (n = 12) | 29.75±1.58 | 11.31±0.21 |
| 7 (n = 12) | 28.58±1.56 | 10.97±0.19 |
| 21 (n = 15) | 28.82±1.35 | 10.94±0.18 |
| Total (n = 42) | 29.28±0.80 | 11.11±0.11 |

*Data are presented as Mean±SEM, There is no significant differences between weight and length of sampled fish at different sampling times ($p<0.05$)

For each fish, gill sections derived from the second arch of the gills on the right hand side of the head were taken. Tissues were fixed at 4°C in phosphate-buffered 2% paraformaldehyde plus 2% glutaraldehyde (pH 7.4) for at least 16 h. Fixed gill filaments were washed twice in 0.1 M phosphate buffer (pH 7.4) and dehydrated in ascending concentrations of ethanol from 50% to absolute, then in 100% acetone (Chen *et al*., 2004; Lin *et al*., 2004; Kelly *et al*., 1999a, b). The tissue pieces were then critical point dried using liquid carbon dioxide and mounted on copper stubs using double sided non-conductive tape. Samples were given a fine coating of gold using a sputter coater (Edwards, model SC7620). The preparation was then examined under a LEO 1455VP scanning electron microscope at 15 kV (Kelly *et al*., 1999a).

Photomicrographs of 4 afferent filament surfaces from each fish were taken parallel to the stub at the point of separation from the septum. Different subtypes of MR cells were identified under SEM observation according to size and morphology of the apical surfaces (Chang *et al*.,

2001). The MRC exposure numbers were conducted using photomicrograph images taken at 2000x mag (Kelly *et al*., 1999a, b). The counting of the MRC apical outcrops was carried out on 19264-37466 $\mu m^2$ surfaces (3 fish per salinity, 4 measurements per fish). Mean density for each fish were calculated and used for statistical analysis.

Data on opening numbers of branchial MRCs per $mm^2$ at the afferent surface of the filament are presented as Mean±SEM. Differences in gill MRC density among treatments were analyzed using one-way analysis of variance (ANOVA) (SPSS 11.5). Throughout the study Duncan post hoc test was performed when appropriate, as indicated by significance using ANOVA and significance was accepted when $p<0.05$ (Schreiber and Specker, 2000).

## RESULTS

No mortality was observed in yellow seabream exposed to an immediate salinity change in hypoosmotic and hyperosmotic media (5, 20 and 60‰) and during gradual adaptation to hypoosmotic environment (FW) during the 21 days experimental period.

Three regions were distinctly seen on the gill filament; the afferent (trailing) edge, interlamellar region and the efferent (leading) edge of the filament (Fig. 1). The surface area of the branchial epithelia were covered with a mosaic of polygonal pavement epithelial cells (PVC), however the extent of PVCs in the interlamellar region in the proximity of the afferent edge is less than other regions. The PVCs generally showed large polygonal surfaces. The apical membrane of Pavement cells is characterized by the presence of microvilli and or microplicae (micro ridges). The MR cells are usually found on the afferent edge of the gill filaments as well as in the interlamellar region. Their numbers decrease towards the efferent edge of the gill filament and they were completely absent from the efferent filament surface (Fig. 2). The MR cell outcrops were large ovoid shaped cells and were found between two or more PVCs.

**Apical surface morphology of MR cells:** Apical membrane morphology of MR cells showed different types of these cells from deep-hole type, formed by the deep invagination of the apical membrane set below the neighboring cells to the wavy convex type. In some cases the MR cells were of the shallow basin type, in which the apical membranes either possessed microvilli of varying density. Apical membranes were found either at the same level or slightly higher than PVC surface with branched longer microvilli (wavy convex subtype) (Fig. 3).

