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STUDIES ON THE CHLORIDE CELLS OF THE GILLS OF FRESH WATER FISH *(OREOCHROMIS NILOTICUS)* AND MARINE WATER FISH *(SPARUS AURATA)*

El-Gharbawy S.M.*[1], El-Bargeesy G. A.[1], El-Saba A.A.[1], Khattab M.A.[1] and Bulefa M.H.[2]

[1]Department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University. Egypt.
[2]Department of Histology and Anatomy, Faculty of veterinary medicine, Omer El-Mukhtar University (El-bieda-Libya).

***Corresponding Author: El-Gharbawy S.M.**
Department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University. Egypt.

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ABSTRACT
Concerning, the distribution of chloride cells of both species; they were most frequently found in the filament interlamellar regions; particularly concentrated at the bases of the secondary lamellae; adjacent to their vascular supply. However, such chloride cells might also appear in some of the secondary lamellar epithelium especially in *Sparus aurata*. Histochemicaliy, chloride cells reacted negatively with PAS, alcian blue pH1 and pH 2.5 and alcian blue pH 2.5–PAS combination techniques. In the semi-thin sections, these chloride cells were easily distinguished with toluidine blue by their lighter staining ability than those of adjacent cells and by their granular cytoplasm. At the ultrastructural level, the chloride cells of *Sparus aurata* were much bigger in size and had more mitochondria than in *Oreochromis niloticus*. Moreover, most of the apical membrane of the chloride cells was in contact with the external medium and located between the pavement cells**.** Meanwhile, some of the apical surfaces of chloride cells of *Oreochromis niloticus* were partially covered by lateral cytoplasmic extensions of adjacent pavement cells, so only a small area of the cell is exposed to the aquatic milieu. Another ultrastructural feature of chloride cells is the intracytoplasmic tubules and vesicles. These tubules were often more apparent in *Oreochromis niloticus* than in the *Sparus aurata*. Also the mitochondria of both species had closely packed cristae and their mitochondrial matrix varied greatly in their electro-density especially in *Oreochromis niloticus***.**

1-INTRODUCTION
Fish are at the top of the aquatic food chain **(Moyle and Cech 1996)** and are a valuable source of proteins **(Santos *et al*., 2011)**. They have some unique anatomical and physical characteristics that are different from mammals; however, they still possess the same organ system that is present in other animals. Organ system of fish varies to some extent from that of mammals due to the aquatic environment they live in **(Guidetti and Boero, 2004).** Nile Tilapia (*Oreochromis niloticus*) is the most important fresh water fish in the Nile River in Egypt. Accordingly, this fish species have a great economic importance **(Ashraf *et al.*, 2014).** Gilthead sea bream (*Sparus aurata*) is species of great economical importance for the Mediterranean marine culture industry. Their rapid growth rates, good productivity per unit volume of water and economic food conversion; make sea bream a suitable fish for the needs of modern aquaculture **(Stephanuis, 1996).**

Gills are one of the major organs conducting the internal ionic and acid-base regulation, with specialized chloride cells as the major cells carrying out active transport of ions **(Hwang *et al.*, 2011)**. Furthermore, the gill epithelium is a sight of gaseous exchange, ionic regulation, acid-base balance and nitrogenous waste excretion **(Aresan, 2015)**. **Samajdar and Mandal (2017)** suggested that the chloride cells participate in osmo-regulation other than respiration.

The anatomy and morphology of the fish gills of several fish species had been studied by many authors. However; none of the available literature was dealing with the normal structure of the gills of *Sparus aurata* fish. Moreover, there were a paucity of histological studies have centered their attention to the chloride cells of the gills of *Oreochromis niloticus* fish.

The present study was conducted to elucidate more light on the light and ultrastructural features of the chloride cells in the gills of both *Oreochromis niloticus* as a model of freshwater fish and *Sparus aurata* as a marine water fish.

2-MATERIAL AND METHODS
The total of 40 apparent healthy; 20 adult samples of Tilapia nilotica *Oreochromis niloticus* fish from fisheries of the Fayoum city and 20 adult samples of gilthead sea

bream *Sparus aurata*, were obtained from some private fisheries near the Mediterranean sea in port Said, Damietta. This study adhered to ethical requirement to animal welfare in Egypt.

For Light Microscopic Studies; gills samples from both species were collected and fixed immediately in 10% neutral buffered formalin and Bouin's fixatives. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of (5-7 µm) thick were obtained, mounted on clean glass slides and stained with: Harris haematoxylin and eosin (H & E) stain, Crossmon trichrome stain, Gomori's reticulin method, Periodic acid Schiff (PAS), Alcian blue (pH 2.5), Alcian blue (pH 1.0) and Periodic acid Schiff –alcian blue (pH 2.5) combination. The aforementioned stains were conducted as outlined by **Bancroft and Stevens (2010).**

For transmission electron microscopy (TEM); small pieces of (1mm) of the gills were fixed in paraformaldehyde-gluteraldehyde in phosphate buffer **(Mc Dowell and Trump, 1976).** Specimens were post fixed in 1 % osmium tetra oxide, washed in 0.1 M phosphate buffer (pH 7.3), dehydrated in ascending grades of ethanol and embedded in Ebon araldite mixture**.** Semi thin sections (1µm) were cut and stained with toluidine blue. Ultrathin sections were cut and stained with Uranyl acetate and lead citrate **(Hayat, 1986).** Sections were examined with a JEOL 1010 transmission electron microscope at Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt

3-RESULTS

The gills of *Oreochromis niloticus* and *Sparus aurata* were formed of rows primary lamellae or gill filaments. A series of alternately arranged; secondary or respiratory lamellae arose from each filament; including the inter-lamellar regions in-between **(Fig.1)**. However, in *Oreochromis niloticus*, the secondary lamellae appeared short and the free end of each lamella located away from the free end of the opposite one **(Figs. 1 and 2)**. On the other hand, the secondary lamellae of *Sparus aurata* were long and their free ends came close to the free ends of the opposite ones and might touch them; forming small channels **(Fig.3)**.

