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Microscopic Observation on Gill Structure of Juvenile *Pseudosciaena crocea* under Different Salinities

[1]Ruanchengxu, [1]Yuanchonggui and [2]Wudefeng
[1]College of Biological Science and Technology, Fuzhou University, Fuzhou, 350108, China
[2]Life Science College, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

Abstract: The characteristics and differences of gill structure of juvenile pseudosciaena crocea under different water salinities are studied through electron microscopic observation. Results show that the structures of gill of juvenile pseudosciaena crocea are similar to those of other teleosts. When the salinity of water is at 6°, the epithelia cells on secondary filament of juvenile *Pseudosciaena crocea* are smooth, the number of chloride cells are less than that of high salinities, the number of mitochondria and tubular network of chloride cells are also few. When the salinity of water is at 16°, the epithelia cells on secondary filament of juvenile *Pseudosciaena crocea* are less smooth, the number of chloride cells will be increased. It can be found that chloride cell combined with accessory cells are turned into the multicellular compleses, the number of mitochondria is increased and the mitochondrial cristae and tubular network became more complicated; when the salinity of water is at 26°, the epithelia cells on secondary filament of juvenile *Pseudosciaena crocea* are not smooth, the number of chloride cells is abundant and is mainly located in the secondary filament part, many chloride cell combined with accessory cells forms the multicellular compleses, the number of mitochondria is numerous, the mitochondrial cristae and tubular network are also complicated.

Key words: Gill, salinity, light microscope, transmission electron microscope, juvenile *Pseudosciaena crocea*

INTRODUCTION

Most coastal fishes have strong ability of adapting the salinity changes of water. There many mechanism of seawater fish in adapting and adjusting the osmotic pressure, as known that the fish gills are one of the most important regulating organ (Lin, 1999; Evans *et al*., 2005), the quantity of gill chloride cell secretion, structure and activity of Na^+/K^+ATP are closely related with salinity changes of water (Daborn *et al*., 2001; Martinez-Alvarez *et al*., 2005). Academics have studied the influence of salinity on gill morphological structure and physiological changes of sturgeon (*Acipenser naccarii*), (*Acipenser schrenckii*), (*Lateolabrax japonicus*), (*Oreochromisco aureus*) (Martinez-Alvarez *et al*., 2005; Carmona *et al*., 2004; Hou *et al*., 2006; Wang and Hu, 2009; Jiang *et al*., 1998).

Pseudosciaena crocea is a coastal migratory fish and has strong adapting ability to water salinity (Cheung *et al*., 1979; Shenyanglv, 2007; Zhu and Wu, 1985). *Pseudosciaena crocea* belongs to Perciformes and Sciaenidae and Pseudosciaena also is known as cucumber fish, yellow croaker, etc. (Zhu and Wu, 1985), its meat is tender delicious and has high content of protein and unsaturated fatty acid, it is one of main marine fish in china.

Many extensive research about *Pseudosciaena crocea* have been performed in recent years (Zhou *et al*., 2008; Lin *et al*., 2002; Ning *et al*., 2007), but influence of salinity change on gill structure of *Pseudosciaena crocea* has not been reported. In the study, it adopts the light microscope and transmission electron microscopy in the observation and research of the gill tissue structure changes under different water salinity.

MATERIALS AND METHODS

Fishes in the experiment: test fishes are bought from Ningde city Fujian province, they are from Guanjing yang area and they belong to *Pseudosciaena crocea* of Fujian-Guangdong family. Their body length is about 1cm and they are breeding in the laboratory. In the experiment, the fishes have physical robustness are selected.

Management of breeding: The experiments are conducted in the aquatic animals box, its size is 60×50×50 cm and water depth is 40 cm, the *Pseudosciaena crocea* are feeding in three groups, respectively, in the water salinity of 6, 16 and 26°, each group has 30 young fishes of *Pseudosciaena crocea*.

Corresponding Author: Yuanchonggui, College of Biological Science and Technology, Fuzhou University, Fuzhou, 350108, China

In the study, in the group of the salinity 6 and 16°, every day° of water salinity are changed with 2~3°, after the 5 and 10 days, the experimental water salinity is reached.

In the youth period of the *Pseudosciaena crocea*, the feeding baits are mainly involves branch angle class, after entering the juvenile fish period,the feed are mainly the heavy feeds. In the breeding process water salinity is not changed, in order to the keep dynamic microorganism balance between the water and pseudo sciaena crocea, every 5 days, the bottom sediments are cleaned up with siphon method, water temperature of the aquaculture is controlled by heating rods at the temperature 25±1°C, the aeration oxygenation is adopted in order to keep the dissolved oxygen is larger than 0.5 mg L^{-1}. The sampling is made after the pseudosciaena crocea are raised for 95 days.

