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A comparative study on gill histology and ultrastructure of the sea bass (*Dicentrarchus labrax*) inhabiting brackish, marine and hyper-saline waters

Sanaa E. Abdel Samei[1], Hoda M. Shoman[1] and **Ahmad M. Azab[2*]**
1. Zoology Department, Faculty of Science (Girls), Al-Azhar University, Cairo
2. Zoology Department, Faculty of Science (Boys), Al-Azhar University, Cairo
[*]Corresponding author: amazab2000@yahoo.com

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ABSTRACT

The present work was carried out to study the gill histology and ultrastructure of the seabass, *Dicentrarchus labrax*; and to compare between these structures in fish specimens inhabiting waters with different salinities. Fish specimens of the sea bass (*D. labrax*) were collected from Gholion Pond at Kafr El-Shiekh Governorate (Salinity: 18-20 ‰); Timsah Lake at Ismailia Governorate (salinity: 35-37‰) and Bardawil Lagoon at Al-Arish, North Sinai Governorate (Salinity: 64 ‰).

Histological examination showed that the chloride cells in brackish waters are located within the epithelium of the inter-lamellar regions and secondary lamellae, but in marine and hypersaline waters they are located within the primary epithelium of the inter-lamellar regions. The flat elongated pavement cells of secondary lamellar epithelium in hypersaline specimens are more flattened than those in brackish and marine waters. The ultrastructure examination showed that chloride cells in specimens of brackish water are large; polygonal and ovoid in shape with a little population of mitochondria. While the chloride cells in specimens from marine and hypersaline waters displayed more rich populations of mitochondria. In marine and hypersaline specimens, each lamellar epithelium mostly consists of one layer of flat elongated pavement cell, while it consists of two layers in brackish water specimens. Results of statistical analysis to gill characteristic counts and measurements of *D. labrax* inhabiting waters with different salinities revealed that there are statistically significant differences between groups for most counts and measurements as well as their ratios, except that of the pillar system diameter. Generally, most of the multiple comparisons (brackish versus marine; brackish versus hypersaline or marine versus hypersaline) are significantly varied

INTRODUCTION

In their natural environment, seabass (*Dicentrarchus labrax*) fish face strong salinity variations due to their seasonal life cycle transition from seawater "SW" to brackish and even freshwater "FW" **(Varsamos *et al.*, 2001; Varsamos *et al.*, 2002** and **Lorin-Nebel *et al.*, 2006).** Asian seabass (*Lates calcarifer*) is a protandrous fish and tolerate a wide range of salinities **(Mozanzadeh *et al.*, 2021).**

Euryhaline fishes demonstrate a remarkable ability to adapt to opposing osmotic challenges, from hypoosmotic surroundings in freshwater, to hyperosmotic surroundings in seawater. A surprising number of species can go further, maintaining osmotic homeostasis under hypersaline conditions of up to two or three time's seawater salinity, or even higher **(Nordlie, 2006)**

Gills are the primary osmoregulatory organ in teleosts (**Brown, 1992; Laurent *et al.*, 1994 a & b)**. The general structure of gill is composed of gill rakers, gill arches and gill filaments where the epithelial cells are located **(Senarat *et al.*, 2018).** Morphological, histological and physiological characterizations of the gill epithelial cells (or respiratory epithelium) have demonstrated that these cells are designed for gas-exchange surface, systemic maintenance of blood acid-base balance and ionic regulation (**Goss *et al.*, 1998; Sturla *et al.*, 2001; Garcia *et al.*, 2015** and **Neurasteh *et al.*, 2017*).*** Teleost osmoregulation and chloride cells (CCs) have been reviewed extensively **(Sakamoto *et al.*, 2001).**

Chloride cells are the main site of ion absorption and secretion, and play a crucial role in adaptation to freshwater and seawater conditions **(Katoh & Kaneko, 2003** and **Pritchard, 2003).** Several studies revealed that in response to external salinity CCs undergo morphological transformation and changes in location and number **(Sakamoto *et al.*, 2000, 2001** and **Katoh & Kaneko, 2003)**.

So, the present work was carried out to study the histological and ultrastructure of the gills in seabass, *Dicenrarchus labrax*; and to compare between these structural properties in fish specimens inhabiting waters with different salinities.

MATERIALS AND METHODS

1. Collection of samples

The current study was conducted on fifteen mature fish specimens of sea-bass (*Dicentrarchus labrax*). These specimens were collected during the period from January 2020 to April 2021, from Gholion Pond, Kafr El-Shiekh Governorate (Salinity: 18-20 ‰); Timsah Lake, at Ismailia, Ismailia Governorate (salinity: 35 - 37‰) and Bardawil Lagoon, at Al-Arish, North Sinai Governorate (Salinity: 64-68 ‰).

2. Morphology of gill arch

In the laboratory, specimens were carefully dissected, operculum was removed, the first gill arch in the left side of the fish was cut off from the rest of the gill and immersed in 70% ethyl alcohol and 3% Alizarin Red for 24 hours. Then, it was washed in 1% KOH for 2 hours. The gill arches were microscopically examined and photographed using a digital camera mounted on a dissecting microscope.

3. Histological studies

The collected specimens of *D. labrax* were dissected, operculum was removed and gills were separated, the first gill arch in the right side of the fish was cut off from the rest gill arches and immediately fixed in 10% formalin for 24 hours. Then, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in wax (M.P.: 58°C). Transverse sections were cut at 4-6 µ in thickness; sections were hydrated and stained with Harris`s haematoxylin and eosin. Then sections dehydrated, cleared, mounted with Canada balsam and covered for routine histological examination. Sections were examined under light microscope and photographed by digital camera mounted on light microscope.