The gill filaments were supported by bundles of collagen fibers **(Fig.4)**, as well as, cartilaginous plates **(Fig.1).** These plates were covered on both sides by a thick perichondrium formed of dense regular fibrous connective tissue. Each chondrocyte situated in a lacuna surrounded by closely packed darkly stained capsules. The longitudinal axis of these capsules was perpendicular to the cartilaginous surface **(Fig. 5)**. In addition, a network of reticular fibers was also demonstrated in the core of the filament **(Fig.6)** but, no elastic fibers could be encountered. Moreover, blood vessel; contained some nucleated red blood cells, were extended within the filaments and gave rise to small branches that entered the secondary lamellae **(Fig.7)**.

In both species, a simple epithelium; made up of pavement cells covered the free part of the secondary lamellae while; a stratified squamous epithelium covered the gill filaments including the interlamellar regions. Such stratified epithelium composed of four or more cellular layers. It contained superficial pavement cells, intermediate cells as well as, basal undifferentiated cells. Beside these cells; chloride cells and mucous cells could be observed within this multilayered epithelium. Concerning chloride cells, of the gill filament of *Oreochromis niloticus*, they were visible among the epithelial cells of the inter**-**lamellar regions. They were mostly localized at the bases of secondary lamellae; adjacent to their origin from the gill filament and near their vascular side. These chloride cells were usually located apically in the gill filament epithelium under or among the pavement cells and appeared ovoid in shape with light basophilic cytoplasm and spherical or ovoid large eccentric nuclei **(Fig.8)**.

The chloride cells of *Sparus aurata* occupied the same situation as that of *Oreochromis niloticus*. In addition, they were usually located beside the mucous cells. These chloride cells were generally smaller than the mucous cells and appeared irregular or ovoid in shape with light acidophilic cytoplasm with ovoid or irregular eccentrically situated large nuclei **(Fig.9)**.However, in both species such chloride cells might also appear in some of the secondary lamellar epithelium specially in *Sparus aurata* **(Fig.10)**.In the semi-thin sections, these chloride cells were easily distinguished with toluidine blue by their lighter stain ability than those of adjacent cells and their granular cytoplasm **(Fig.11).**

From the histochemical techniques, it appeared that the mucous cells were the only cells that gave a positive reactivities with alcian blue pH 2.5, alcian blue pH 1 **(Fig.12)**, PAS **(Fig.13)** and alcian blue pH 2.5–PAS combination stains **(Fig.14)**. However, the cartilaginous plates also exhibited positive reactivities with these stains. On the other hand, the other cell types either lining the gill filaments or the secondary lamellae; including the chloride cells reacted negatively with all the histochemical stains used in this study.

By electron microscopy, the chloride cells of *Oreochromis niloticus* were ovoid or irregular in shape with irregularly shaped nuclei and numerous mitochondria were distributed throughout most of the cytoplasm. These mitochondria varied greatly in size and in shape from round to ovoid to elongated and irregular with closely packed cristae. The mitochondrial matrix also varied greatly in their electron density **(Fig.15).** Moreover, an extensive intracytoplasmic tubules and vesicles occurred between the mitochondria **(Fig.16).** Some of the apical surfaces of these chloride cells were exposed directly to the pharyngeal water; by an apical area or pit that opened directly toward the gill cavity between the long cytoplasmic extensions of the superficial pavement cells **(Fig.17).**

Meanwhile, the chloride cells of *Sparus aurata* were much bigger in size with more numerous mitochondria than those in their *Oreochromis niloticus* counterparts **(Fig.18)**. Furthermore, their apical membrane was in contact with the external medium and located between the surface epithelial cells **(Fig.19).**Some of these chloride cells were irregular in shaped with ovoid or irregular eccentric nuclei **(Fig.18).** Other cells appeared as inverted pyramidal or triangular in shaped and exhibited a basal nucleus and a mitochondria-rich cytoplasm. These mitochondria occupied most of the cytoplasm and had different shape and sizes and their matrix varied greatly in their electron density. Such nuclei were much more electron lucent than that of and had a peripheral heterochromatin. Intercellular spaces could be observed, between the chloride cells and adjacent epithelial cells. In addition, the intracytoplasmic tubules were often less apparent than in the *Oreochromis niloticus* fish. Moreover, cytoplasmic vesicles of various sizes contained an electron dense material was also observed **(Fig.19)**.

