A comparative study on gill histology and ultrastructure of the flathead grey mullet, *Mugil cephalus* inhabiting marine and hyper-saline waters

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ABSTRACT

The present study aimed to investigate the characteristics in gill histology and ultrastructure of the flathead grey mullet, *Mugil cephalus*; as well as to compare between these structures in fish specimens inhabiting waters with different salinities. Fish specimens of *M. cephalus* were collected from Great bitter Lake at Ismailia Governorate (salinity: 38-45‰) and Bardawil Lagoon at Al-Arish, North Sinai Governorate (Salinity: 64-68 ‰). Histological examination showed that the chloride cells in gills of *M. cephalus* inhabiting marine waters (Great Bitter Lake) are located within the epithelium of the inter-lamellar regions and secondary lamellae; but in hyper saline waters (Bardawil Lagoon) fishes, chloride cells are located only within the primary epithelium of the inter-lamellar regions. The flat elongated pavement cells of secondary lamellar epithelium in marine fishes are more flattened than that in hypersaline water fishes. In each epithelium of the two opposite sides of secondary lamellae, two layers of cells were observed in hypersaline specimens, in which they overlap and interdigitate in a complex manner; but in marine specimens, only one layer of pavement cells was observed. The ultrastructure examination showed that chloride cells in specimens of marine water are large; ovoid or rounded in shape with a rich population of mitochondria. While, chloride cells in fishes inhabited hypersaline water displayed more rich populations of mitochondria. In the present study, statistical analysis to gill morphometric characteristic counts and measurements of gills in *M. cephalus* inhabiting marine water and hypersaline water revealed that there are statistically significant differences between these groups for most counts and measurements as well as their ratios.

INTRODUCTION

Osmoregulation is one of the most vital functions to all euryhaline fish (**Evans, 2008**), which provide hyper osmo-regulatory and hypo osmo-regulatory abilities to keep osmolality of body fluids within a narrow physiological range in freshwater (FW) and seawater (SW) fish, respectively (**Kang *et al.,* 2013**). Flathead grey mullet, *Mugil cephalus* is a euryhaline fish species that can accommodate in both freshwater and saltwater and it is a crucial economically fish. 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** and **Li *et al.,* 2017).**

The gills are composed of four holobranchs. Each holobranch contains several gill lamellae which act as gas exchange barrier as a highly vascularized layer of pillar cells surround them (**Wilson & Laurent, 2002).**

The gills have many functions including; acid-base balance, gas exchange, excretion of nitrogenous waste products and osmoregulation **(Evans et al., 2005).** The mitochondria-rich cells (chloride cells) act as ionic regulators while the function of pavement cells is a gas exchange **(Perry, 1998; Evans et al., 2005; Hwang et al., 2011).** On euryhaline fish, the mitochondria-rich cells (MRCs) have the property of change from freshwater kind to the saltwater kind and vice versa as a method for acclimation of gills to change of water salinity **(Ching et al., 2013).**

So, the present work was carried out to study the histology and ultrastructure properties of the gills in flathead grey mullet, *Mugil cephalus*; 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 flathead grey mullet, *Mugil cephalus*. These specimens were collected from Great Bitter Lake at Ismailia Governorate (salinity: 38-45‰) 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 *M. cephalus* 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 (TEM) study:

Small specimens (0.5*0.5 cm) were immediately cut off from gill filaments of *M. cephalus* samples. 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 *Mugil cephalus* 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)**.

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 marine waters, the chloride cells are located within the epithelium of the inter-lamellar regions and secondary lamellae; but in 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 marine specimens, it is more flattened than that in hyper saline 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 Alcian- PAS reaction, indicating the presence of basic muco-polysaccharides. These mucus cells in gills of marine specimens are more numerous than that of hyper saline specimens **(Fig. 1)**.

2. Effect of different salinities on gill ultrastructure

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

2.1. Secondary lamellae

The secondary gill lamella of *M. cephalus* 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 were observed in hyper saline specimens, in which they overlap and interdigitate in a complex manner; but in marine specimens, only one layer of pavement cells was observed. The flat elongated pavement cells characterize the external layer. They have really flattened nuclei, characterized by more electron dense peripheral heterochromatin in hyper saline water specimens than that in marine water ones. 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 **(Fig. 3).**

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 hyper saline water specimens **(Fig. 3)**. A decrease in the thickness of the pillar cell flanges of marine water specimens was observed in contrast to the thickness of flanges in hyper saline waters.