4. Gill morphometric characteristic measurements and counts

The digital photographs of gill arches and histological photographs were used to record the characteristic morphometric counts of different gill parts and measuring the morphometric measurements using the Image ProPlus Program.

5. Transmission electron microscopy study:

Small specimens (0.5*0.5 cm) were immediately cut off from gill filaments of *D. labrax* fishes. These specimens were fixed in 2.5% glutraldehyde in 0.1 M cacodylate buffer at (pH 7.4) for about five hours followed by washing in phosphate buffer (pH 7.4) and post fixed in buffered 1% osmium tetraoxide. The specimens were washed thoroughly in buffer and dehyderated in ascending series of cold ethyl alcohol; cleared in propylene oxide and mounted in epoxy resin.

Semi-thin sections (0.5 - 1.0 µ) parallel to the long axis of the primary filament were obtained by using LKB Ultra-tome. Sections were stained by Toluidine blue, examined by light microscope and photographed by digital camera mounted on light microscope.

Ultrathin sections were cut with (Leica Ultra cut UCT microtome) mounted on grids; stained with uranyl acetate and lead citrate. Examination and imaging were carried out using a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo.

6. Statistical analyses of data:

The main effects and interactions were examined using ANOVAs (the corresponding multivariate tests had high power). Analysis of variance (ANOVA) applied using Holm-sidak method to refuse the null hypnosis and confirm the presence of significant variance between different levels of factors. The probabilities of Epsilon corrected F values (Greenhouse-Geisser Epsilon) were calculated to compensate for deviations from univariate assumptions. The analysis becomes available using SigmaPlot V12.5 software.

RESULTS

1. Effect of different salinities on gill structure:

Histological examination revealed that each gill arch in the gills of *Dicentrarchus labrax* bears a double row of gill filaments (non-respiratory primary lamellae); and each filament carries two rows of secondary lamellae (respiratory secondary lamellae). The gill epithelium of the primary filament is composed of a multilayered filament epithelium (primary epithelium). The epithelium of secondary lamellae is composed of bi-layered lamellar epithelium (secondary epithelium). The primary epithelium covers the filament including the inter-lamellar region, while the secondary epithelium covers the free part of the secondary lamella **(Fig. 1)**.

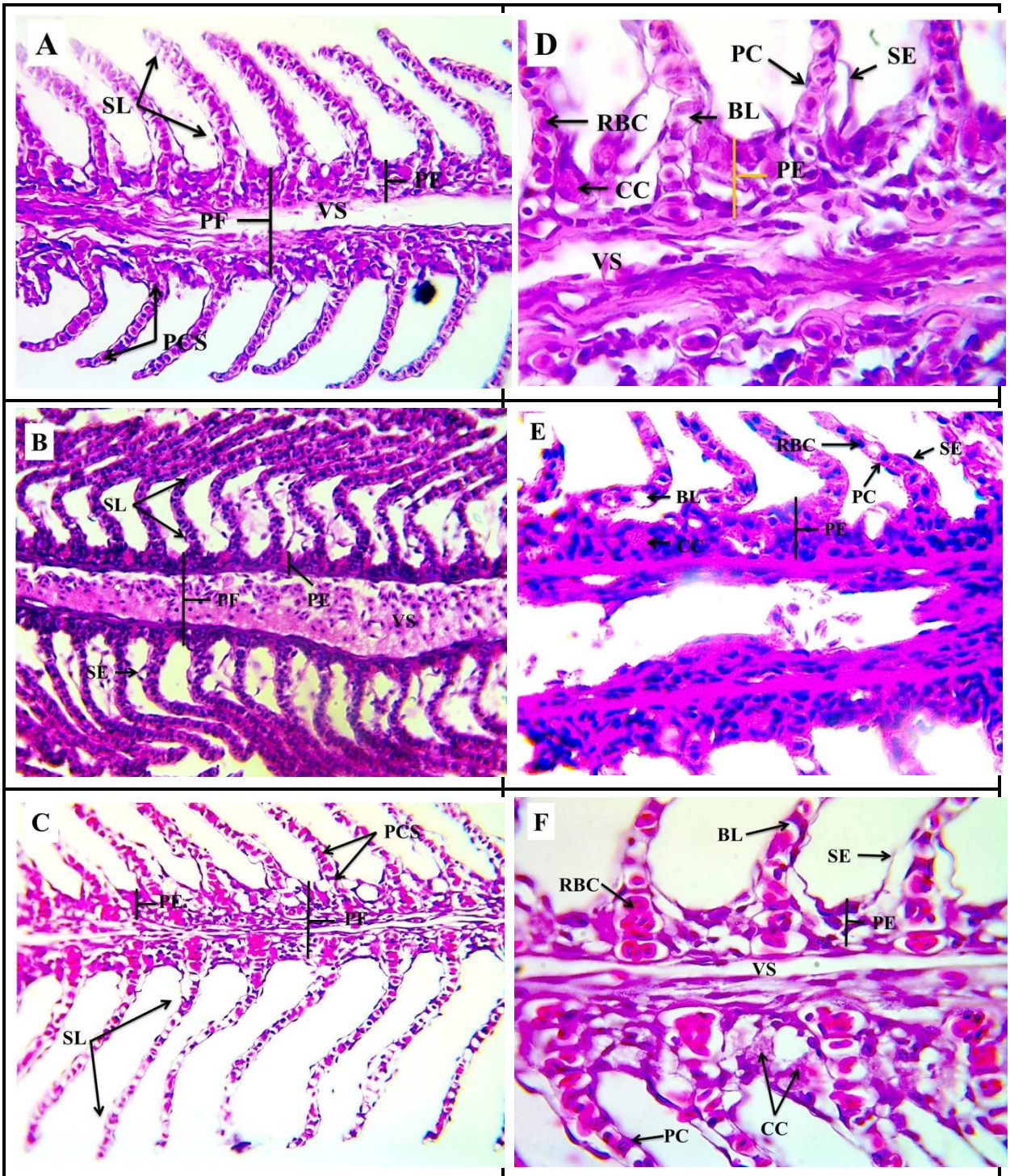

Fig. (1). Photomicrographs of cross-section through a gill filament of seabass (*D. labrax*) from brackish water (A&D), marine water (B&E) and hypersaline water (C&F); showing the different regions of the filament epithelium and associated vasculature.