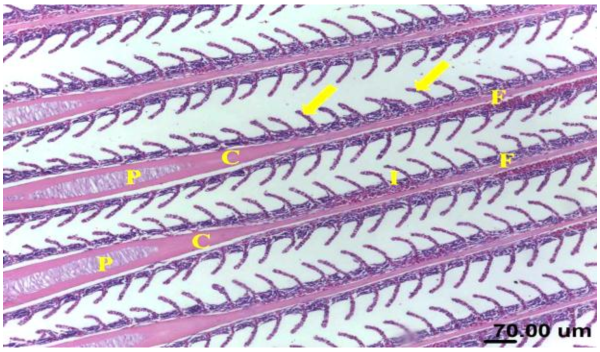

Fig.(1): A photomicrograph of the gills of *Oreochromis niloticus* showing gill filaments (F), secondary lamellae (Arrows) and inter-lamellar regions (I).Notice the collagen fibers (C) and cartilaginous plates (P) in the core of the filaments H&E stain, x 100.

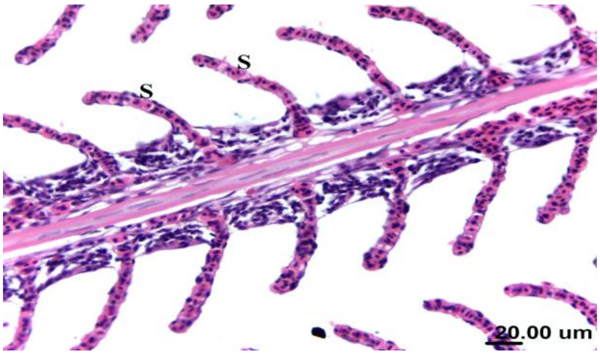

Fig.(2): A photomicrograph of the gills of *Oreochromis niloticus* showing the short secondary lamellae (S) and the free end of each lamella located away from the free end of the opposite one. H&E stain, x 100.

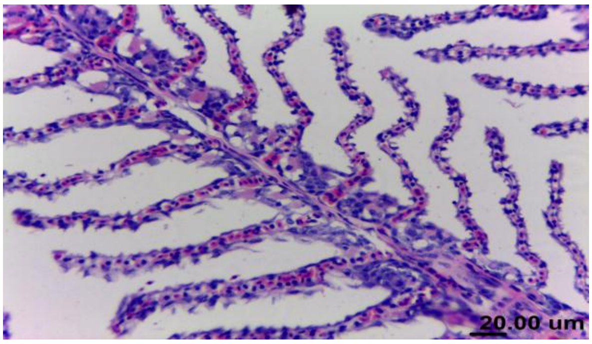

Fig.(3): A photomicrograph of the gills of *Sparus aurata* showing tall secondary lamellae (S) and their free ends came close to the free ends of the opposite ones and might touch them. H&E stain, x 100.

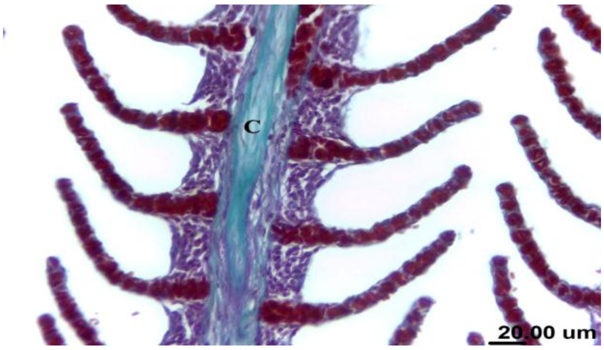

Fig.(4): A photomicrograph of the gills of *Oreochromis niloticus* showing collagen bundles (C) supporting the gill filament. H&E stain, x 400.

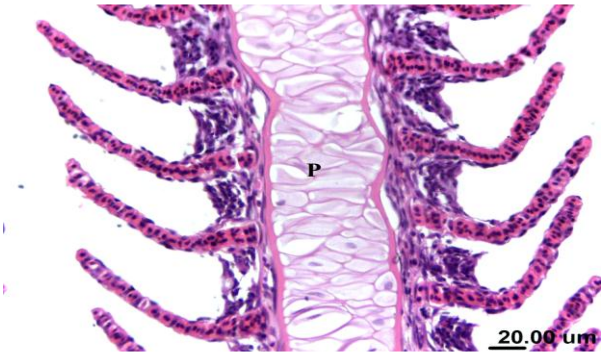

Fig.(5): A photomicrograph of the gills of *Oreochromis niloticus* showing cartilaginous plate (P) within the core of the gill filament. H&E stain, x1000.

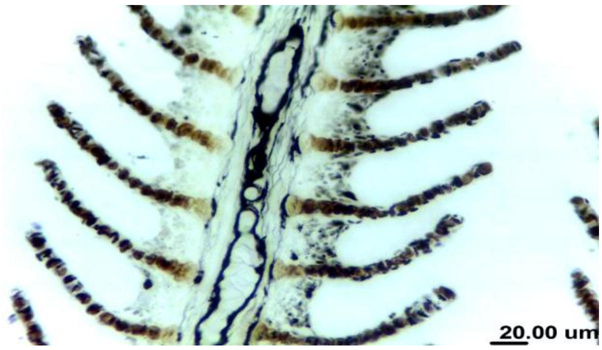

Fig.(6): A photomicrograph of the gills of *Oreochromis niloticus* showing the reticular fibers in the core of the gill filament. H&E stain, x400.