In control, the apical membrane of MR cells exhibited crypts of different depth showing microvilli. The MR cells at the same level with PVCs were rarely seen in gill epithelium in fish exposed to sea water (Fig. 4). Apical morphology of MRCs in gill epithelium exposed to 60‰ salinity for 24 h was ovoid and larger than that of gill epithelium in the control group. Alteration in size of the apical openings of MR cells was more in the interlamellar region of the gill epithelium. This increase in size of apical openings was distinct on days 7 and 21 (Fig. 5A). Within 24 h of exposure to water at 20‰ salinity, MR cell exposure numbers were decreased and the apical

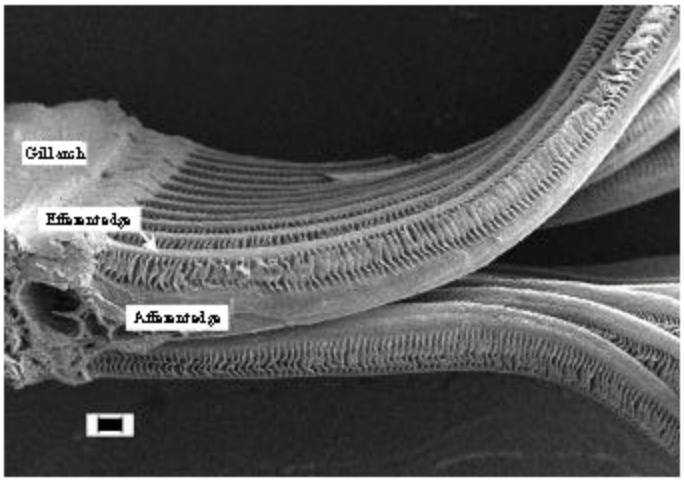


Fig. 1: Scanning electron micrographs of a portion of a gill arch from yellowfin sea bream (*Acanthopagrus latus*) including two hemibranches which radiate off it. In a side profile of a filament the afferent edge, Efferent edge and lamellae (that located between these two edges) can be distinguished (scale bar = 100 μm)

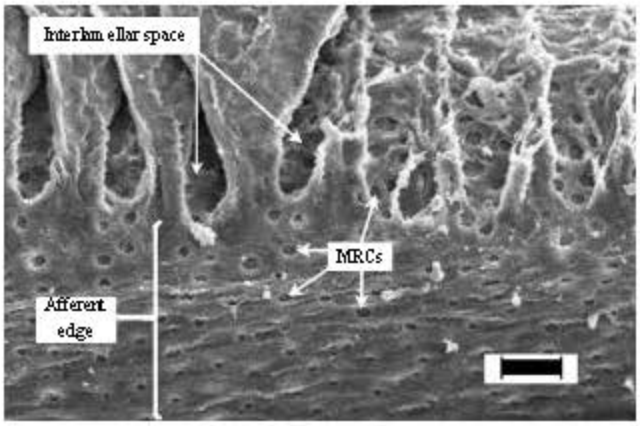


Fig. 2: MR cell openings on the afferent edge and interlamellar spaces. Note that the MRC outcrops in the interlamellar space is more abundant toward the afferent edge. (Scale bar = 20 μm)

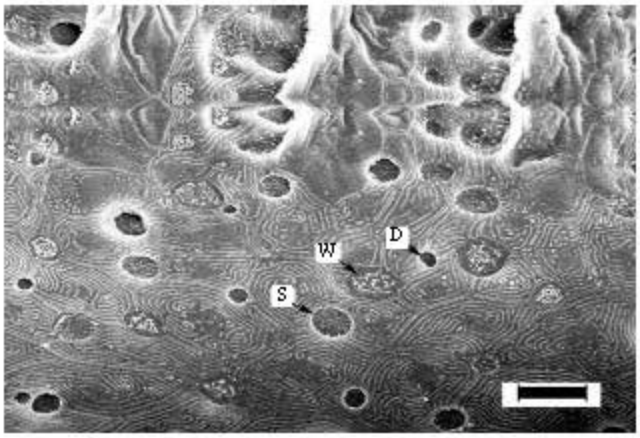


Fig. 3: Various MRC subtypes according to the apical opening morphology recognizable under scanning electron microscope. D: Deep hole MR cells, S : Shallow basin MR cells, W: Wavy convex MR cells (scale bar = 10 μm)