BL: Blood lacuna; CC: Chloride cell; PC: Pillar cell; PCS: Pillar cell system; PE: Primary epithelium; PF: Primary filament; RBC: Red blood cell; SE: Secondary epithelium; SL: Secondary lamella and VS: Venus sinus. {H & E; A, B & C X 400 - D, E & F: X1000}.

The filament epithelium contains several cell types, such as superficial pavement cells, basal epithelial cells, mucous cells, and mitochondria-rich chloride cells. Chloride cell usually is covered by the superficial pavement cell **(Fig. 1)**. In brackish waters, the chloride cells are located within the epithelium of the inter-lamellar regions and secondary lamellae; but in marine and hyper saline waters they are located within the primary epithelium of the inter-lamellar regions and resting on the pillar capillary basal lamina **(Fig. 2)**.

The secondary gill lamella consists of two secondary epithelia resting on basal lamina separated by a central pillar cell system. Each secondary epithelium consists of two layers of cells, which overlap and interdigitate in a complex manner **(Fig. 1)**. The flat elongated pavement cells characterize the external layer. In hyper saline specimens, it is more flattened than that in brackish and marine waters **(Fig. 2)**.

Results showed that the mucus cells are apically located in the primary epithelium. They are characterized by the presence of large amount of mucus. Mucous cells attain dark blue color with Toluidine blue stain in semithin sections **(Fig. 2)**.

2. Effect of different salinities on gill ultrastructure

In semi thin sections, chloride cells in fishes of marine and hyper saline waters are large; rounded or ovoid in shape, while they are polygonal in brackish waters (**Fig. 2**). Inter-lamellar chloride cells in hyper saline and marine waters have large apical pits. While in brackish water, chloride cells frequently haven't these apical pits (**Fig. 2**).

2.1. Secondary lamellae

The secondary gill lamella of ***D. labrax*** consists of two epithelia resting on basal lamina separated by a central pillar cell system **(Fig. 3)**.

In each epithelium of the two opposite sides, two layers of cells was observed in brackish water, which overlap and interdigitate in a complex manner. The flat elongated pavement cells characterize the external layer. They have really flattened nuclei, characterized by dense peripheral heterochromatin. The plasma membrane of these cells sometimes forms a system of micro-ridges and gives a fuzzy coat appearance on the surface of lamellae. The cells forming the innermost layer are also flat with elongated nuclei. Their cytoplasmic matrix is less electron dense than that of pavement ones. These cells are joined together with finger-like cytoplasmic projections. Mitochondria may be also seen **(Fig. 3A)**.

In marine and hyper saline specimens, each lamellar epithelium mostly consists of one layer of flat elongated pavement cell. The plasma membrane of these cells sometimes forms a system of micro-ridges and gives a fuzzy coat appearance on the surface of lamellae. They have really flattened nuclei, characterized by more electron dense peripheral heterochromatin in marine water than that in hyper saline water **(Figs. 3B & C)**.

The central pillar cell system is made of the nucleated body forming the pillar cell and the lateral cytoplasmic processes or flanges. The pillar cell body contains bundles of microfilaments located in the cell body periphery and constituting the prominent feature of the pillar cell cytoplasm. The lateral cytoplasmic processes of each pillar cell stretch out to touch those of adjacent pillar cells. The spaces between pillar cells form blood lacunae, through which blood cells flow. The intercellular spaces were also detected in brackish and marine waters **(Figs. 3A, B & C)**. A decrease in the thickness of the pillar cell flanges of hyper saline water was observed in contrast to the thickness of flanges in brackish and marine waters.