Preparation of sample: Sample preparation of the Lens, in each experimental groups, the *Pseudosciaena crocea* with body quality of 1.48±0.08 g and length of 4.95±0.14 cm is selected, the live gill is cut and then put in the Bouin's fluid, then put the grill in the refrigerator with temperature of 4°C.

The gradient ethanol dehydration is performed to the gill sample fixed in the Bouin's liquid and makes it transparent through salicylic acid methyl; finally embed a single gill filament with paraffin. The slice thickness is about 3 microns, it is stained with H.E and photographs are gotten through the Motic BA210 optical microscope. Sample preparation of the TEM (Transmission Electron Microscope), after sampling, it is fixed in the 3% glutaraldehyde and 1% osmic acid fixed, after 1.5 h later, it is rinsed for 3times with PBS (pH 7.2) and then it is dyed with 70% alcohol saturated uranium acetate. After gradient dehydration of alcohol and acetone, it is embedded with medium 618 epoxy resin. Thickness of ultrathin section is about 80 nm; staining time of the uranium acetate and lead citrate is about 5 min. The observation and photography are conducted with TE, its type is JEM1010.

RESULTS

Microstructure of juvenile *Pseudosciaena crocea* gill: After the observation of microstructure of *Pseudosciaena crocea* gill, it can be seen that its gills structure is similar to most of the ocean fish gills, gill filaments are aligned small pieces parallel to the longitudinal axis of gill filament on both sides of the gill, gill is vertical to the longitudinal axis of gill filaments and it is arranged in parallel. The gill filament trunk is formed

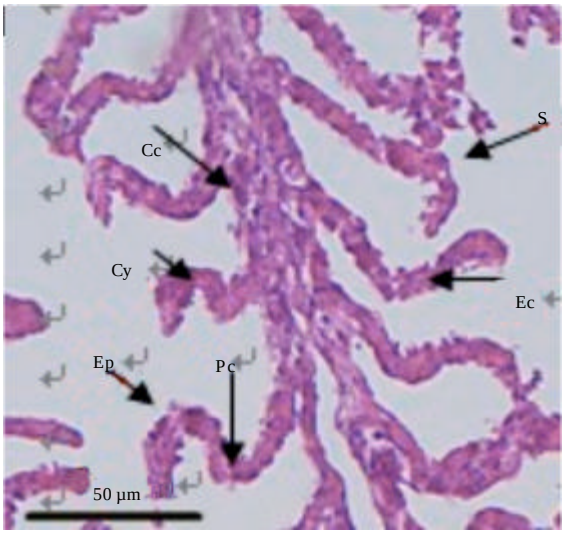

Fig. 1: Primary filament and secondary filaments, the No. of chloride cells is less, in 6° of salinity. Jc: Junctional complex, Ep: Epithelia cell

by gill filaments cartilage, gill filament epithelium and the central venous sinus, gill filament epithelium is the multilayer epithelium and it is made up of epithelial cells, cells that make mucus cells and secrete chloride, etc. Gill is mainly composed of epithelial cells, dice column cells and capillary network, etc., as shown in Fig. 1-3.

The group of salinity 6, excretive chloride cell number of gills is small and it distributes in the gill and gill dice base, epithelial cells are closely packed, the body is large (Fig. 1). The group with salinity 16°, the chlorine cell number of gills is increased slightly and the color is deepened (Fig. 2). The group of salinity 26°, the number of secrete chloride cells is large and is mainly distributed in the gills in adjacent state base and some dice and its cell body is bigger, staining colors of H.E is deepen (Fig. 3).

Ultrastructure of *Pseudosciaena crocea* gill: Through transmission electron microscopy, it can be seen that the *Pseudosciaena crocea* rlarvae gills surface is mainly covered by epithelial cells, its epithelial cells are flat, cell surface is covered with mucus and sugar calyx. The cells have the dense black vesicles as shown in Fig. 4-6, epithelial cells are connected compactness as shown in Fig. 7. In the base of gill, there exists some excretive chlorine cells and in the parallel arrangement of gill there also has some excretive chlorine cells. Excretive chlorine cells and excretive mucous cells are connection with epithelial cells closely as shown in Fig. 8, 9.