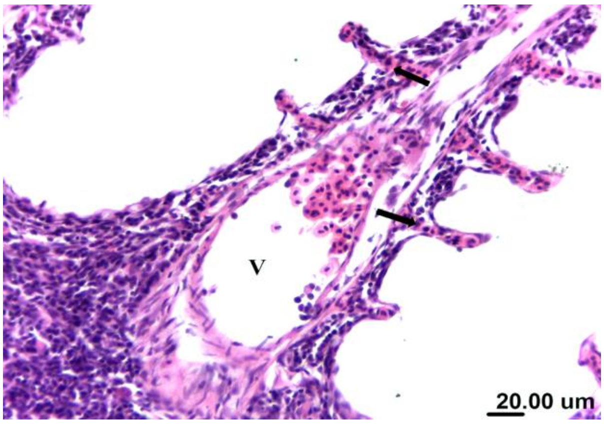

Fig.(7): A photomicrograph of the gills of *Oreochromis niloticus* showing blood vessel (V) in the gill filament and gave side branches (Arrows) to the secondary lamellae. H&E stain, x400.

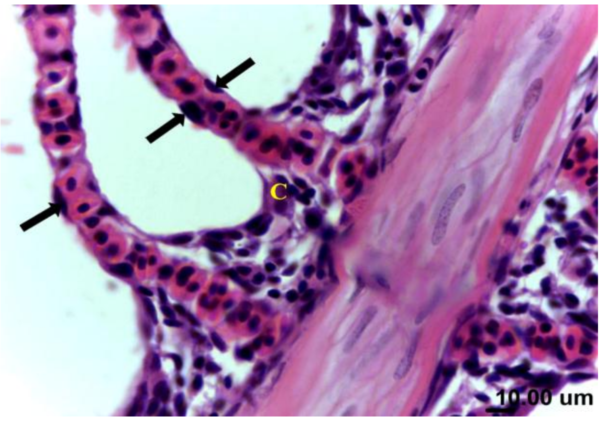

Fig.(8): A photomicrograph of the gills of *Oreochromis niloticus* to show a simple epithelium (arrows) covers the secondary lamellae and a stratified squamous epithelium including chloride cells (C) covers the gill filaments. H&E stain, x1000.

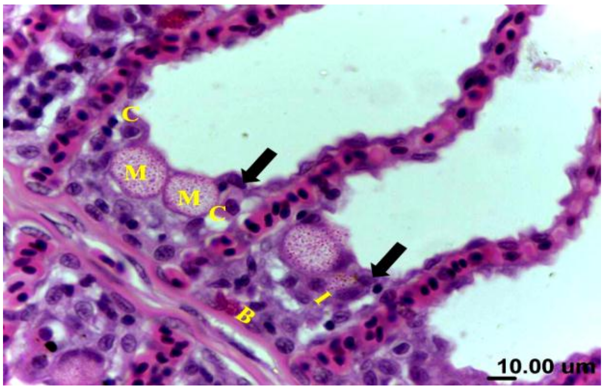

Fig.(9): a photomicrograph of the gills of *Sparus aurata* showing pavement cells (Arrows), intermediate (I) and basal cells(B) of the gill filament epithelium. Notice mucous cells (M) and chloride cells (C). H&E stain, x1000.

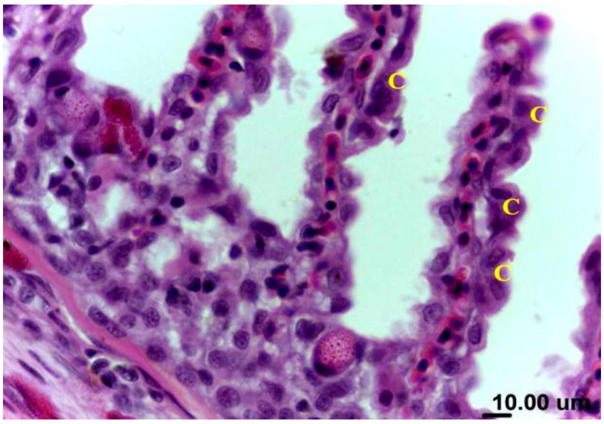

Fig.(10): A photomicrograph of the gills of *Sparus aurata* to show chloride cell (C) in the epithelium of secondary lamellae. H&E stain, x1000.

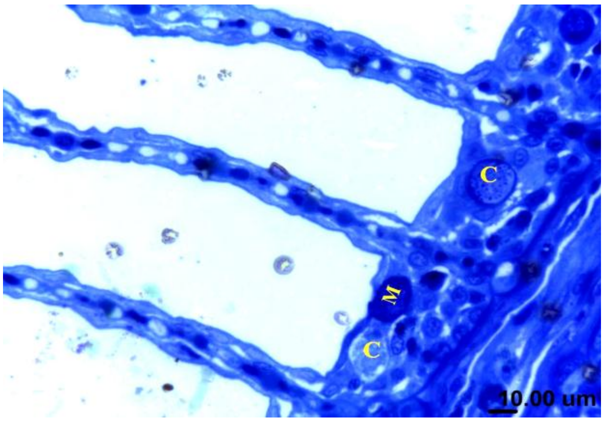

Fig.(11): A photomicrograph of the gills of *Sparus aurata* illustrating the lightly stained granular cytoplasm of the chloride cells (C). Notice mucous cells (M). Toluidine blue stain, x1000.