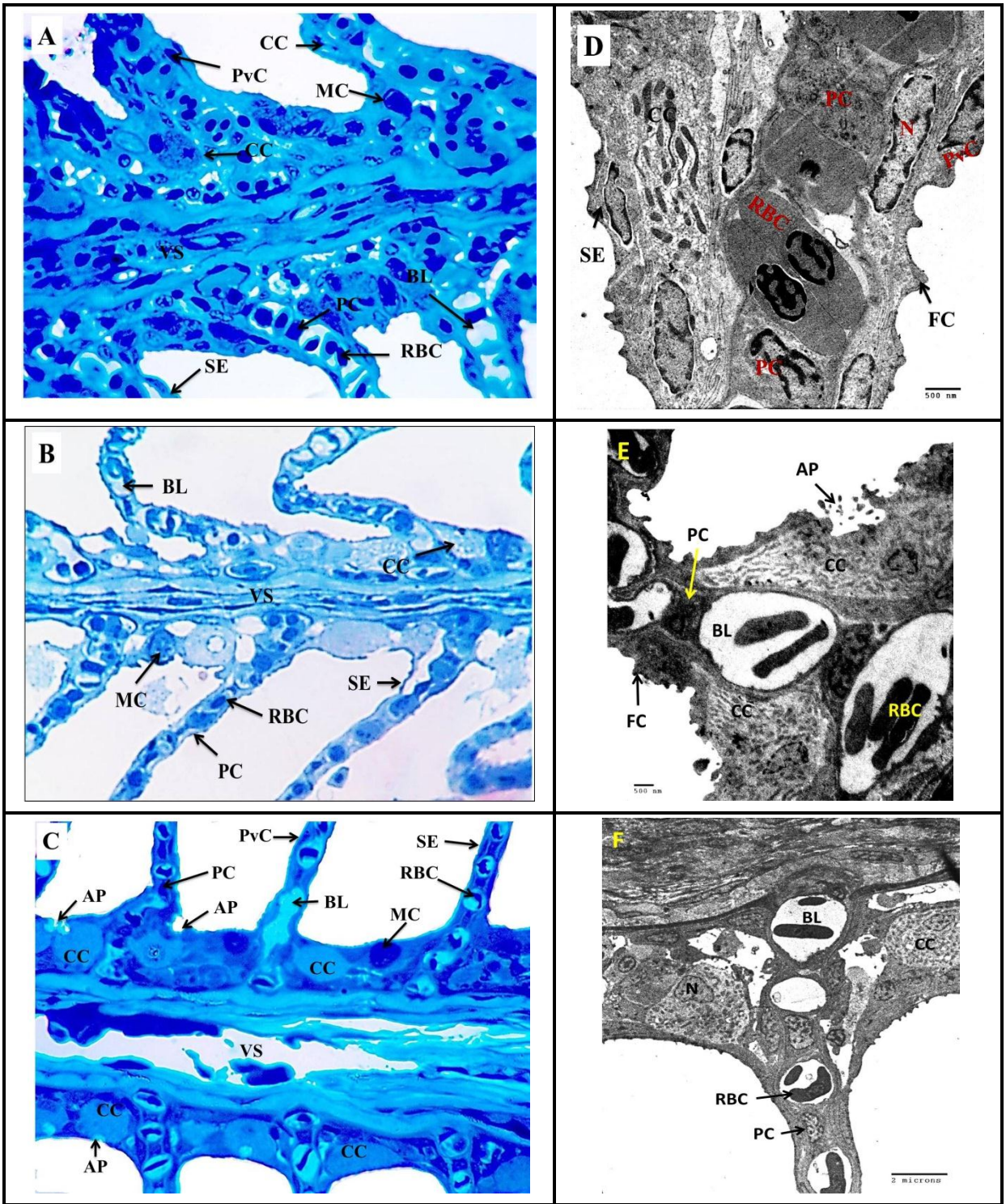

Fig. (2). Photomicrographs of cross-section through a gill filament of seabass (*D. labrax*) from brackish water (A&D), marine water (B&E) and hypersaline water (C&F).

AP: Apical pit; BL: Blood lacuna; CC: Chloride cell; FC: Fuzzy coat; MC: Mucus cell; N: Nucleus; PC: Pilar cell; PCF: Pillar cell flanges; PCS: Pillar cell system; PE: Primary epithelium; PF: Primary filament; PvC: Pavement cell; RBC: Red blood cell; SE: Secondary epithelium; SL: Secondary lamella and VS: Venus sinus. {A, B & C: semithin stained with toluidine blue, X1000; D, E & F: (TEM) Uranyl acetate & lead citrate}.

2.2. Chloride cell

Most of the inter-lamellar chloride cells in gill filament are found exposing a little surface to the external medium. While, some chloride cells are not in contact with the external medium and they have sheets of pavement cells covering their apical surfaces **(Fig. 2D, E & F)**..

The ultrastructure examination showed that chloride cells in specimens of brackish water are large; polygonal and ovoid in shape; usually covered by the superficial pavement cells. They display a little population of mitochondria, evenly distributed throughout the cytoplasm. These mitochondria are variable in size and shape. Vacuolization of the cytoplasm and multi-vesicular bodies were also observed in some chloride cells. They have really large nuclei, characterized by dense peripheral heterochromatin in some chloride cell, but other chloride cells have nuclei characterized by light peripheral heterochromatin **(Figs. 2D & 3D)**.