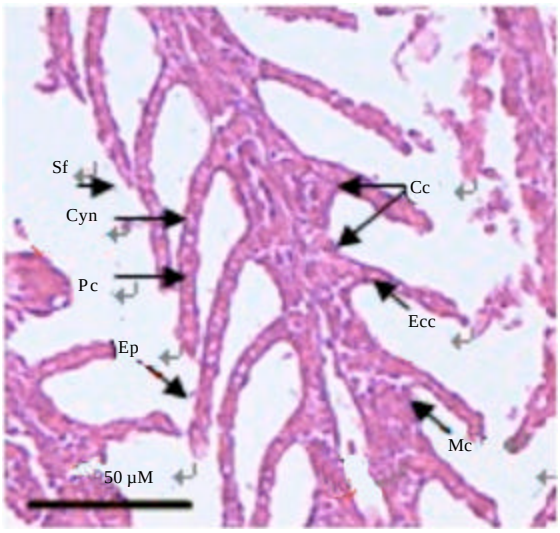

Fig. 2: No. of chloride cells are increased in 16° of salinity. Sf: Secondary filament, Cvn: Capillary vessel net, Pc: Pillar cell, Ep: Epithelia cell, Cb: Cartilage bar, Ec: Erythrocyte, Cc: Chloride cell, Mc: Mucous cell

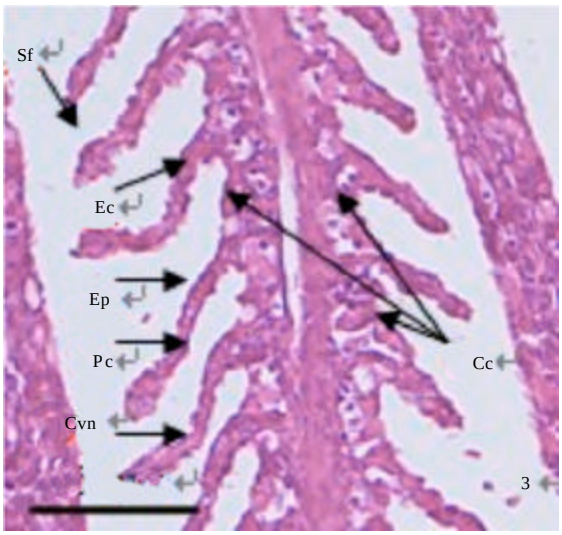

Fig. 3: No. of chloride cells is more and mainly located in the base of the secondary filament in 26° of salinity. Sf: Secondary filament, Cb: Cartilage bar, Ep: Epithelia cell, Pc: Pillar cell, Ec: Erythrocyte, Cvn: Capillary vessel net, Cc: Chloride cell, Mc: Mucous cell

Excretive chlorine cells presents the shape of circular or ovoid or elongated, excretive chlorine cells on small

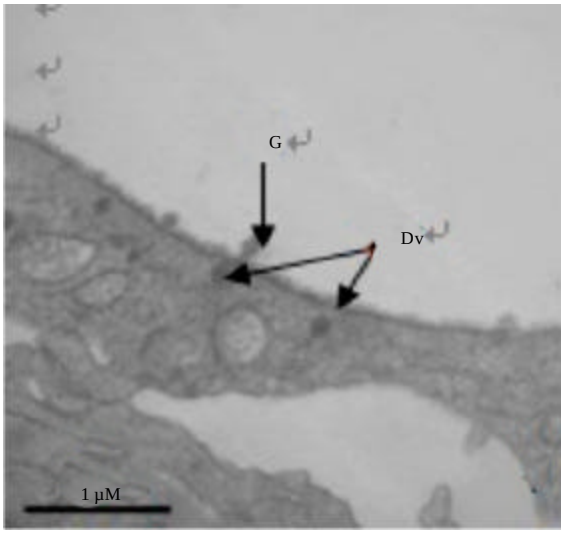

Fig. 4: Epithelia cells are smooth in 6° of salinity. Dv: Dark vesicle; G: Glycocalyx

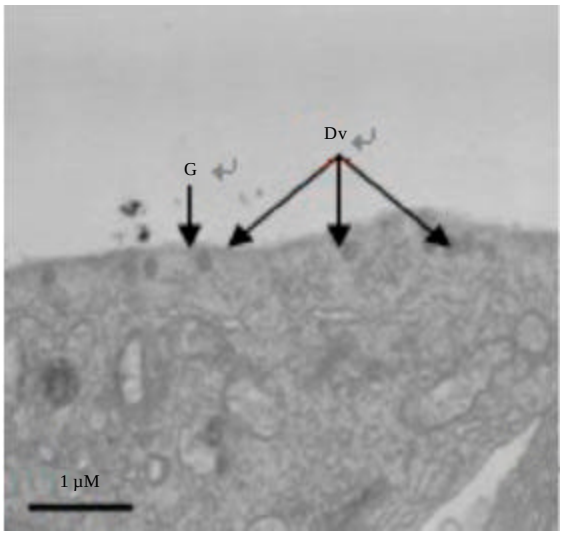

Fig. 5: Epithelia cells are less smooth in 16° of salinity. Dv: Dark vesicle, G: Glycocalyx