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. 2 C&D)**.

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. These chloride cells display a rich population of mitochondria, evenly distributed throughout the cytoplasm. These mitochondria are large in size **(Figs. 2C & 3C)**.

In specimens of hyper saline waters, chloride cells are large; ovoid and elongated in shape, usually covered by the superficial pavement cells. They have really large nuclei,

characterized by dense peripheral heterochromatin. 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. 2D & 3D)**.
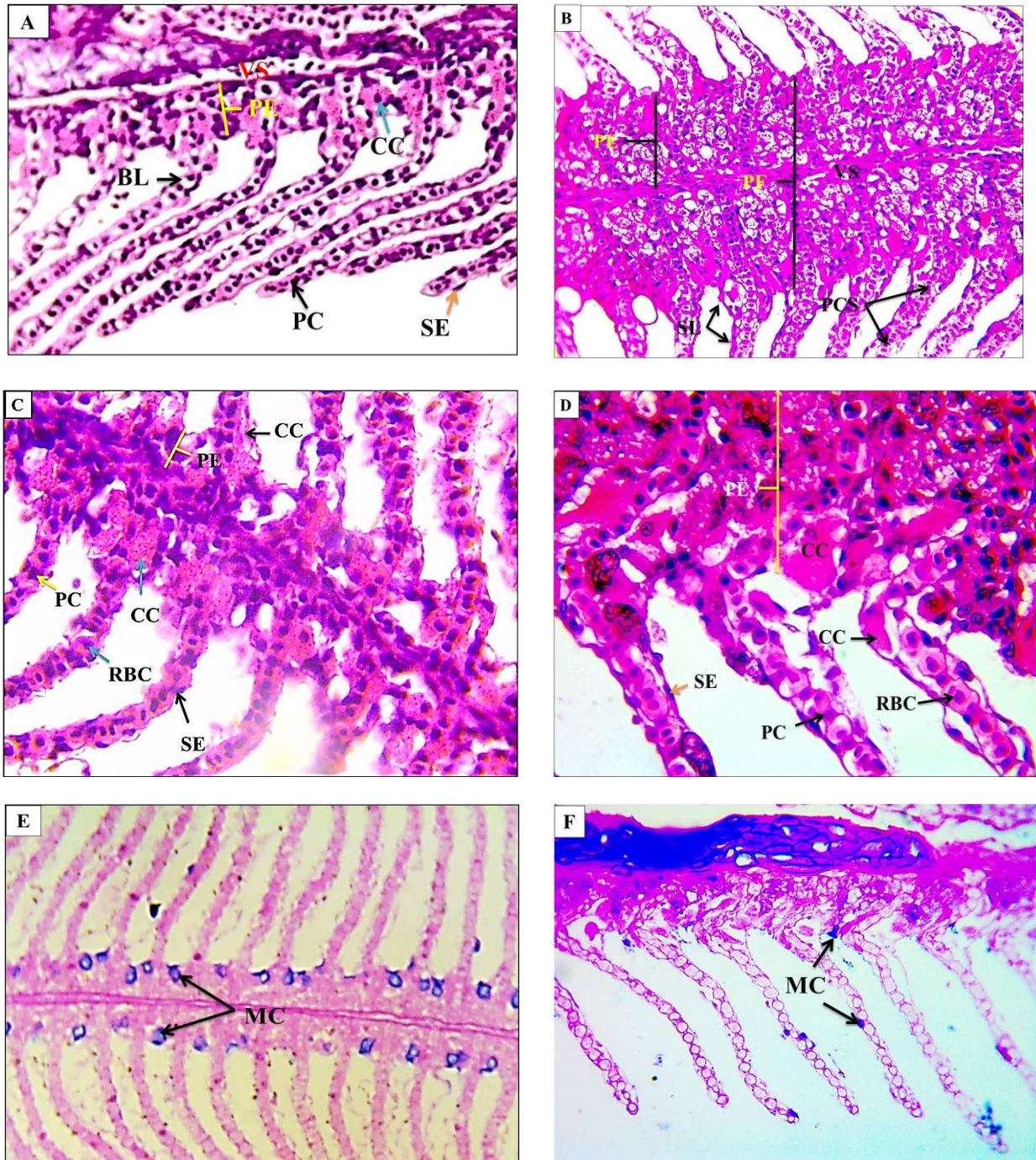