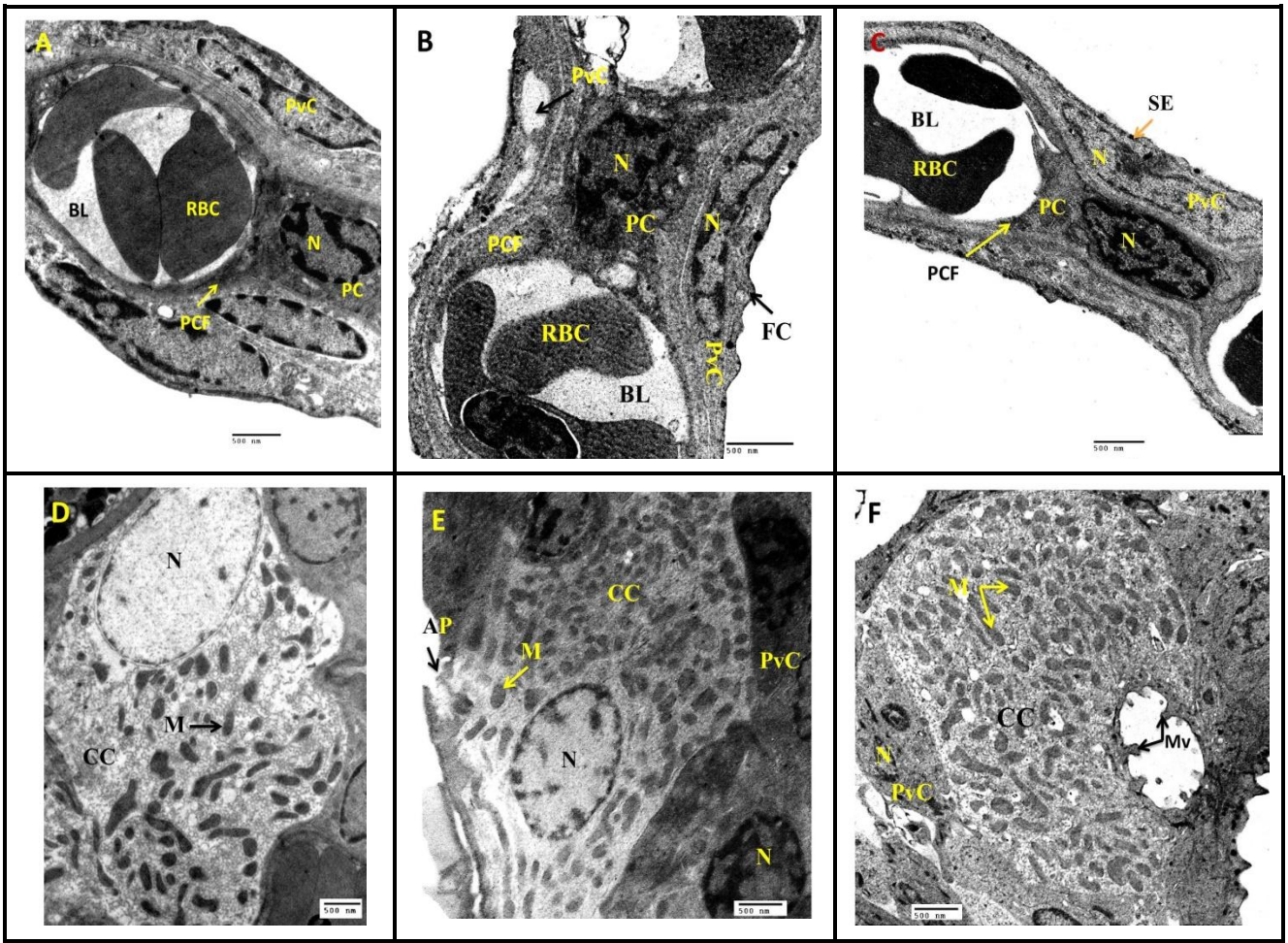

Fig. (3). Photomicrographs to TEM in cross-sections through gill lamellae (A, B & C) and chloride cells in gill filaments (D, E & F) of seabass (*D. labrax*) from brackish water (A&D), marine water (B&E) and hypersaline water (C&F).

AP: Apical pit; BL: Blood lacuna; CC: Chloride cell; FC: Fuzzy coat; MC: Mucus cell; N: Nucleus; PC: Pillar cell; PCF: Pillar cell flanges; PCS: Pillar cell system; PE: Primary epithelium; PF: Primary filament; PvC: Pavement cell; RBC: Red blood cell; SE: Secondary epithelium; SL: Secondary lamella and VS: Venus sinus.

In specimens of marine water, a conspicuous feature of many chloride cells is the presence of an apical pit opening to the external environment. The pit is usually located between two superficial pavement cells. The pit may contain finely granulated material. Chloride cells are large; polygonal in shape. They have really large nuclei, characterized by dense peripheral heterochromatin. These chloride cells display a rich population of mitochondria, evenly distributed throughout the cytoplasm. These mitochondria are small in size **(Figs. 2 E & 3 E)**.

In specimens of hyper saline waters, chloride cells are large; ovoid and rounded in shape. Most chloride cells of the gill filament had a greatly hypertrophied tubular system that formed a dense network of anastomosed tubules. These chloride cells display a very rich population of mitochondria found in cytoplasm. These mitochondria are small in size and variable in shape **(Figs. 2 F & 3 F).**

3. Effect of different salinities on gill morphometric characters;

The number of mucus cells was higher in gill filaments of brackish water specimens (22.4 ± 2.07) than those of marine (19.4 ± 4.16) and hypersaline (13.8 ± 4.66) specimens. In contrast, the number of chloride cells was higher in gill filaments of hypersaline specimens (36.0 ± 1.00) than those of brackish water (16.0 ± 2.65) and marine (20.7 ± 1.15) specimens. In the same manner, the number of mitochondria of the chloride cells was higher in gill filaments of hypersaline water specimens (196.7 ± 28.3) than those of brackish (102.0 ± 2.00) and marine (121.3 ± 25.4) specimens **(Table 1)**.

The results showed that the primary gill filament length **(PFL)** in marine water specimens was longer (11000 ± 948.7 µ) than that of brackish water specimens (8500 ± 894.4 µ) and those in hyper saline specimens (6166 ± 816.5 µ). But, the ratio of gill filament length to total body length was higher in brackish and marine fishes than that in hypersaline fishes **(Table 1)**.

It was found that the primary filament thickness **(PFT)** in brackish water specimens was thicker (86.4 ± 4.79 µ) than that of marine water (57.9 ± 4.81 µ) and those in hyper saline (57.8 ± 1.62 µ) specimens. Also, the ratio of primary filament thickness **(PFT)** to filament length **(PFL)** was higher in brackish water specimens (1.03 ± 0.056) than that of marine (0.53 ± 0.045) and hyper saline (0.94 ± 0.026) specimens **(Table 1)**.

The present results showed that the primary epithelial thickness **(PET)** in brackish water specimens was thicker (17.99 ± 3.882 µ) than that of marine water (9.78 ± 2.015 µ) and those in hyper saline (16.69 ± 5.070 µ) specimens. In contrast, the ratio of primary epithelium thickness to filament thickness **(PET/PFT)** was higher in hyper saline water specimens (28.88 ± 8.773) than that of brackish (20.83 ± 4.496) and marine (16.89 ± 3.637) specimens **(Table 1)**.