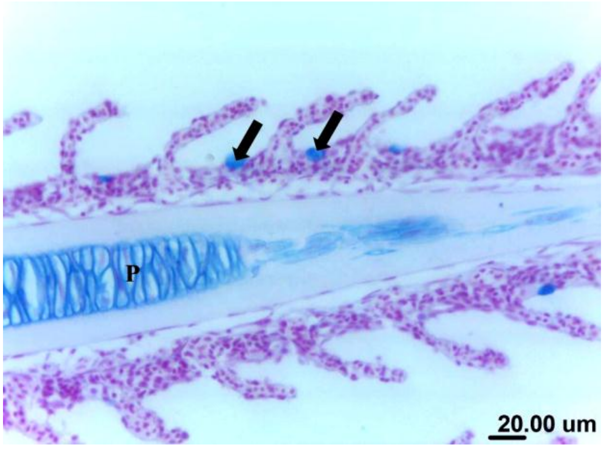

Fig.(12): A photomicrograph of the gills of *Oreochromis niloticus* showing that the mucous cells (Arrows) were the only cells gave a positive reactivity with alcian blue. Notice that, the cartilaginous plate (P) also exhibited positive reactivity. Alcian blue PH1stain, x400.

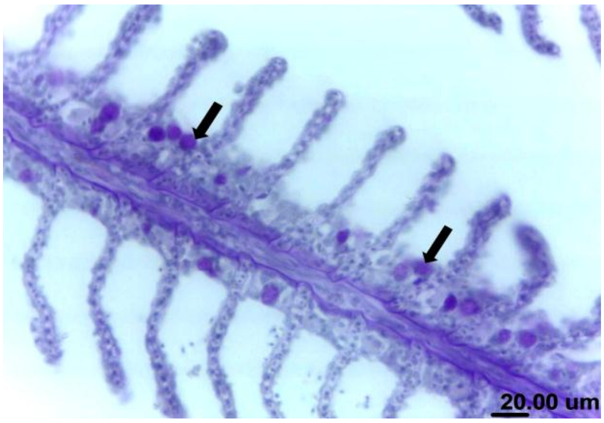

Fig.(13): A photomicrograph of the gills of *Sparus aurata* to show that the mucous cells (Arrows) were the only cells gave a positive reactivity with PAS. PAS stain, x1000.

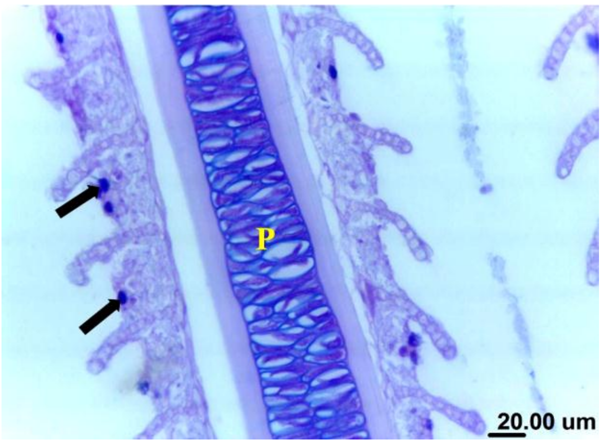

Fig.(14): A photomicrograph of the gills *of Oreochromis niloticus* to show that the mucous cells (Arrows) were the only cells gave a positive reactivity with combined alcian blue–PAS. Notice that, the cartilaginous plate (P) also gave positive reactivity. Alcian blue pH 2.5–PAS combination stain, x1000.

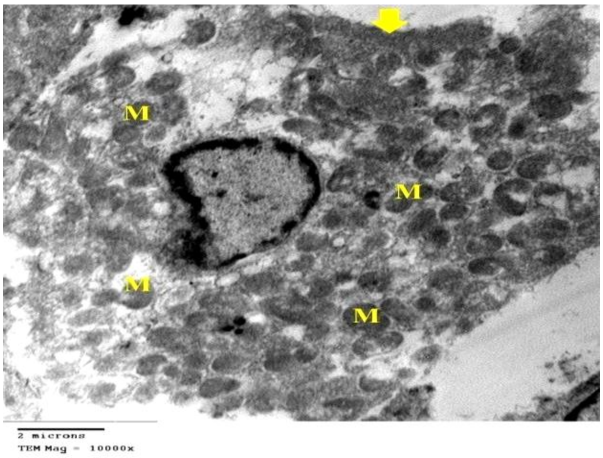

Fig.(15): A transmission electron micrograph of the filament epithelium of *Oreochromis niloticus* gills to show mitochondria of various shapes and sizes electrodensity (M) scattered throughout the cytoplasm of the chloride cell. Uranyl acetate and lead citrate stain, x 10000.

Fig.(16): A transmission electron micrograph of the filament epithelium of *Oreochromis niloticus* gills to show intracytoplasmic tubules (T) and vesicles (V) between the mitochondria (M). Uranyl acetate and lead citrate stain, x 25000.

Fig.(17): A transmission electron micrograph of the filament epithelium of *Oreochromis niloticus* gills to show that an apical pit exposed (P) between the cytoplasmic extensions (Arrows) of pavement cells. Uranyl acetate and lead citrate stain, x 20000.