Fig. (1). Photomicrographs of cross-section through a gill filament of *M. cephalus* from marine water (A, C & E), hypersaline water (B, D & F); showing the different regions of the filament epithelium and associated vasculature.

BL: Blood lacuna; **CC**: Chloride cell; **MC**: mucus 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**: X 400, **C&D**: X1000; Alcian-PAS, **E&F**: X 400}.
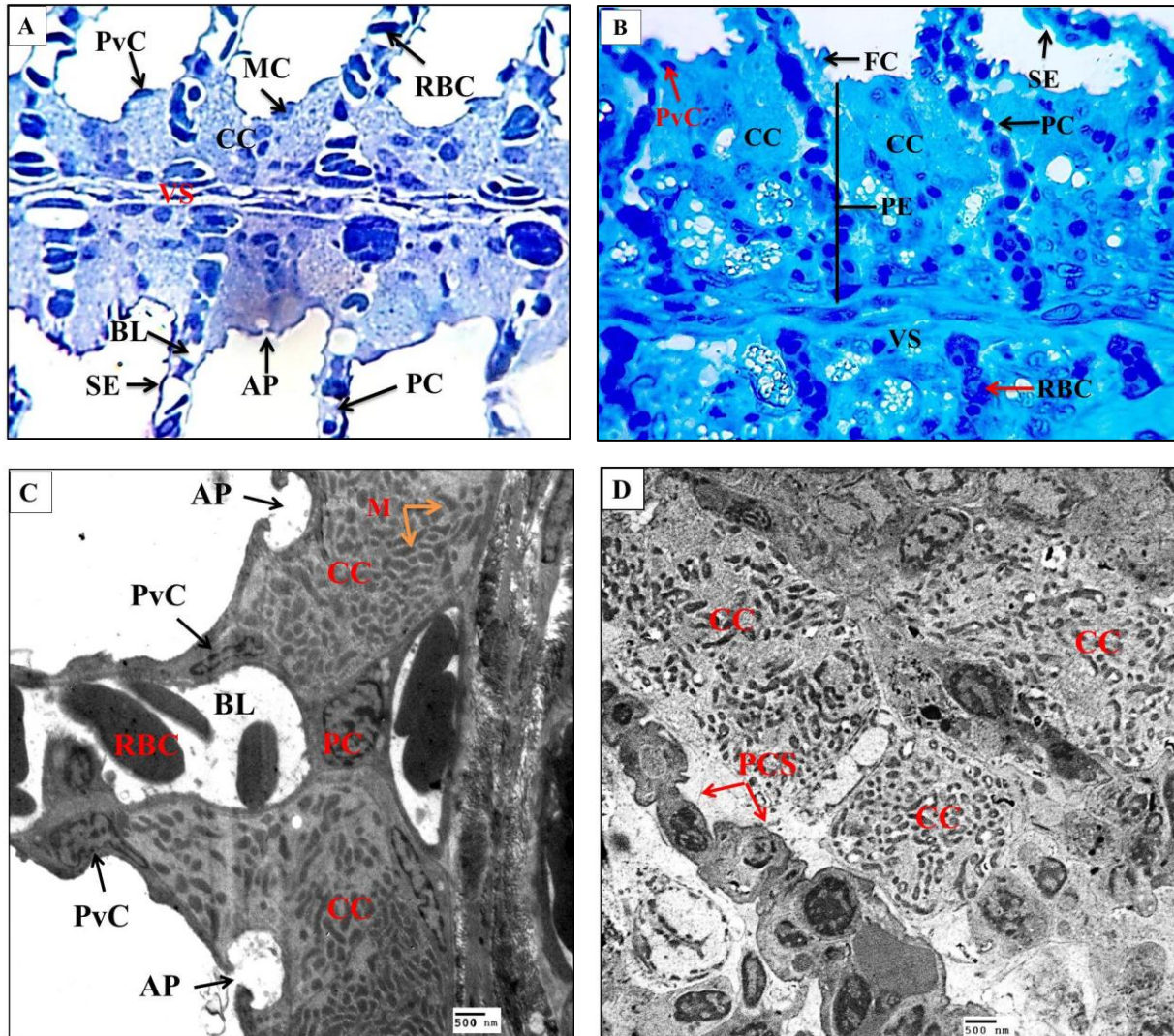
Fig. (2). Photomicrographs of cross-section through a gill filament of *M. cephalus* from marine water (A&C) and hyper saline water (B&D).