It was found that the secondary lamellar length **(SLL)** in marine water specimens was taller (160.1 ± 11.97 µ) than that of brackish water (92.6 ± 9.45 µ) and those in hyper saline (113.4 ± 4.36 µ) specimens. But, the ratio of secondary lamellar length to filament length **(SLL/PFL)** was higher in hyper saline water specimens (1.84 ± 0.071) than that of marine (1.46 ± 0.109) and brackish water (0.53 ± 0.045) specimens **(Table 1)**.

The present results showed that the secondary epithelial thickness **(SLT)** in brackish water specimens was thicker (8.27 ± 1.125 µ) than that of marine water (6.75 ± 1.098 µ) and those in hyper saline (5.14 ± 1.047 µ) specimens. Also, the ratio of secondary epithelial

thickness to lamellar thickness **(SLT/SLL)** was higher in brackish water specimens (8.9 ± 1.21) than that of marine (4.2 ± 0.69) and hyper saline (4.5 ± 0.92) specimens **(Table 1)**.

The present results showed that the inter-lamellar space **(ILS)** in hyper saline water specimens was wider (23.06 ± 1.663 µ) than that of brackish water (17.89 ± 3.704 µ) and those in marine (17.70 ± 3.761 µ) specimens. Also, ratio of inter-lamellar space to secondary lamellar length **(SLT/SLL)** was higher in hyper saline water specimens (20.33 ± 1.466) than that of brackish (19.32 ± 4.001) and marine (11.06 ± 2.350) specimens **(Table 1)**.

It was found that the pillar system diameter **(PSD)** in marine water specimens was slightly higher (2.166 ± 0.754 µ) than that of brackish water (1.956 ± 0.451 µ) and those in hyper saline (2.098 ± 0.031 µ) specimens. But, the ratio of pilar system diameter to secondary epithelial thickness **(PSD/SLT)** was higher in hyper saline water specimens (40.82 ± 5.869) than that of brackish (23.65 ± 5.458) and marine (32.13 ± 11.172) specimens **(Table 1)**.

Results of statistical analysis to gill characteristic counts and measurements of *Dicentrarchus labrax* inhabiting waters with different salinities are presented in **Table (2)**. These results revealed that there are statistically significant differences between groups for all counts and measurements as well as their ratios, except that of the pillar system diameter. Generally, most of the multiple comparisons (brackish versus marine; brackish versus hypersaline or marine versus hypersaline) are statistically significant varied **(Table 2)**.

Table (1): Gill characteristic measurements and counts of *D. labrax* inhabiting waters with different salinities (Data expressed as Mean ± SD)

Gill characteristics		Brackish water	Marine water	Hyper-saline water
Counts	**Mucus cell number (MC)**	22.4 ± 2.07	19.4 ± 4.16	13.8 ± 4.66
	Chloride cell number (CC)	16.0 ± 2.65	20.7 ± 1.15	36.0 ± 1.00
	Mitochondria number (M)	102.0 ± 2.00	121.3 ± 25.4	196.7 ± 28.3
Measurements µ	**Primary filament length, PFL**	8500 ± 894.4	11000 ± 948.7	6166 ± 816.5
	Primary filament thick, PFT	86.35± 4.792	57.88 ± 4.814	57.79 ± 1.617
	Filament epithelial thick, PET	17.99 ± 3.882	9.78 ± 2.015	16.69 ± 5.070
	Secondary lamellar length, SLL	92.6 ± 9.45	160.1 ± 11.97	113.5 ± 4.36
	Secondary epithelial thick, SLT	8.27 ± 1.125	6.75 ± 1.098	5.14 ± 1.047
	Inter-lamellar space, ILS	17.89 ± 3.704	17.70 ± 3.761	23.06 ± 1.663
	Pillar system diameter, PSD	1.96 ± 0.451	2.17 ± 0.754	2.10 ± 0.031
Morphometric ratios (%)	**PFL/Body length (BL)**	3.09 ± 0.225	2.97 ± 0.256	2.20 ± 0.292
	PFT / PFL	1.02 ± 0.056	0.53 ± 0.045	0.94 ± 0.026
	PET / PFT	20.83 ± 4.496	16.89 ± 3.637	28.88 ± 8.773
	SLL / PFL	1.09 ± 0.111	1.46 ± 0.109	1.84 ± 0.071
	SLT / SLL	8.93 ± 1.21	4.21 ± 0.69	4.48 ± 0.92
	ILS / SLL	19.32 ± 4.001	11.06 ± 2.350	20.33 ± 1.466
	PSD / SLT	23.46 ± 5.46	32.13 ± 11.172	40.82 ± 5.869

Table (2): Statistical analysis to gill characteristic counts and measurements of *D. labrax* inhabiting waters with different salinities

Gill characteristics		Analysis of Variance (Between groups)	Multiple Comparison (Holm-Sidak method)		
			Brackish vs Marine	Brackish vs Hypersaline	Marine vs Hypersaline
Counts	MC	*	NS	*	NS
	CC	**	*	**	**
	M	**	NS	**	*
Measurements (µ)	PFL	**	**	**	**
	PFT	**	**	**	NS
	PET	**	**	NS	**
	SLL	**	**	**	**
	SLT	**	**	**	**
	ILS	**	NS	**	**
	PSD	NS	--	--	--
Morphometric ratios (%)	PFL/ BL	**	NS	**	**
	PFT / PFL	**	**	**	**
	PET / PFT	**	NS	**	**
	SLL / PFL	**	**	**	**
	SLT / SLL	**	**	**	NS
	ILS / SLL	**	**	NS	**
	PSD / SLT	**	**	**	**

DISCUSSION

Gills are the chief osmoregulatory organs in fish and which are highly sensitive to many factors, such as changes in salinity, pollution and stress (**Kultz *et al.*, 1995** and **Uchida *et al.*, 1996).** Gills are also the primary corridor for molecular exchange between the internal environment of fish and their external medium **(Olson, 1996),** such as gas transfer, acid-base regulation **(Randall *et al.*, 1982)** and ionic regulation **(Eddy, 1982).**

The primary and the secondary lamellae of the gills represent 2 general types of epithelium **(Laurent & Dunel, 1980** and **Laurent, 1984)** that mainly contain 3 cell types: the chloride cells, the pavement cells and the mucous cells. According to **Wilson *et al*. (2000),** the gills among different groups of fish possess marked differences in their gross anatomy. However, the cells that compose the gill epithelia are very similar.

