Vol. 16, No. 2, Summer, 2020, 47-53
DOI: 10.22055/ivj.2019.194902.2168
Journal Homepage: http://www.ivj.ir/

Original Article

Comparative histomorphometry of dorsal, ventral and lateral skin in macroscopy, microscopy and free scale fish

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Received: 19.07.2019 Accepted: 27.11.2019

Abstract
To perform this study, three species of fish, macroscopic scaled fish as *Otolithes rube*r, microscopic scaled fish as *Huso huso* and free scaled fish as *Pangasius hypophthalmus* were prepared and sampling of different parts of the dorsal, ventral and lateral surface was carried out. Routine procedures of tissues preparation were followed and paraffin blocks were cut at 4-6 micron, stained with H&E and studied under a light microscope. Results by (H&E) staining showed that epidermis formed non keratinized stratified squamous epithelium with mucous goblets like cells, taste bud and club cells. Mucous cells were along with the superficial cells layers and their distributions varied. In histomorphometry studies, the highest number of mucous cells were seen in dorsal regions, and the lowest were seen in the ventral region. The goblet cells were mostly secreted in the middle layers and they were drawn to the surface layer of skin. The club cells, with a large nucleus, mostly founded in deep and intermediate layers. These cells were not seen in the lateral surfaces of all three species. Taste buds was seen on ventral surface and in catfish the number of them was high. According to histometric results, except for goblet cells, in all other studied cases, there were significant differences in all three species in dorsal, ventral and lateral surface.

Keywords: Skin, *Otolithes rube*r, *Huso huso*, *Pangasius hypophthalmus,* Histomorphometry

Introduction
Skin is the first line of defence against diseases and contains epidermal cells and different proportions of mucus and proteins that protect it from scratching or drying (Park et al. 2003; Chong et al. 2005). Meanwhile, the skin with mucus plays an important role in the health of fish. Mucosal secretion cells are single-cellular exocrine glands that have created a viscous surface. Goblet cells and alarm mucous cells are responsible for the secretion of mucus in the epidermis of the fish (Sivakumar 2000). The skin of fish is fresher, non-keratinized, flexible and dynamic than mammals, and are considered as a key structure in fish anatomy studies. The skin of fish lacks

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cuticle secretion, but it has mucus. The fish lack the creatine layer on their epidermis. A cuticle consists of mucus, mucopolysaccharide, immunoglobulin, and fatty acids, that cover their surface (Scillitani et al. 2012). In mature fish, the skin has two layers of epidermis and dermis. The epidermis consists of a variable-thickness fused layer epithelium. Exterior epidermis cells retain their division. Other cells in the epidermis of goblet or mucosa cells are responsible for the secretion of the cuticle, eosinophilic club cells or alarm cells (in most fish species), functionally unspecified granular cells, ion cells, migratory cells, leukocytes, macrophages, free migratory cells, toxic cells, and sacciform cells are in the epidermis of some species (Fontenot and Neiffer 2004). The epidermis has cells that are connected to the basement membrane (Sharifpour 2004). Also, mucosal or goblet cells, apocrine and other types of secretion, ion and wandering cells and leukocytes, macrophages, migratory cells, venom cells, and some kind of sacciform cells in some species in the epidermis. The epidermis is derived from embryonic ectoderm. The epidermis, the outermost layer of the skin, is naturally secreted by epithelial surface cells, and mostly by mucosa goblet cells (Nakamura 2002). Unlike mammals, the epidermis is active in fish. In the pelagic fish, the epidermis is thicker in the back of the body than the rest of the body, while in the demersal fish, the epidermis is thicker in the ventral surface. The outer part of the epidermis, which contains non-keratinized stratified squamous cells, but in some fish, such as Asian catfish, it's keratinized (Harvey and Bathy 2002, Hausen 2005). The epidermis has no blood vessels and so it can damage the skin without causing obvious bleeding. The appearance of the *Otolithes ruber* fish indicates that their prominent feature is having a very advanced lateral line that reaches the bottom of the caudal fin (Pinky et al. 2008). Also, these fish have a large dorsal fin that reaches their tail. The shape of the sturgeon fits its quick moves and living in a fairly large depth so that the body is long and gradually narrowing from head to tail. The present studied catfish is a shark catfish or pangasius (*Pangasius hypophthalmus*). It is an ornamental fish of freshwater; Thailand is their main habitat (Damasceno 2012; Kim 2001). Because the skin is protective, it's used for detecting diseases in cytological experiments, and the anatomy and histology of a species are not generalized to other species (Kim and Lien 2000). This study can answer many questions in the field of pathology, physiology, and histology in the studied species. To the best of author knowledge, there's no report available about Otolithes ruber, Huso huso, and pangasius striped catfish.