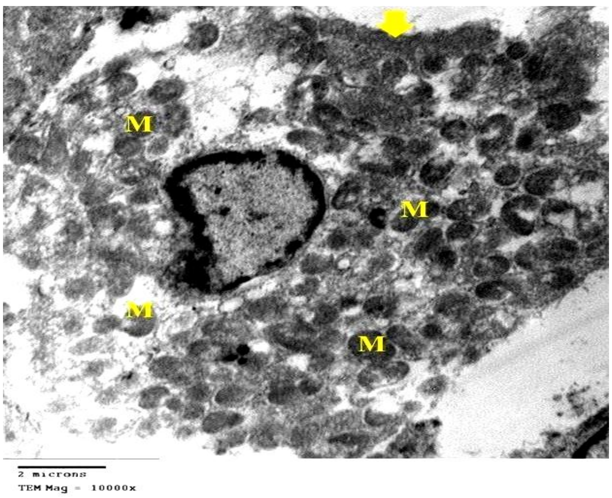

Fig.(18): A transmission electron micrograph of the filament epithelium of *Sparus aurata* gills to show large irregular shaped chloride cells mostly occupied

by mitochondria (M).Notice that, their apical membrane (Arrow) was in contact with the external medium. Uranyl acetate and lead citrate stain, x 10000.

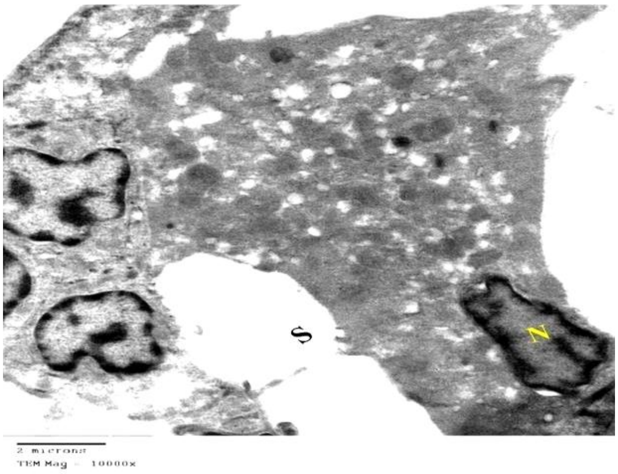

Fig.(19): A transmission electron micrograph of the filament epithelium of *Sparus aurata* gills to show inverted pyramidal or triangular shaped chloride cells with electron lucent basal nucleus (N). Notice the intercellular space (S). Uranyl acetate and lead citrate stain, x 10000.

4-DISCUSSION

The gills of both species were formed of rows of gill filaments with secondary or respiratory lamellae, arose from each filament. However, in *Oreochromis niloticus*, the secondary lamellae appeared short and the free end of each lamella located away from the free end of the opposite one. On the other hand, the free ends of the secondary lamellae of *Sparus aurata* came close to the free ends of the opposite ones and might touch them. **Diaz et al. (2005)** revealed that the gill filaments serve for support of the secondary lamella**. Wilson and Laurent (2002)** speculated that, the outer margins of the secondary lamellae forming a sieve-like arrangement for the respiratory water to pass through. However, **Mandal (2017)** added that the secondary lamellae increase the respiratory surface area.

The gill filaments were supported by bundles of collagen fibers, as well as, cartilaginous plates. Similar results were observed in *Odontesthes bonariensis* **(Vigliano *et al.*, 2006)**. And in *Catla Catla* **(Faheem *et al.*, 2016).** However, the presence of cartilages could be conductive to form a channel that would function to direct water flow and hence would be of enhanced survival value to the organism **(Shah *et al.*, 1990** and **El-Habback *et al.*, 1997).** Whereas, in *Catla catla* the gill filaments were supported by a bony structure **(Drishya *et al.*, 2016)**. While, **El-Habback *et al.* (1997)** found that, the gill filaments of *Oreochromis niloticus* are supported by branching system of cartilaginous plates and striated muscles. **Cong *et al.* (2019)** confirmed that, longitudinal muscles are present at the bottom of the filaments of *Ruditapes philippinarum*. The present work also showed that, the core of the gill filament contained a network of reticular fibers but, no elastic fibers could be encountered. **Wilson and Laurent (2002)** claimed that in the *lampreys* and *elasmobranchs*, the filaments are supported by an inter-branchial septum containing a vertical sheet of connective tissue. On the other hand, **Diaz *et al.* (2010)** observed that, the gill filaments are supported by fibro elastic tissue.

In both species, a simple epithelium; made up of pavement cells covered the free part of the secondary lamellae while; a stratified squamous epithelium covered the gill filaments including the interlamellar regions. Similar observations were recorded by many authors in different fish species **(Olson, 2002; Carmona *et al*., 2004; Diaz *et al*., 2005; Sorour and Al-Harbey, 2012; Mokhtar and Abd-Elhafeez, 2013; Drishya *et al.*, 2016; Franchini *et al.*, 2016** and **Mandal, 2017)**. However, **Wilson and Laurent (2002)** suggested that the gill filament epithelium supports the lamellae. Meanwhile, the lamellar epithelium forms a barrier between the fish's blood and the surrounding water **(Winkaler *et al*., 2001** and **Fatna *et al*., 2003).**

Aresan (2015) stated that the gill epithelium of *Tilapia mossambica* is a sight of gaseous exchange, ionic regulation, acid-base balance and nitrogenous waste excretion.