AP: Apical pit; **BL**: Blood lacuna; **CC**: Chloride cell; **FC**: Fuzzy coat; **M**: mitochondria; **MC**: Mucus cell; **PC**: Pillar cell; **PE**: Primary epithelium; **PvC**: Pavement cell; **RBC**: Red blood cell; **SE**: Secondary epithelium; and **VS**: Venus sinus. {**A& B**: semi-thin stained with toluidine blue, X1000; **C& D**: TEM, Uranyl acetate & lead citrate}
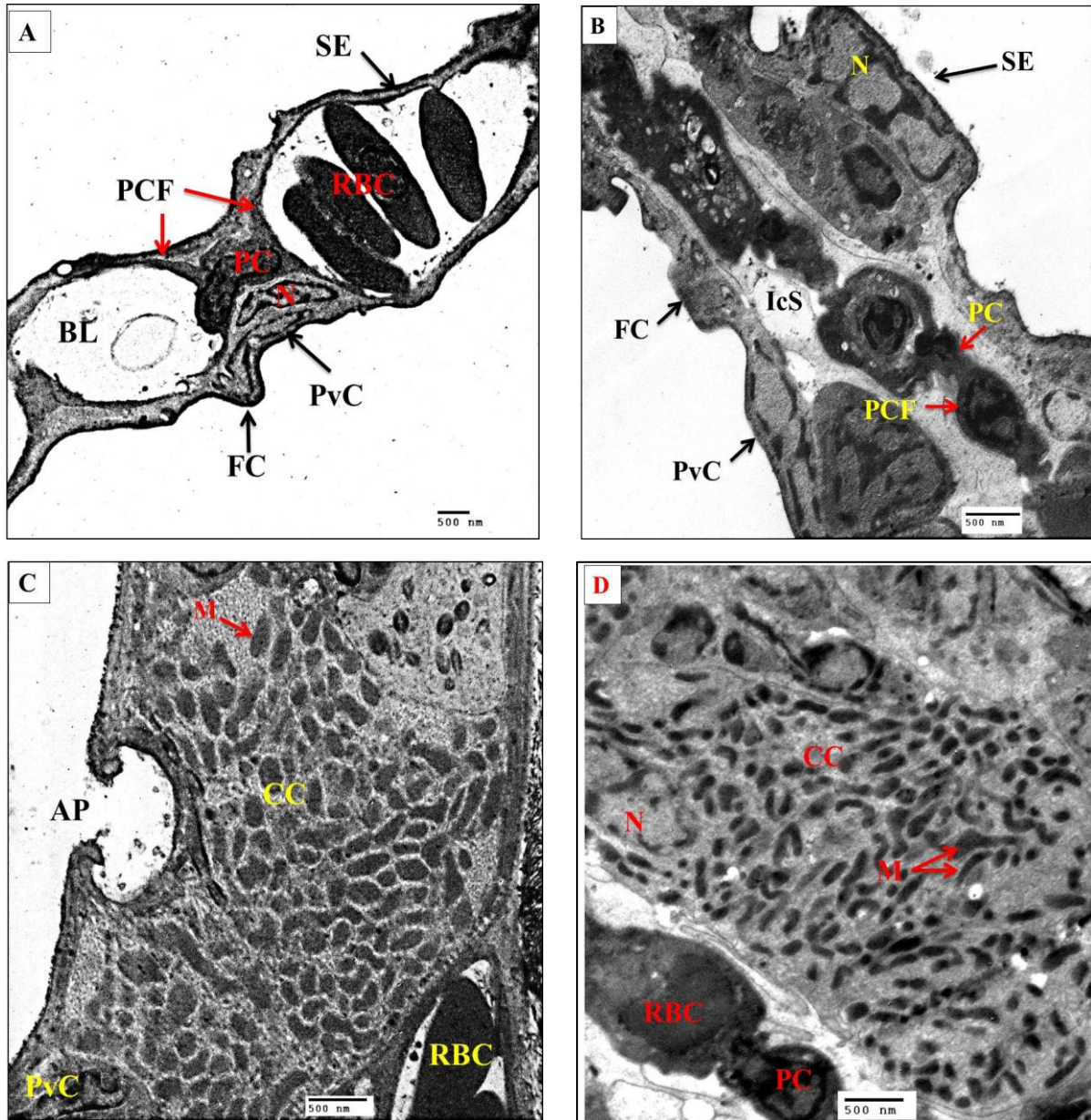
Fig. (3): Photomicrographs of TEM in cross-section through a gill filament of *M. cephalus* from marine water (A&C) and hyper saline water (B&D).