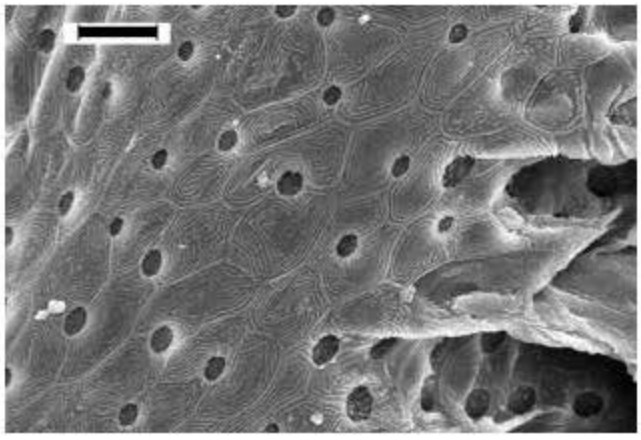

Fig. 4: Scanning electron micrograph of afferent and interlamellar surface from sea bream in SW condition (42‰). (Scale bar = 10 μm)

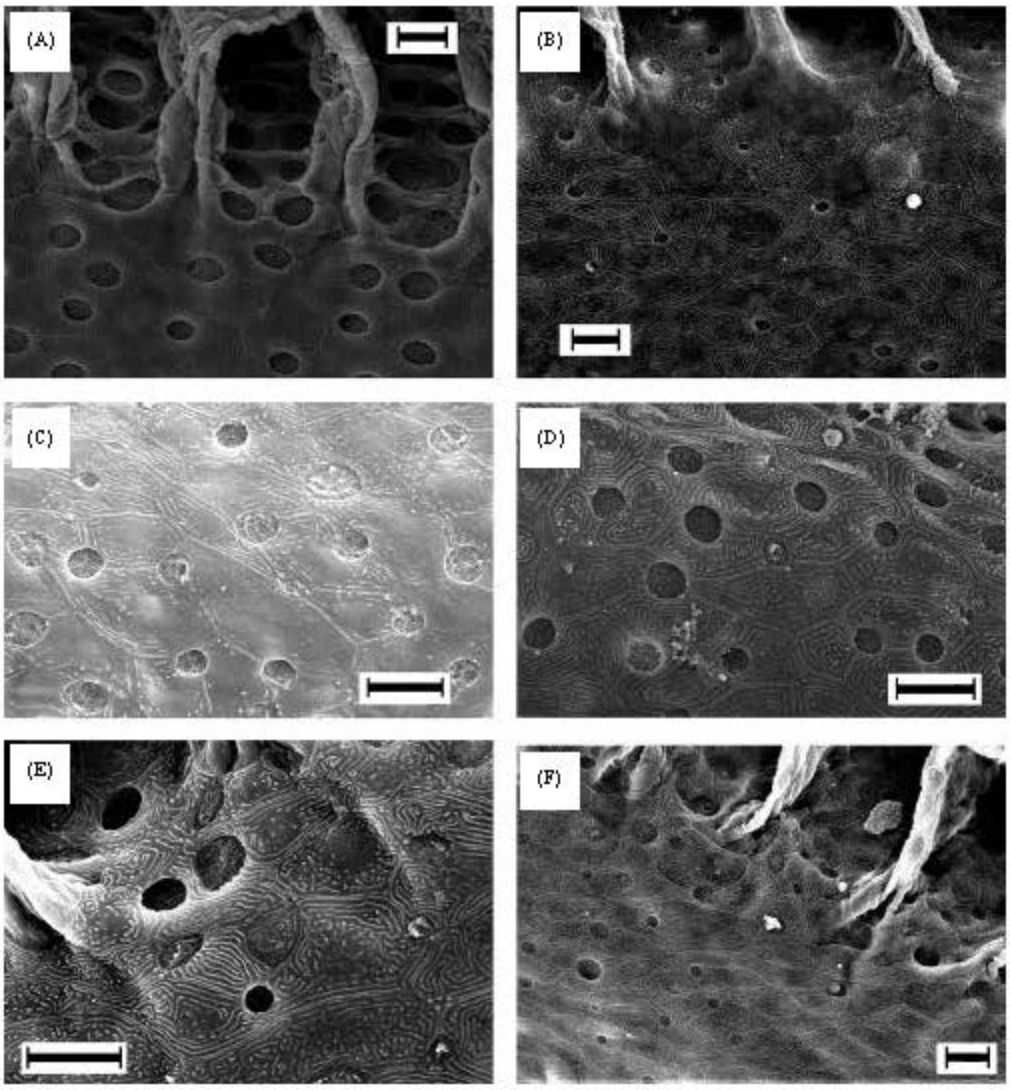


Fig. 5: SEM images of the surface of gill filaments from *Acanthopagrus latus* acclimated to different hyperosmotic (60‰ and 20‰) and hypoosmotic (5 and 1‰) environments. All views show changes in MRC apical morphologies during adaptations to 60‰ after 7 days (A), 20‰ after 24 h, 7 and 21 days (B, C and D, respectively), 5‰ after 24 h (E) and 1‰ after 21 days (F). For more details refer to the text (scale bars=10 μm)

membrane of MR cells were elevated (Fig. 5B). Within 7 days the apical membrane was transformed to aprotrusive convex apical membrane close to the surface of PVCs. A few shallow basin MR cells were still seen (Fig. 5C). But after 21 days exposure the depth and size of apical openings increased once again and were almost similar to that of controls (Fig. 5D). In the experimental group exposed to 5‰ salinity, transformation of MRCs was very rapid and apical membranes of most MR cells were transformed to a protrusive convex apical membrane up to the surface of PVCs within 24 h (Fig. 5E). The number of shallow basin MRCs increased once again after 7 days. In 7 and 21 day specimens, protrusive apical membranes at the same level as neighboring PVCs on the afferent edge were more common close to or near-by the lamellae while shallow basin subtype were more abundant at the edge (away from the lamellae) (Fig. 3). In the experimental group of 1‰ transformations observed after 21 days adaptation were similar to those reported for 5‰ during the same period (Fig. 5F). In experimental groups

exposed to 1 and 5‰ salinities, size and depth of apical membrane of MR cells on the afferent edge of the gill filament and in the interlamellar region were much lower than that of controls.

**MRC exposure densities:** Apical opening densities of MR cells in control group during different sampling times were 1250.46±40.65 to 1421.06±35.35 which showed no significant differences among the experimental groups studied. No significant differences were observed between MRC density in the experimental group exposed to 60‰ and in that in the control group after the same period. Significant reduction in MRC opening densities were observed in experimental groups with 5 and 20‰ salinities as compared to that in the experimental group 60‰ and in controls after 24 h exposure ($p<0.01$) although no significant differences existed between the experimental groups 5 and 20‰ within 24 h exposure. On day 7, MRC density in the experimental group 20‰ (1486.07±39.22) increased significantly as compared to that on day one at the same treatment ($p<0.05$), but was not significantly different from that reported in experimental groups 5, 60‰ and in control on the same day. No significant differences ($p>0.05$) were detected in MRC exposure densities among the various experimental groups at the end of the experimental period (day 21). MRC density in experimental groups 5 and 20‰ on day 21 were not increased significantly as compared to day 7 (Fig. 6).