pieces of gills chlorine cells is the shape of ovoid. Excretive chlorine cells of the gill base present the elongated shape. Number of the mitochondria in the excretive chloride is large, they present round, ovoid, bending shape, etc., they have a variety of forms. There has a tiny tube intracellular distribution, its expansion can form the endoplasmic reticulum cisternae and they are doped with tiny pipe system as shown in Fig. 10-18.

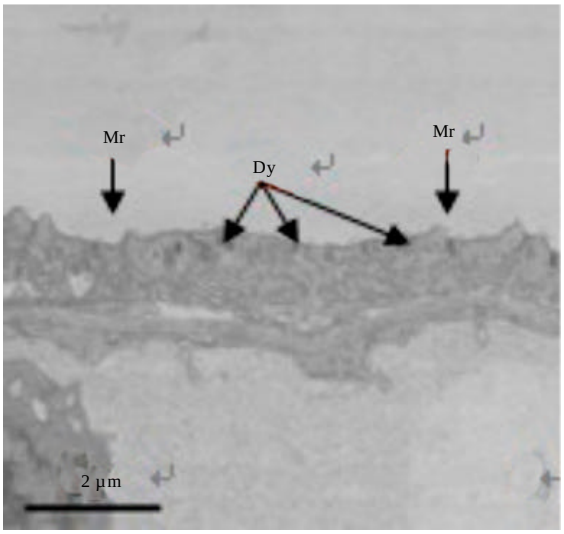

Fig. 6: Epithelia cells are less smooth in 26° of salinity. Mr: Microridges, Dv: Dark vesicle

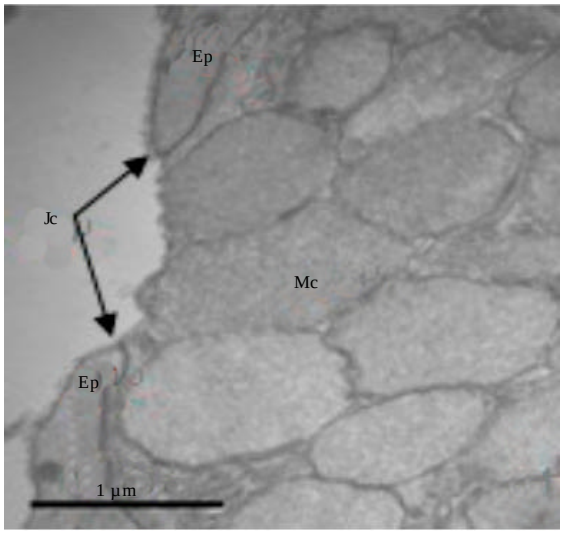

Fig. 8: Junctional complex between mucous cell and epithelia cells. Ep: Epithelia cell, Mc: Mucous cell, Jc: Junctional complex

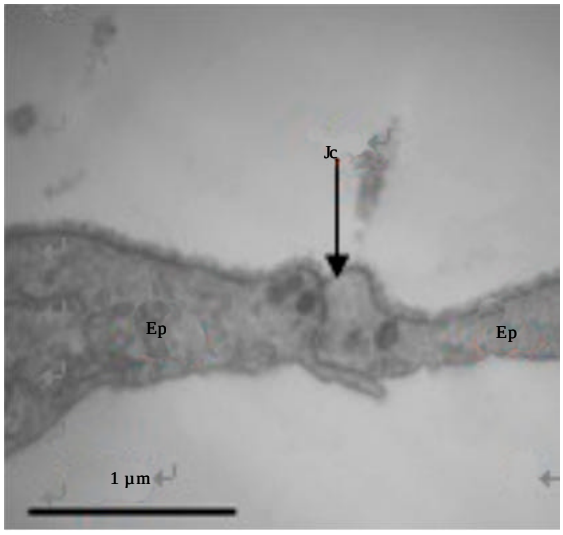

Fig. 7: Junctional complex between epithelia cells. Ep: Epithelia cell, Jc: Junctional complex