AP: Apical pit; **BL**: Blood lacuna; **CC**: Chloride cell; **FC**: Fuzzy coat IcS: Inter cellular space; **M**: mitochondria; **MC**: Mucus cell; **N**: Nucleus; **PC**: Pillar cell; **PCF**: Pillar cell flanges; **PE**: Primary epithelium; **PvC**: Pavement cell; **RBC**: Red blood cell; **SE**: Secondary epithelium; and **VS**: Venus sinus. {Uranyl acetate & lead citrate}

3. Effect of different salinities on gill morphometric characters;

The number of mucus cells was higher in gill filaments of marine water fishes "52.4 ± 2.966" than those of hyper saline "24.4 ± 6.427". In contrast, the number of chloride cells was higher in gill filaments of hyper saline fishes "51±5.196" than those of marine water fishes "33± 6.245". In the same manner, the number of mitochondria of the chloride cells was higher in gill filaments of hyper saline water fishes "207.667 ± 41.308" than those of marine fishes "124.33 ± 43.466" **(Table 1)**.

The results showed that the primary gill filament length **(PFL)** in marine water fishes was longer "6166.667±816.497μ" than that of hyper saline fishes "1591.667 ± 801.041μ" But, the ratio of gill filament length to total body length was higher in hyper saline fishes "5.895± 0.297 %" than that in marine fishes "3.524± 0.467 %" (**(Table 1)**.

It was found that the primary filament thickness **(PFT)** in hyper saline water fishes was thicker "147.08±13.923μ" than that of marine water fishes "48.938±7.299μ". Also, the ratio of primary filament thickness **(PFT)** to filament length **(PFL)** was higher in hyper saline water fishes "0.92± 0.087 %" than that of marine water fishes "0.79± 0.118 %" **(Table 1)**.

The present results showed that the primary epithelial thickness **(PET)** in hyper saline water fishes was thicker (59.72± 12.918μ) than that of marine water fishes "18.88± 3.079μ". Also the ratio of primary epithelium thickness to filament thickness **(PET/PFT)** was higher in hyper saline water fishes "40.60± 8.78" than that of marine water fishes "38.57± 6.29 %" **(Table 1)**. It was found that the secondary lamellar length **(SLL)** in hyper saline water fishes was taller "161.275± 8.064 μ" than that of marine water fishes "151.654± 10.512μ". In contrast, the ratio of secondary lamellar length to filament length **(SLL/PFL)** was higher in marine water fishes "2.46± 0.170 %" than that of hyper saline water fishes "1.01± 0.051 %" **(Table 1)**.

The present results showed that the secondary epithelial thickness **(SLT)** in hyper saline water fishes was thicker "10.344± 1.700μ" than that of marine water fishes "8.572± 0.872μ". Also, the ratio of secondary epithelial thickness to lamellar thickness **(SLT/SLL)** was higher in hyper saline water fishes "6.41± 1.05 %" than that of marine fishes "5.65± 0.57 %" **(Table 1)**.