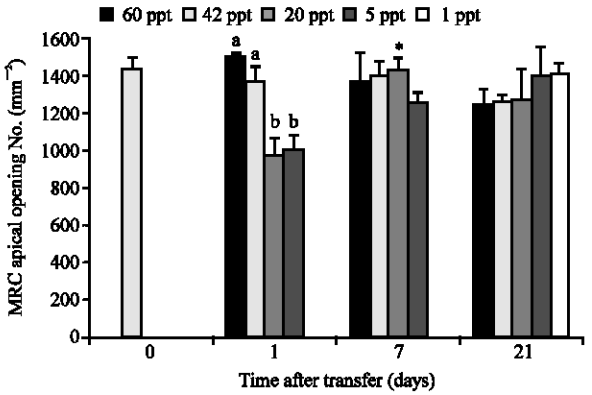


Fig. 6: Number of MRC openings on the afferent side of gill filaments of sea bream transferred from 42 to 60‰, 20, 5 and 1‰. Vertical line indicates Standard Error of the Mean (SEM) (No. = 3 per treatment). Different letter(s) (a and b), denotes significant difference ($p<0.05$) between concurrent sampling points. *Denotes significant difference ($p<0.05$) between current and preceding sampling point in the same treatment

## DISCUSSION

Short and long-term responses to direct transfer to various salinities on transformations in apical openings of MR cells were studied. The results of this study reveal that morphological changes in MRCs reflect the ionic and osmotic requirements of the animal. In this study, yellowfin seabream was able to tolerate direct exposure to salinities ranging from 5 to 60‰ without showing mortality. These results conform to previous findings related to osmoregulatory potentials of other sparid species (Fielder *et al*., 2007; Kelly and Woo, 1999; Kelly *et al*., 1999a, b). Yellowfin sea breams were also able to tolerate gradual decrease in salinity in the surrounding medium from seawater (42‰) to FW (1‰) through 10 days and successfully show temporary adaptation to FW at the end of the experimental period without exhibiting mortality. This is the shortest period reported for adaptation to FW in a true marine fish in an acceptable experimental duration. Kelly *et al*. (1999b) reported adaptation of black seabream for 21 days (*Mylio macrocephalus*) to FW after 8 months acclimation with 6‰ salinity.

Like in silver seabream (*Sparus sarba*) (Kelly and Woo, 1999), apical openings of MR cells in yellowfin seabream are not found on the efferent edge of gill filaments, but are more common on the afferent edge and interlamellar region of gill filaments (showing increased density toward the afferent edge). Therefore, as is done in the case of most teleosts, it is appropriate to study MR cells on branchial epithelium in the yellowfin seabream. However, in some teleosts such as milkfish (*Chanos chanos*), MR cells rarely occurred on the trailing (afferent) edge of gill filaments (Chen *et al*., 2004). Two MR cell subtypes (α and β) were described in freshwater stenohaline fish or euryhaline fish acclimated to freshwater on the basis of cell shape, apical membrane morphology, associated subapical structures, extent of tubular system and anatomic location on gill filaments. On the whole, MRcells in seawater (or chloride cell) are identified as αMRCs, while the βMR cells are specific of FW species (Pisam *et al*., 1993; Shikano and Fujio, 1998; Chang *et al*., 2001; Hirose *et al*., 2003; Kaneko and Katoh, 2004; Evans *et al*., 2005). In the present study, three subtypes of MR cells (shallow basin, deep hole and wavy convex) were considered to describe the ultrastructural characteristics of apical surface of MR cells using scanning electron microscopy. In a previous study on tilapia (*Oreochromis mossambicus*) adapted to freshwater, MRCs were categorized by the same three subtypes (Chang *et al*., 2001).