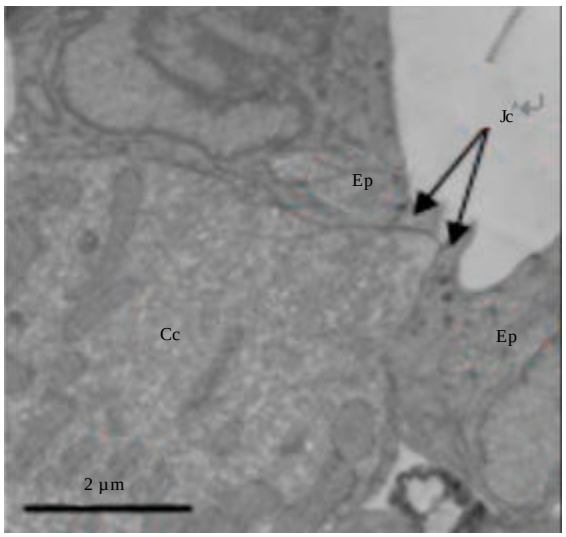

Fig. 9: Junctional complex between chloride cell and epithelia cells. Ep: Epithelia cell, Cc: Chloride cell, Jc: Junctional complex

In the test group of salinity 6, the surface of gill epithelium cell is smooth as shown in Fig. 4; in the gill base and gill epithelium, there have the distribution of secrete chloride cell, the excretive chloride cell in the gill is ovoid and possesses median nucleus are as shown Fig. 10; chlorine cells of gill base are elongated rectangle and the base nuclear as shown Fig. 11. Number of Intracellular mitochondria is relatively less than high salinity group, the mitochondria are ovoid, small pipe system is less as shown in Fig. 12.

In the group of 16° salinity, the epithelial cells of the small piece of test group are less flat (as shown in Fig. 14; excretive chloride cells is increased compared with

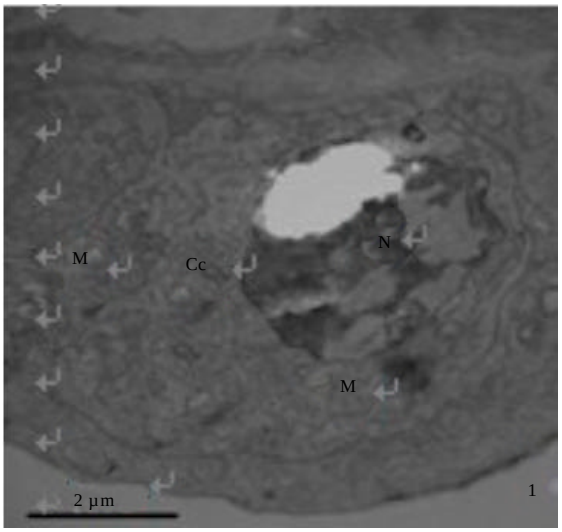

Fig. 10: Chloride cell on secondary filament are oval, the No. of mitochondria is less, nucleus is located in the middle, in 6° of salinity. Cc: Chloride cell, N: Nucleus, M: Mitochondria

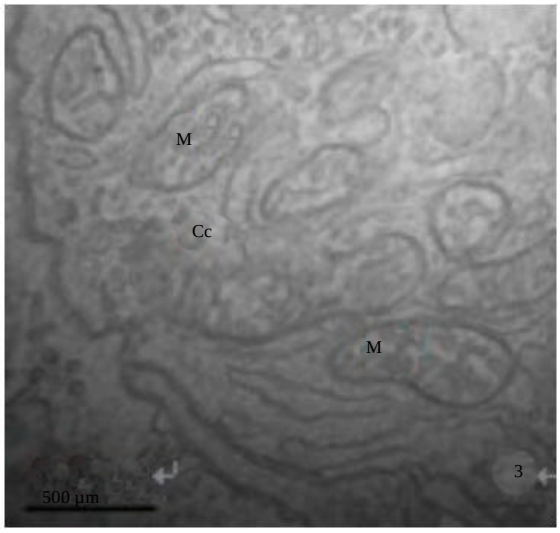

Fig. 12: Mitochondria of chloride cells are oval in 6° of salinity. Cc: Chloride cell, M: Mitochondria

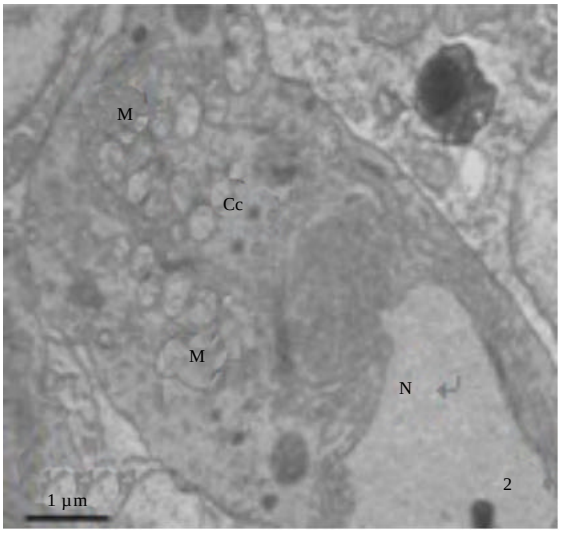

Fig. 11: Six degrees of salinity, the chloride cells are elongated, the No. of mitochondria of chloride cells on the base region of secondary filament are less, nucleus is located in the base. Cc: Chloride cell, N: Nucleus, M: Mitochondria