In the present study, it was found that each gill arch of *D. Labrax* bears a double row of gill filaments and each filament carries two rows of secondary lamellae. A multi-layered epithelium covers the filament, while a bi-layered epithelium covers the free part of the secondary lamella. Thus, the general gill morphology of *D. Labrax* is not markedly varied from that previously reported for most other fishes **(Laurent & Dunel, 1980; Jagoe & Haines, 1983; Cioni *et al*., 1991; Perera, 1993** and **Cardoso *et al*., 1996).**

In agreement with earlier reports **(Laurent & Dunel, 1980** and **Laurent, 1984),** the presence of chloride cells is the main feature of the filament epithelium. In brackish water specimens, the chloride cells are located within the epithelium of the inter-lamellar regions and secondary lamellae; but in marine and hyper saline waters specimens they are located

only within the primary epithelium of the inter-lamellar regions. The presence of chloride cells on lamellar epithelia have been previously reported by many authors **(Cataldi *et al*., 1995; Cardoso *et al*., 1996** and **Fernandes *et al*., 1998).** It was suggested that the increased number of chloride cells located in the secondary lamellae may be involved in ion regulation in freshwater fishes **(Perry & Wood, 1985; Bornacin *et al*., 1987** and **Avella *et al*., 1993).** Whereas the increased number of chloride cells in the filaments in seawater fishes was suggested as teleost adaptation to elevated external salinity **(Karnaky *et al*., 1976; Thomson & Sargent, 1977** and **Laurent & Dunel, 1980);** and it may be responsible for salt extrusion in hypertonic medium **(Keys & Wilmer, 1932). Laurent (1984)** postulated two possibilities to explain the reason that chloride cells invade the lamellar epithelium: (1) The migration of chloride cells from gill filament epithelium, where no more room is available or (2) A differentiation of resident stem cells.

In the present study, the secondary gill lamella of *D. labrax* consists of two epithelia resting on basal lamina separated by a central pillar cell system. In each epithelium of the two opposite sides, two layers of cells was observed in specimens of brackish water, which overlapped and interdigitated in a complex manner. The flat elongated pavement cells characterize the external layer. The plasma membrane of these cells sometimes forms a system of micro-ridges and gives a fuzzy coat appearance on the surface of lamellae. In marine and hyper saline specimens, each lamellar epithelium mostly consists of one layer of flat elongated pavement cell. The plasma membrane of these cells sometimes forms a system of micro-ridges and gives a fuzzy coat appearance on the surface of lamellae.

These results didn't agree with **Mir *et al.* (2011)** who concluded that pavement cells located on the primary lamellae of the ***Schizothorax curvifrons*** bear microridges on their apical plasma membrane which were coated with a thin film of glycocalyx. However, microridges were not seen on the apical plasma membrane of pavement cells of the secondary lamellae.

The absence of microridges on the surface of secondary lamellae has been reported in *Hoplias malabaricus* **(Moron & Fernandes, 1996),** *Solea senagalensis* **(Arellano *et al*., 2004**), Micropogonias furnieri **(Diaz *et al*., 2005).** Nevertheless**, Fernandes & Perna-Martins (2001)** reported the presence of microridges on the pavement cells of secondary lamellae of *Hypostomus plecostomus*. The surface microridges may mechanically facilitate the adhesion of water molecules favoring the respiratory gases to diffuse from water to blood and vice versa **(Rajbanshi, 1977)** and some turbulences and mixing of water caused by the apical cell projections in the water flow could be advantageous for gas exchange **(Lewis & Potter, 1976** and **Hughes, 1978).** The glycocalyx that coat the microridges may probably contribute to the retention of mucous by reducing the abrasive action of particles suspended in water.

Kultz *et al.* (1995) speculated that the absence of microridges on the pavement cells of secondary lamellae could facilitate gas exchange by minimizing the mucous layer on the epithelial surface and by enhancing the water flow through the secondary lamellae. The pavement cells of freshwater teleost gills have been implicated by many authors **(Perry & Laurent, 1993; Perry, 1997; Goss *et al*., 1998; Wilson *et al*., 2000 and Evans *et al*., 2005)** in ion uptake, acid-base regulation and osmotic regulation.

The central pillar cell system is made of the nucleated body forming the pillar cell and the lateral cytoplasmic processes or flanges. The flanges are kept thin to minimize the distances between blood and water for diffusion **(Newstead, 1967).**

The present study showed that the intercellular spaces were detected in gill specimens of brackish and marine waters. These results didn't agree with that described by **(Azab *et al*.,**

1999) who recorded the appearance of intercellular spaces between the epithelia and basal lamina of the secondary lamellae in the hypersaline-adapted fishes. These intercellular spaces communicate with the corresponding intercellular spaces of filament epithelium, a feature that may be an important implication in consideration of trans-branchial fluid movement **(Laurent & Dunel, 1980).** On the other hand, the swelling of the intercellular spaces with infiltration of leukocytes was parallel to those alterations seen by **Skidmore & Tovell (1972)** when fishes were exposed to pollutants.