Chang *et al.* (2001) reported deep hole MR cell in freshwater adapted tilapia to be similar to αMRC and shallow basin MR cell to be similar to βMR cell. However, in the present study, only shallow basin MR cells were reported on gill filaments at 60‰. Also at 42‰ (control) most of the MR cells were of shallow basin type and only a few deep hole MRCs were observed. In diluted hypertonic (in the early days of adaptation) and hypotonic media (20, 5‰ and FW) MR cells with all three types of apical surfaces occurred on the gill filament. Hence considering that previous research stresses on the presence of only the α subtype MR cell in marine fish or euryhaline fish adapted to SW (Evans *et al.*, 2005), the present study considers the deep hole and shallow basin MR cells to be similar to αMR cell. As there is not enough information available to document this relationship, the photomicrographs available from SEM should be compared with photomicrographs available from Transmission Electron Microscopy (TEM) under similar conditions. It is likely that species, natural environment of the species and type of adaptation (from higher to lower salinities or vice versa) are effective in the two MR cell classifications.

Rapid responses in MR cells to sudden changes in salinitiy occurred in gill filaments in yellowfin seabream so that transformations in ultrastructure and MR cell densities were observed within 24 h. Similar transformations were previously reported in silver seabream by Kelly and Woo (1999). Although, significant differences were not detected in MRC density after 7 and 21 days in the different experimental groups and the control group ($p<0.05$), ultrastructural differences were observed between the different groups studied. Increase in the density of the apical openings of MR cells within 24 h of exposure to 60‰ was not significantly different from that in the control group. But functional apical surface area and the ratio of this area to the epithelium area on the afferent edge of the filament increased substantially. The extensive apical surface area was observed even on day 21. Deep hole MR cells were not observed at this salinity although they were seen in small numbers in the control group suggesting the transformation to the shallow basin subtype by the protrusion of the apical membrane.

After 24 h exposure to 5 and 20‰ salinities, with regard to the decrease in mean area of apical surface area of MR cells and a steady decline in MRC density, MR cells occupied a smaller area on the gill epithelium as compared to that in the control group. However, after 21 days exposure to 20‰, apical surface area and density of the apical openings of MR cells in gill epithelium were almost similar to that in the control group. This temporary transformation in apical surface morphology in 20‰ environment is indicative of the primary and vital role of gills in response to osmotic shocks. Also, since the external environment is still hyperosmotic, the density and morphology of apical membrane of chloride cells return to their normal state. Hence, changes in number and distribution patterns of enzymes and ion transporting proteins in the apical membrane of SW MR cells are capable of meeting the physiological needs of fish adapted to 20‰ without changing density and ultrastructure of apical surface of these cells as compared to that in full strength seawater (42‰) and/or the need for further adaptations in other organs such as kidneys (Fielder *et al.*, 2007). It is also evident from the results of the present study that shallow basin MR cells are characteristic of seawater MR cells and can fulfill the physiological needs of marine teleosts or euryhaline teleosts adapted to seawater and hyperosmostic environments. It is likely that the function of these cells in hypoosmotic conditions follows the model presented by Evans (2008) (Fig. 7) showing functional and morphological transformation of MR cells and changes in the type and location of enzymes and other transporting proteins in the cell membrane. In the present study the