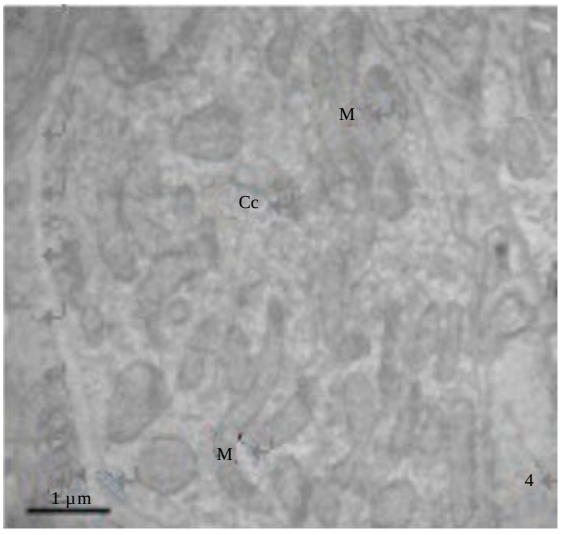

Fig. 13: Number of mitochondria is increased in 16° of salinity. Cc: Chloride cell, M: Mitochondria

the group of salinity 6° and the number of mitochondria in cells is also increased as shown in Fig. 13 and it can be seen that a excretive chloride cell can combine with other attached cells form the cell complex, on the top of excretive chloride cells is the cave and it can form the top alveolus with attached cells and epithelial cells (they are as shown in Fig. 14; the number of elongated mitochondria is also increased, inner crest of mitochondrial is relatively rich, the small pipe system is more advanced as shown in Fig. 15.

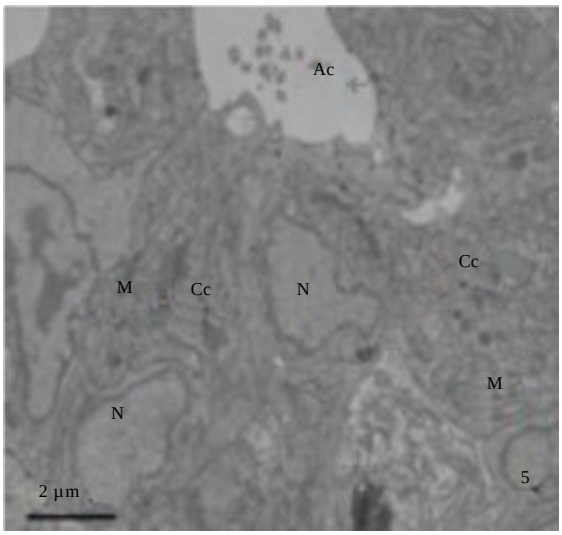

Fig. 14: Chloride cell on the base region of secondary filament combined with accessory cells into multicellular compleses, form apical crypt in 16° of salinity. Cc: Chloride cell, N: Nucleus, M: Mitochondria; Ac: Apical crypt

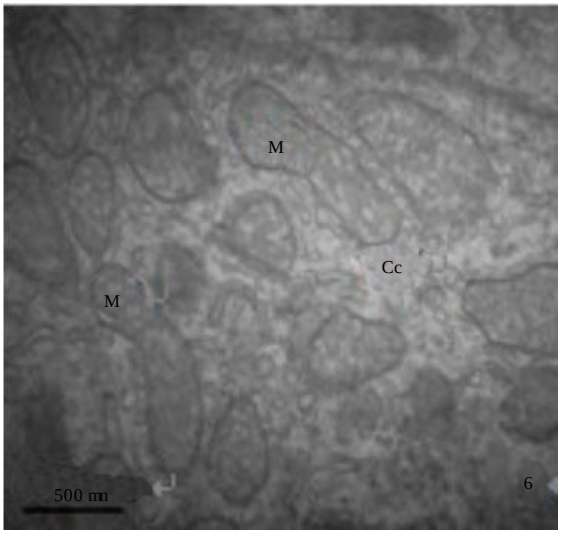

Fig. 15: Mitochondria are elongated, the No. of mitochondrial cristae is rich in 16° of salinity. Cc: Chloride cell, M: Mitochondria