In the present study, chloride cells in specimens of brackish water usually covered by the superficial pavement cells. These results agreed with that described by **(Azab *et al*., 1999)** who reported that apical pit was not observed in the chloride cells of freshwater-adapted fishes of *A. dispar*. However, many authors observed the apical pit in the chloride cells of some freshwater-adapted fishes including tilapias **(Fishelson, 1980** and **Maina, 1990),** guppies **(Straus, 1963)** and *Fundulus* spp**. (Philpott & Copeland, 1963)**. Furthermore, apical pit has been described in freshwater and seawater adapted *Rivulus marmoratus* at all tested salinities **(King *et al.*, 1989).**

In specimens of marine water, a conspicuous feature of many chloride cells is the presence of an apical pit opening to the external environment. The pit is usually located between two superficial pavement cells. The pit may contain finely granulated material. These results agreed with that described by **(Azab *et al*., 1999)** who reported that in normal (seawater) individuals of *A. dispar*, some of chloride cells open to the external medium through an apical pit. This finding has been generally reported as a distinctive feature of chloride cells in seawater fishes **(Laurent & Dunel, 1980);** or as a structural modification that the chloride cells display when an euryhaline species is transferred from freshwater to seawater **(Laurent, 1984).** Unlike, **Karnaky *et al* (1976) and Foskett *et al* (1981),** who reported that apical pit is often correlated with adaptation to high salinities.

The present study showed that the number of mucus cells was higher in gill filaments of brackish water specimens (22.4 ± 2.07) than those of marine (19.4 ± 4.16) and hypersaline (13.8 ± 4.66) specimens. These results didn't agree with **Sathron *et al.* (2021)** who observed that the density of mucus-secreting cells of *P. mexicana* increases in response to the salinity change from freshwater to meso-saline conditions. It is possible that these cells support the iono-regulation of chloride cells in *P. mexicana*. **Shephard (1989)** suggested that mucus secreting cells trap neighboring cations, creating an ionic gradient. **Franklin (1990)** also showed that the number of mucus cells increased in the sockeye salmon, *Oncorhynchus nerka* after transfer to seawater. In addition to the potential function of supporting chloride cells, mucus-secreting cells had been suggested to play a role in the increase of the blood-to-water diffusion barrier for respiratory gas exchange **(Fernandes *et al.*, 1998; Fernandes & Perna-Martins, 2002**), and consequently reduces oxygen uptake **(Sakuragui *et al.*, 2003)** and carbon dioxide excretion **(Powell & Perry, 1997).**

The present study showed that the number of chloride cells was higher in gill filaments of hypersaline specimens (36.0 ± 1.00) than those of marine (20.7 ± 1.15) and brackish water (16.0 ± 2.65) specimens. In the same manner, the number of mitochondria of the chloride cells was higher in gill filaments of hypersaline water specimens (196.7 ± 28.3) than those of marine (121.3 ± 25.4) and brackish water (102.0 ± 2.00) specimens. These results are in agreement with **Varsamos *et al.* (2002)** who reported that adaptation of sea bass to fresh water (FW) or double salinity of seawater (DSW) is followed by a numerical increase of branchial chloride cells (CC). Such an increase has been reported in most of the studied euryhaline species during their transition from FW to SW but not from SW to FW (**King & Hossler, 1991; Kültz &Jürss, 1993; Avella *et al*., 1993** and **Ura *et al*., 1997).**

This increase was linked to the increased need for ion transport capacity in SW or DSW, as demonstrated by **Marshall** and **Nishioka (1980)** in *Gillichthys mirabilis* exposed to DSW. However, the transfer of some marine species to FW **(Pisam *et al*., 1990)** or of some FW species to SW **(Mattheij & Stroband, 1971** and **Lee *et al.*, 1996)** was followed by a decrease in CC number, but always with a concomitant loss of osmoregulatory ability. Such findings mark the difference between euryhaline and stenohaline species. The sea bass, which is considered to be strongly euryhaline, increases its CC number in both FW and DSW.

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The present work found that the primary filament thickness (PFT) in brackish water specimens was thicker (86.4 ± 4.79 µ) than that of marine water (57.9 ± 4.81 µ) and those in hyper saline (57.8 ± 1.62 µ) specimens. Also, the ratio of primary filament thickness (PFT) to filament length (PFL) was higher in brackish water specimens (1.03 ± 0.056%) than that of marine (0.53 ± 0.045%) and hyper saline (0.94 ± 0.026%) specimens.

The pillar system diameter (PSD) in marine water specimens was slightly higher (2.166 ± 0.754 µ) than that of brackish water (1.956 ± 0.451 µ) and those in hyper saline (2.098 ± 0.031 µ) specimens. But, the ratio of pillar system diameter to secondary epithelial thickness (PSD/SLT) was higher in hyper saline water specimens (40.82 ± 5.869%) than that of brackish (23.65 ± 5.458%) and marine (32.13 ± 11.172%) specimens. These changes were described by **Farrel *et al.* (1980)** as a result of blood pressure raise and emphasized by **Soivio & Tuurala (1981)** who worked on rainbow trout exposed to chronic hypoxia. This modification was interpreted as a result of lamellar vascular distension, which in its turn increases the functional gill surface, decreases the blood-water barrier and adjusts the lamellar orientation in the respiratory water flow **(Laurent, 1984).** Such interpretation may be accepted in the present study, since the solubility of oxygen in water decreases with increased salinity **(Nybakin, 1993).** This explanation was also confirmed by the results of **Hughes (1973).**

CONCLUSION

The present study concluded that chloride cells in the gills of sea bass, *D. labrax* became activated with increasing environmental salinity. Those activated chloride cells may function as sites responsible for salt secretion in environments.

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