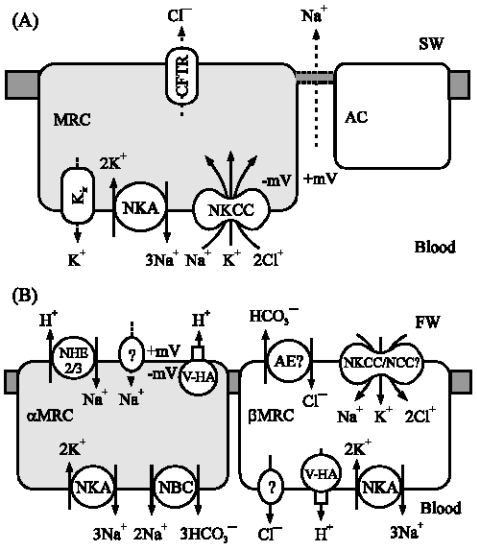


Fig. 7: Working model for the mechanisms of NaCl secretion (A) and uptake (B) by the teleost gill Mitochondria-Rich Cells (MRC). AC: Accessory cell; AE?: undefined $Cl^-/HCO_3^-$ exchanger (AE1 or Pendrin?); CFTR: fish cystic fibrosis transmembrane conductance regulator; $?Cl^-$: undefined $Cl^-$ channel; FW: fresh water; $K_{ir}$: inward rectifying $K^+$ channel; $?Na^+$: undefined $Na^+$ channel; NBC: $Na^+/HCO_3^-$ cotransporter; NCC: $Na^+/K^+$ cotransporter; NHE2/3: $Na^+/H^+$ exchanger; NKA: $Na^+/K^+$-activated ATPase; NKCC: $Na^+/K^+/2Cl^-$ cotransporter; SW: sea water; V-HA: V-type $H^+$-ATPase (Modified from two figures in Evans, 2008)

relative density of different subtypes of MR cells was closely related to the environmental salinity. The presence of all three subtypes of MR cells in hyposmotic environments also reported by Chang *et al.* (2001), Marisa and Perna-Martins (2002) and Moron *et al.* (2003), suggest that MR cells with different morphologies are equally active in such environments but exhibit different ion transporting functions. The high frequency of wavy convex subtype MR cells with numerous microvilli on the apical membrane following hypoosmotic exposure increases apical surface area, which is consistent with its ion absorptive nature (Pisam *et al.*, 1993; Hirose *et al.*, 2003; Varsamos *et al.*, 2005).

Increase in MR cells in gill filament was reported in two freshwater teleosts belonging to the genus *Oreochromis* when transferred to 10 and 20‰ (Avella *et al.*, 1993). Differences in the number and size of chloride cells have also been reported in a sturgeon (*Acipenser oxyrinchus*) after transfer from seawater to freshwater and from freshwater to seawater (Altinok *et al.*, 1998). The saline conditions showed an increase in number of the chloride cells during the early days of adaptation. Similar trends were detected in the present study for the first 24 h after transfer of yellowfin seabream to 20 and 5‰. Although, no significant differences were detected in the frequency of newly differentiated MR cells between hypoosmotic transferred (5‰ and FW) and other experimental groups studied at the end of 21 days, differences in apical membrane morphology were observed. The number of seawater-type MR cells in hyposmotic environments was clearly lower than that in hyperosmotic environments (20, 42 and 60‰). Carmona *et al.* (2004) reports modifications in MR cells of the gill epithelia in Adriatic sturgeon *Acipenser naccarii* following their transfer to seawater. Fish held in freshwater showed less invaginated apical surface as compared to those in seawater. In the present study, protrusion of the apical membrane in yellowfin seabream after 24 h exposure to 20 and 5‰ environments was reported. Presence of the shallow basin MR cells, was maintained even after 21 days of exposure to hypoosmotic environments although deep hole and wavy convex subtypes were also observed in the gill epithelium of fish adapted to these environments.

In conclusion, the results of this study suggest that successful adaptation to a wide range of salinities in yellowfin seabream is not accompanied by mortality and they are able to conform to sudden exposures to hypoosmotic (5‰) as well as to hyperosmotic environments higher than the salinity of seawater (60‰). This species was also able to adapt well to gradual decrease in salinity of the external environment and transfer into FW (1‰). Quantitative and qualitative transformations in apical openings of MR cells were easily detectable (within 24 h). Present findings revealed that it took 7 to 21 days for this species to reach a constant condition in the new salinity. With regard to the short-term transformation in the number of apical openings and MR cell replacement by newly differentiated cells in the gill filament, chloride cell morphology is thought to be a reliable index for adaptability to salinity. This study has demonstrated the ability of yellowfin seabream to adapt to a diverse range of environmental conditions and hence it can be used as a reliable model for osmoregulation in teleosts.

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