In the group of salinity 26°, the surface of small gill epithelial cells is uneven than low salinity group, in test group, the micro crests can be observed as shown in Fig. 6; the number of chloride cells is increased significantly, the cell body becomes larger as shown in

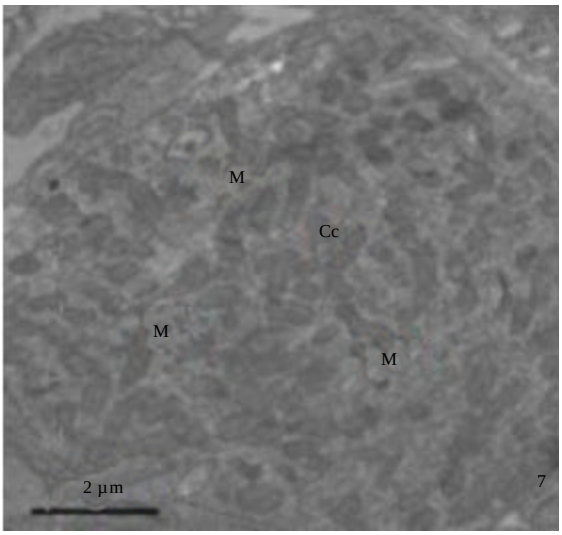

Fig. 16: No. of mitochondria is abundant in 26° of salinity. Cc: Chloride cell, M: Mitochondria

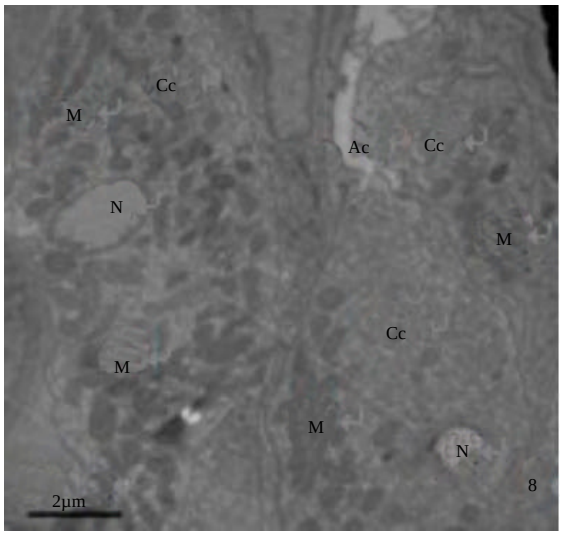

Fig. 17: Chloride cell on the base region of lamellae combined with accessory cells into multicellular compleses, showing apical crypt in 26° of salinity. Cc: Chloride cell, N: Nucleus, M: Mitochondria, Ac: Apical crypt

Fig. 16, it can be found that the chloride cells combined with attached cells form the more complex cells as shown in Fig. 17 the cells are elongated and the quantity of mitochondrial is larger. It possesses rich mitochondria crest and a developed tiny tube system as shown in Fig. 18.

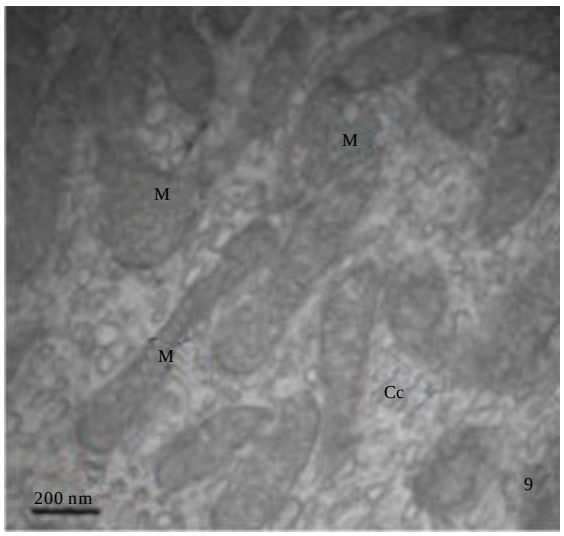

Fig. 18: Mitochondria are elongated, the No. of mitochondrial cristae is abundant. Cc: Chloride cell, M: Mitochondria

DISCUSSION

The *Pseudosciaena crocea* is one of the coastal migratory fish; it has a strong ability to adapt the changing of sea water salinity. Though the cells gills of fish are mainly formed by flat cell and excretive chloride cell (Pisam and Rambourg, 1991; Wilson and Laurent, 2002), but when the salinity of environment is changed, the fish gill epithelial cells may also make changes accordingly (Evans *et al*., 2005).