The present results showed that the inter-lamellar space **(ILS)** in hyper saline water fishes was wider "19.10± 6.25μ" than that of marine water fishes "12.96± 3.85μ". Also, the ratio of inter-lamellar space to secondary lamellar length **(ILS /SLL)** was higher in hyper saline water fishes "11.84± 3.87 %" than that of marine fishes "8.55± 2.54 %" **(Table 1)**.

It was found that the pillar system diameter **(PSD)** in marine water fishes was slightly higher "2.484± 0.6547μ" than that of hyper saline water fishes "2.260± 0.539μ". Also, the ratio of pillar system diameter to secondary epithelial thickness **(PSD/SLT)** was higher in marine water fishes "28.972±7.637 %" than that of hyper saline fishes "21.848±5.214 %" **(Table 1)**.

Results of the statistical analysis to gill characteristic counts and measurements of *Mugil cephalus* inhabiting waters with different salinities are presented in **Table (1)**. These results revealed that there are statistically significant differences between gills of marine water fishes and gills of hyper saline water fishes for all counts and measurements as well as their ratios, except that of the pillar system diameter (PSD). Generally, most of the multiple comparisons (marine versus hyper saline) are statistically significant varied except that of primary epithelial thickness to the primary filament thickness **PET / PFT (Table 1)**.

Table (1): Gill characteristic measurements and counts of *M. cephalus* inhabiting waters with different salinities (Data expressed as mean+standard deviation)

	Gill characteristics	Marine water	Hyper saline water	Significance
Counts	Mucus cell number (MC)	52.4 ± 2.966	24.4 ± 6.427	**
	Chloride cell number (CC)	33± 6.245	51±5.196	*
	Mitochondria number (M)	124.33 ± 43.466	207.667 ± 41.308	*
Measurements (μ)	Primary filament length, PFL	6166.67±816.50	1591.67±801.041	**
	Primary filament thick, PFT	48.938±7.299	147.08±13.923	**
	Filament epithelial thick, PET	18.88± 3.079	59.72± 12.918	**
	Secondary lamellar length, SLL	151.654± 10.512	161.275± 8.064	*
	Secondary epithelial thick, SLT	8.572± 0.872	10.344±1.700	**
	Inter-lamellar space, ILS	12.96± 3.85	19.10± 6.25	**
	Pillar system diameter, PSD	2.484± 0.6547	2.260± 0.539	NS
Morphometric ratios (%)	PFL/Body length (BL)	3.524± 0.467	5.895± 0.297	**
	PFT / PFL	0.79± 0.118	0.92± 0.087	**
	PET / PFT	38.57± 6.29	40.60± 8.78	NS
	SLL / PFL	2.46± 0.170	1.01± 0.051	**
	SLT / SLL	5.65± 0.57	6.41± 1.05	*
	ILS / SLL	8.55± 2.54	11.84± 3.87	*
	PSD / SLT	28.972± 7.637	21.848± 5.214	*

*: $P < 0.05$; **: $P < 0.01$ and NS: $P > 0.05$

DISCUSSION

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**).

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**).

The general gill morphology of *M. cephalus* 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 the present study, it was found that each gill arch of *M cephalus* 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.

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 our study it was found that the chloride cells are located within the epithelium of the inter-lamellar regions and secondary lamellae in gills of *Mugil cephalus* inhabited marine waters. But in hyper saline water specimens, these chloride cells are limted to locate within the primary epithelium of the inter-lamellar regions and resting on the pillar capillary basal lamina. These results are

in agreement withthose reported by **Abdel Samei *et al.* (2021)** in gills of *Dicentrarcus labracus* inhabited brackish water, marine water and hyper saline water. They reported that the chloride cells in specimens inhabited brackish water are located within the epithelium of the inter-lamellar regions and secondary lamellae; but in marine and hyper saline water specimens they are located only within the primary epithelium of the inter-lamellar regions and resting on the pillar capillary basal lamina.

This results didn't agree with **Azab *et al.* (1999)** who concluded that the chloride cells were frequently seen in the inter lamellar regions and at the bases of the secondary lamellae in both of the normal (seawater) and hyper saline-adapted fishes. In freshwater-adapted fishes, chloride cells are not restricted to the gill filaments, but they are also found in some secondary lamellae within epithelia.