Concerning, the distribution of chloride cells, they were most frequently found in the filament interlamellar regions; particularly concentrated at the bases of the secondary lamellae and near their vascular side. Similar distribution was also demonstrated in different fish species **(Carmona *et al.*, 2004; Diaz *et al.*, 2005; Vigliano *et al.*, 2006; Cinar *et al.*, 2009; Hadi and Alwan 2012; Sorour, and AlHarbey, 2012 ; Essien *et al.*, 2013; Mokhtar and Abd-Elhafeez, 2013; Brraich and Kaur, 2015; Franchini *et al.*, 2016** and **Mandal, 2017)**. However, **Verbost *et al.*, (1994)** added that the chloride cells are found in contact with both water; via the apical surface and blood; via the basolateral membrane**.** Moreover, **Sorour, and AlHarbey (2012)** in *Oreochromis niloticus* confirmed that, the bloods vessels are in contact with these chloride cells.

Chloride cells were found to be not exclusive for the stratified epithelium of the gill filaments, as they were also occasionally identified within the epithelial lining of some secondary lamellae especially in *Sparus aurata*. The same findings were also mentioned in *Odontesthes bonariensis* by **Vigliano *et al.*, (2006)**.Moreover, **Diaz *et al.*, (2005)** speculated that, the chloride cells in the secondary lamellar epithelium of *Micropogonias furnieri* is morphologically adapted to gas exchange**.** On the other hand, the chloride cells were only observed in the gill filament epithelium and are absent in the secondary lamellae of *Pseudophoxinus antalyae* **(Cinar *et al.*, 2009),** of *Alosa sapidissima* **(Zydlewski and Mccormick 2001)** of *Labeorohita* **(Brraich and Kaur,**

2015) and in *carassius carassius* **(Franchini *et al.*, 2016)**.However, **Eiras-Stofella *et al*. (2001)** suggested that, this distribution, reduces the thickness of the blood–water diffusion barrier. Meanwhile, **Moron *et al*. (2003)** and **Fernandes *et al*. (2007)** clarified that, *Hoplerythrinus unitaeniatus* has large number of chloride cells, including those in the secondary lamellae while in *Hoplias malabaricus*, living in the same environment, the chloride cells are restricted only to the filament epithelium.

Histochemicaliy, it appeared that all the epithelial cells of the gill filaments and the secondary lamellae; except the mucous cells, reacted negatively with PAS, alcian blue pH 2.5, alcian blue pH 1, PAS and alcian blue pH 2.5–PAS combination stains. On the other hand, **Powell *et al.* (1994)** in *Oncorhynchus mykiss* and **Van der heijden *et al.*, (1997)** in *Oreochromis mossambicus* demonstrated that the membrane surface of the apical pits of chloride cells includes a high level of glycoproteins**.** However, our observations are in agreement with **Franchini *et al.* (2016)** that, in the semi-thin sections, the chloride cells were easily distinguished with toluidine blue by their lighter staining ability than those of adjacent cells as well as, by their granular cytoplasm**.**

At the ultrastructural level, numerous mitochondria were distributed throughout most of the cytoplasm of the chloride cells especially in *Sparus aurata*. However, these chloride cells were often named as mitochondria-rich cells in different fish species **(Wilson** and **Laurent 2002; Evans *et al.*, 2005; Vigliano *et al.*, 2006; Sorour, and AlHarbey, 2012 ; Oguz, 2015; Pereira *et al.*, 2013**; **Raskovic *et al.*, 2013** and **Oguz, 2015).**Whereas, they are described as osmoregulators and termed the primary ion transporting epithelium of the gills in *Carcinus maenas* **(Towle, 1997).**They are also termed ionocytes in *Oreochromis mossambicus* by **Hwang *et al.*, (2011)** and called striated cells in *Ruditapes philippinarum* by **Chen *et al.*, (2019).** However, **Zydlewski *et al.*, (2001)** confirmed that, the chloride cells are identified by morphology and mitochondria-richness, not by function**.**

By electron microscopy, some of the apical surfaces of chloride cells of *Oreochromis niloticus* were partially covered by lateral cytoplasmic extensions of adjacent pavement cells, so only a small area or pit of the cell is exposed to the aquatic milieu. Similar results were recorded in *Odontesthes bonariensis* by **Vigliano *et al.*, (2006).** Meanwhile, the apical membrane of chloride cells of *Sparus aurata* was in contact with the external medium and located between the pavement cells**. Carmona *et al.* (2004)** confirmed that the apical surfaces of the chloride cells of *Acipensern accarii* open to the external environment. In *Labeorohita* these cells have been observed in the spaces between two pavement cells **(Brraich and Kaur 2015)**. Furthermore**, Mandal (2017)** found that, crypts of the chloride cells are found amidst the pavement cells. **Diaz *et al.*, (2005)** the most superficial, layer of the gill filament epithelium of *Micropogonias furnieri* is made up of three cell types; pavement cells, chloride cells and mucous cells**.** Whereas, **Evans *et al.*, (1999)** noticed that, the chloride cells usually have their apical surface above the adjacent pavement cells. However, **Sorour, and AlHarbey (2012)** found that, the apical part of chloride cells in *Oreochromis niloticus* forms a deep pit with clearly developed microvilli**.** These observations appeared to be in contrast with that of **Van der heijden*et al.*,** (**1997)** who described that, all chloride cells of *Oreochromis mossambicus* are overlain and covered by the superficial pavement cells. Also, **Franchini *et al.*, (2016)** mentioned that the chloride cells are embedded in filament multilayered epithelium.