Epithelial cells are covered surface cells in most regions of the gill filament, cell surface covered with mucus, calyx and sugar which can protect the gill filament from damages by the water suspended solids (Wilson and Laurent, 2002; Bartels, 1989), the calyx sugar may be generated by the mucous cells secrete or produced by the epithelial cells of black vesicles (Carmona *et al*., 2004). The close connection between adjacent epithelial cells as well as epithelial cells and other cells, can prevent the leakage of free metal ion (Evans *et al*., 2005; Carmona *et al*., 2004).

In the experiment, in the group of 6 salinity°, the flat squamous cell surface is smooth, with increasing of the salinity, epithelial cells of the surface becomes less smooth, the reason may be that when salinity is lower than the physiological salinity, epithelial cells will be bibulous expanded. Existing of the Micro crest is to increase the surface area thus improve the gas exchange rate (Weixian *et al*., 2000), also it is possibly used to fix slime (Li *et al*., 2009). Some scholar thinks the crest surface covered with mucus can not increase the area of gas exchange (Hughes and Wright, 1970).

The chloride cells are important regulating cell of the seawater fish adapted to different salinity, excretive chloride cells can regulate secretion of Cl^- in the low permeability and it can regulate absorption of Na^+ and Cl^- in the high permeability, excretive chloride cells have lots of mitochondria and it can provide energy for active transport of ion, in the tiny pipe system, there has a lot of intracellular Na^+/K^+-ATPase. These characteristics make chloride cells have the function of the osmotic regulation (Wei and Lushaogou, 2001).

According to the difference location distribution of the chloride cell in the gill filament and ultrastructure distribution and changes adapt to the water, chloride cells can divided in into α type and β type, two types of chloride cell and type of α cells chloride cell body is big and it is long cylindrical or elongated ovoid, cells have low electron density and have the base nuclear.

Its mitochondrion is rich and presents ovoid or elongated a variety of forms, so the intracellular is widely distributed with small pipe system.

Type α excretive chlorine cells can combine with one or more cells together to form a more complex, α type chloride cell membrane is sag on the top, together with the around attached cells and epithelial cells form a nest at the top which can perform the function of sea water adapting.

Type β excretive chloride cells are often located in neighboring small pieces of gill filament. It usually exists alone, the cells are small and have the shape of ovoid. The electron density of cell is high, in the nuclear, intracellular mitochondria number is less, the small tube system are not too rich (Wei and Lushaogou, 2001; Kaneko *et al*., 2002; Hootman and Philpott, 1980; Sardet *et al*.,1979).

Recent studies have shown that the total number of excretive chloride cells of euryhaline fishes are basically unchanged but the β-type excretive chloride cells can be turned into alpha type, therefore in the process of its adaptation to seawater, the quantity of α type excretive chlorine cells will be increased, in the adapting process of fresh water, chloride cells β type will be also increased.

In this experiment, with the increase of salinity, chlorine cell body becomes large, the number of intracellular mitochondria and mitochondrial crest are also increased, small pipe system is richer, these results are consistent with other scholar's research results (Martinez-Alvarez *et al*., 2005; Carmona *et al*., 2004; Hou *et al*., 2006).

It is shown that with the increase of osmotic pressure, the excretive chloride cells function is strengthen, cell

metabolism level is improved. In the experimental group of salinity 6°, the excretive chloride cell type on the small pieces of gills basic is conform to beta type excretive chlorine cell, the characteristics of the gill base chlorine cells have the features of alpha-type excretive chlorine cells, but it mostly exists alone and it has no multi cellular complex which manifests the features of excretive chloride cells which is adapt to the water environment. In the experiment group of 26° of salinity, it can be found that the excretive chloride cells have the characteristics of type α excretive chloride cells and a large number of α type are form multicellular complex type of excretive chlorine cells which also manifests the characteristic of excretive chlorine cells adapting to the water environment.

All above analysis indicates that the large *Pseudosciaena crocea* under different salinity breeding conditions, the structure adaptive changes of young fish gills have taken place, excretive chloride cells grow into the form adapting to environmental water salinity.

The conclusion that *Pseudosciaena crocea* larvae changes has a strong ability of physiological structure in adapting to different water salinity and it can be formed to the gill structure of low salinity of water. It provides a strong theoretical basis for desalination water breeding of *Pseudosciaena crocea*.

In the observation experiment, the network management system and the microvilli are not found in the excretive chlorine cells (Hou *et al*., 2006); it is may be due to the experimental fishes are in different water varieties or the fishes are too small.

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