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 *M. cephalus* 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 were observed in hyper saline specimens, they overlap and interdigitate in a complex manner; but in marine specimens, only one layer of pavement cells was observed. These results didn't agree with that obtained by **Abdel Samei *et al.* (2021)** who concluded that each secondary lamellar epithelium, in gills of *D. labracus* inhabited hypersaline water, mostly consists of one layer of flat elongated pavement cell.

The flat elongated pavement cells have really flattened nuclei, characterized by more electron dense peripheral heterochromatin in hyper saline water than that in marine water. 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 recorded that microridges were not seen on the apical plasma membrane of pavement cells of the secondary lamellae in the gills of *Schizothorax curvifrons*. While agreed with **Abdel Samei *etal.* (2021)** who concluded that the plasma membrane of these pavement 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.

The absence of microridges on the surface of secondary lamellae has been showed 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.

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 (**Kültz *et al*., 1995**). 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.

Newstead, (1967) suggested that 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.

The present work showed that the intercellular spaces were detected in gill specimens of hyper saline waters. These results agreed 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. But it didn't agreed with **Abdel Samei *et al*. (2021)** who found that the intercellular spaces were also detected in brackish and marine waters.

Laurent & Dunel, (1980) suggested that the 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. 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 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)** reported that apical pit is often correlated with adaptation to high salinities.

In specimens of hyper saline waters, chloride cells usually covered by the superficial pavement cells. These results agreed with that described by (**Azab *et al*., 1999**) who reported that some interlamellar chloride cells were not in contact with the external medium and had sheets of pavement cells covering their apical surfaces.

The number of mucus cells in gill filaments of *M. cephalus* was higher in gill filaments of marine water specimens (52.4 ± 2.966) than those of hyper saline (24.4 ± 6.427). These results agreed with that described by **Abdel Samei *et al*. (2021)** who found that the number of mucus cells of *D. labracus* was higher in gill filaments of marine water specimens than those of hypersaline 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** and **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 hyper saline specimens (51 ± 5.196) than those of marine water (33 ± 6.245) specimens. In the same manner, the number of mitochondria of the chloride cells was higher in gill filaments of hyper saline water specimens (207.667 ± 41.308) than those of marine (124.33 ± 43.466) specimens. **Varsamos *et al.* (2002)** 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).**

Sathron *et al.* (2021) reported that change in chloride cell density contribute to fish adaptation to different salinity since chloride cells regulate the transport of major ions (i.e., Na^+ /Cl^- and Ca^+) to maintain a biologically suitable hydro-mineral balance (**Flik *et al.*, 1984 and Laurent & Dunel, 1980**). Consistent with this concept, several documents showed apparent increase in gill chloride cells density in killifish, *Fundulus heteroclitus* (**Lima & Kültz, 2004**), the Japanese eel *Anguilla japonica* (**Wong & Chan, 2001**) and the sturgeon, *Acipenser naccarii* (**Martínez-Álvares *et al., 2002***).

The present work found that primary filament thickness **(PFT)** in hyper saline water specimens was significantly thicker ($147.08\pm13.923\mu$) than that of marine water specimens ($48.938\pm7.299\mu$). Also, the ratio of primary filament thickness **(PFT)** to filament length **(PFL)** was higher in hyper saline water specimens (0.92 ± 0.087) than that of marine water (0.79 ± 0.118) specimens. These results didn't agree with **Abdel Samei *et al*. (2021)** who concluded that the primary filament thickness in brackish water specimens was thicker than that of marine water and those in hyper saline specimens.

The pillar system diameter **(PSD)** in marine water specimens was slightly higher ($2.484\pm 0.6547\mu$) than that of hyper saline water ($2.260\pm 0.539\mu$) specimens. Also, the ratio of pillar system diameter to secondary epithelial thickness **(PSD/SLT)** was higher in marine water specimens (28.972 ± 7.637) than that of hyper saline (21.848 ± 5.214) specimens. These results agree with **Abdel Samei *et al*. (2021)** who repoeted the pillar system diameter **(PSD)** in marine water specimens was slightly higher than that of brackish water ± and those in hyper saline 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**).

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