The present study implies that, the chloride cells of *Sparus aurata* were much bigger in size and had more mitochondria than those in their *Oreochromis niloticus* equivalents. The same statement was given by **Carmona *et al.*, (2004)** in *Acipenser naccarii***.** However, **Zydlewski *et al.* (2001)** inferred that the enlargement of chloride cells increases the surface area for enzyme pump insertion. The same authors added that, the chloride cells are rich in the enzyme which is essential for ion excretion in seawater acclimated fish. Moreover, the numerous mitochondria may provide the energy required for activetrans epithelial ion transport against the large electrochemical gradients exists between the external medium and the blood **(Verbost *et al.*, 1994).** Also, for the active transport of ions; chloride cells are endowed with a large amount of mitochondria. This characteristic is typical of chloride cells of marine fish **(Olson, 2002),** and, therefore, they may be related to the salinity levels of the aquatic milieu **(Vigliano *et al.*, 2006)**. Generally, the inter specific chloride cell variability between the species was related to inter specific rate of ion uptake and may reflect differences in their efficiency to maintain ion balance; ratio between ion gain/loss, as suggested by (**Moron *et al.*, 2003).** However, in sea water, the amount of chloride ions transported through the chloride cells increases significantly **(Zadunaisky *et al.*, 1995).** Moreover, the hyperosmotic environment of the lake may be advantageous to fish by causing an increase in the quantity of ions that they secrete, which enhances their resistance to negative environmental impacts **(Oguz 2015).**

These mitochondria of both species had closely packed cristae and their mitochondrial matrix varied greatly in their electrodensity especially in *Oreochromis niloticus***. Carmona *et al.* (2004)** reported that**,** the most notable ultrastructural features of the chloride cells in both the fresh and sea water fish are the great abundance of mitochondria with electro-dense matrix and abundant tubular cristae. **Franchini (2009)** showed the presence of two types of chloride cells; light and dark cells, in freshwater adapted fish. However, intercellular spaces could be observed herein, between the chloride cells and adjacent epithelial cells especially in the *Sparus aurata*. **AL-Amoudi and Aguis, (1991)** suggested that, the presence of such intercellular spaces indicated a similar process of salt excretion in sea and fresh water fish.

Another consistent ultrastructural feature of chloride cells in the present work is the intracytoplasmic tubules and vesicles. These tubules were often less apparent in the *Sparus aurata* than in the *Oreochromis niloticus* fish. **AL-Amoudi and Aguis, (1991)** found that these vesicles seemed to contain an electron dense material and they had no apparent relationship with the tubules. **Van der heijden *et al.*, (1997)** noticed a decrease in these tubules, after seawater adaptation compared with freshwater levels. **Franchini (2009)** confirmed that, this intracellular tubule is not restricted to the gills of seawater adapted fish. **Towle, (1997)** speculated that, under low salinity such tubules initiate a systemic ion uptake across the chloride cells. **Olson (2002)** clamed that these tubules and associated vesicles are provided by invaginations of the basolateral membrane. Moreover, **Vigliano *et al.*, (2006)** described that, the mitochondria are associated with these extensive network of tubules and vesicles. While**, Carmona *et al.* (2004)** observed that, this network of smooth-walled tubules is connected with basolateral plasma membrane. However, **El-Habback *et al.* (1997)** suggested that, these intracytoplasmic tubules could be as an equivalent to the smooth endoplasmic reticulum**.** Moreover, they are described as a suite of cellular and molecular mechanisms **(Towle, 1997)** and as an extensive tubular system **(Carmona *et al.*, 2004)** and a well-developed membrane system **(Diaz *et al.*, 2005)**. While**,** they are termed multicellular complexes **(Franchini, 2016).** From the aforementioned and our findings, it can be argued that chloride cells in the present work probably either contact directly to the outer environment through the apical surfaces or using the intracytoplasmic tubules.

5-CONCLUSION

It can be concluded that, the chloride cells of the gills of marine water *Sparus aurata* fish showed marked ultrastructural differences from those of the freshwater *Oreochromis niloticus* fish; the chloride cells were much more bigger in size with a great abundance of mitochondria in their cytoplasm. Moreover, most of the apical surfaces of the chloride cells were exposed directly to the pharyngeal water. On the other hand, some chloride cells of *Oreochromis niloticus* fish were connected to the external environment by an apical pit that opened directly toward the gill cavity between the long cytoplasmic extensions of the superficial pavement cells**.** Also, the chloride cells of this fish had a more apparent intracytoplasmic tubules and vesicles**.** However, these differences may reflect the relatively different osmotic problems of the two species to perform their osmoregulatory function. Also the mitochondria of both species had closely packed cristae and their mitochondrial matrix varied greatly in their electrodensity especially in *Oreochromis niloticus***.**

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