Materials and Method

To do this research, 5 number of each species prepared from three different centers. Mature *Otolithes ruber*, with length of 55cm were sampled from Baharkan coast of Hindijan. One-year-old *Huso huso*, was taken from sturgeon farming center in Dezful suburb with an approximate length of 75cm. Pangasius catfish was provided from an ornamental fish breeding center in Behbahan with a length of 65 cm. Specimens were taken from different parts of the body (dorsal, ventral, and lateral) in size of 0.5 cm and removed in Bouin's fixative. The process of decalcification was done for *Huso huso* and catfish by formic acid. Next steps including dehydration in various degrees of alcohol and clarified by xylene and paraffin embedding were performed with a tissue processor. Following embedding in paraffin, cross and longitudinal sections of 4-6 µm were cut by a Leica RM2255 microtome and collected on glass slides and stained with Hematoxylin and Eosin (H & E), and then observed and photographed using an Olympus BX50 light microscope (Japan) equipped with a Dino-Lite lens together with Dino-Capture software installed on the computer. In the next step, data (epiderm thickness, goblet cells, taste bud and club number) were analyzed using SPSS, ANOVA, Tukey test and $P<0.05$ were considered statistically significant (Reverter et al. 2018, Guardiola et al. 2015).

Results

The results of the microscopic studies on the different parts of skin indicated that the skin is mainly composed of epidermis and dermis. On some parts of body club cells and taste buds were not observed, and the thickness of the epidermis was higher in some parts than in other areas. Goblet cells were observed in high numbers in many body parts of some species. In all three species studied club cells were observed in some parts. Based on microscopic studies, no taste bud was seen on the dorsal part, but the number of goblet cells in the dorsal part was noticeable. Goblet cells in the dorsum of *Otolithes ruber*, in the upper and middle layers of the epidermis, were bold and fraught with mucous observed in high number (673/1 ± 6/13). However, the number of goblet cells in the dorsal surface of sturgeon had the largest number (871/1±14) and it was obvious that some of them were migrating to the surface and they were ready for mucosal secretion. The number of goblet cells in the catfish was the smallest in total. Also, several club cells were seen in all three species, but their number in catfish was higher than the other two. In the microscopic study of the lateral region of this part, epidermis, dermis, hypodermis, especially the thickness of the dermis was detected. A Dermis is a place for scales and blood vessels, and thickening in the lateral region is justifiable. Also, in the skin around the ventral area number of goblet cells, especially alarm cells, were higher in the ventral part of catfish. Lack of scale and alarm cell function, i.e. responsible for fear hormone secretion, is justifiable in catfish (Fig. A1-A3; B1-B3; C1-C3). According to histometric results of 100µm of the epidermis, the number of club cells in comparison to goblet cells was significantly different in the ventral surface, and it was reported more clearly in catfish. According to the results of the investigation of the dorsal, lateral and ventral areas, three species of fish, *Otolithes ruber* with macroscopic scales, *Huso huso* with microscopic scales and pangasius catfish without scales it was found that the taste buds are in the ventral surface. Club cells were seen in the dorsal and abdomen. Goblet cells were seen in large numbers in the body parts of three species, and alarm cells are higher in some body parts of catfish than other species (Table 1-3).

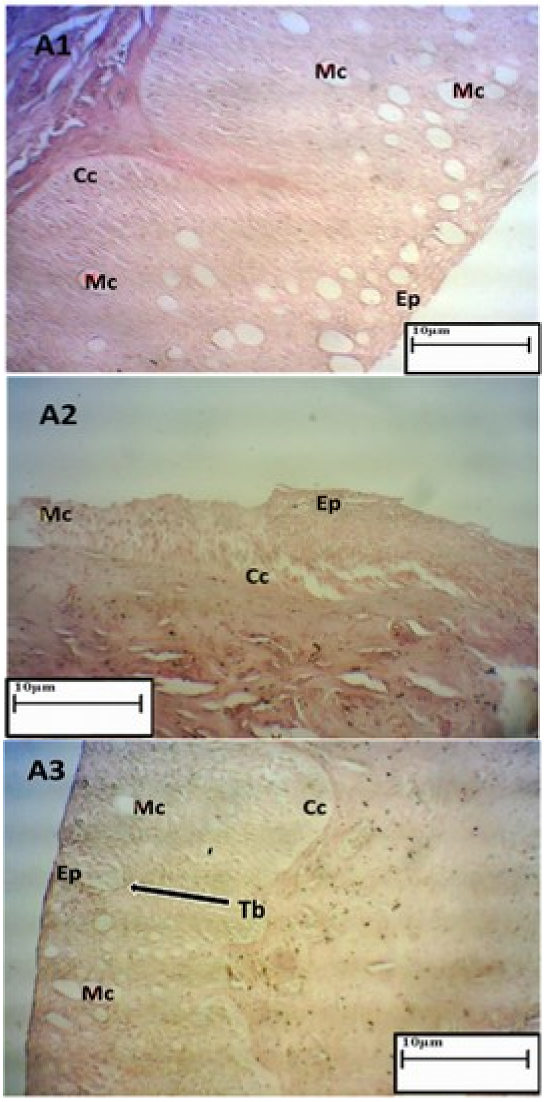

Fig. A1-A3: Photomicrograph of the skin of dorsal surface of *Otolithes ruber*, *Huso huso* and *Pangasius hypophthalmus* showing epidermis (Ep), goblet cells (Mc), taste bud (Tb) and club cell (Cc) (H&E).

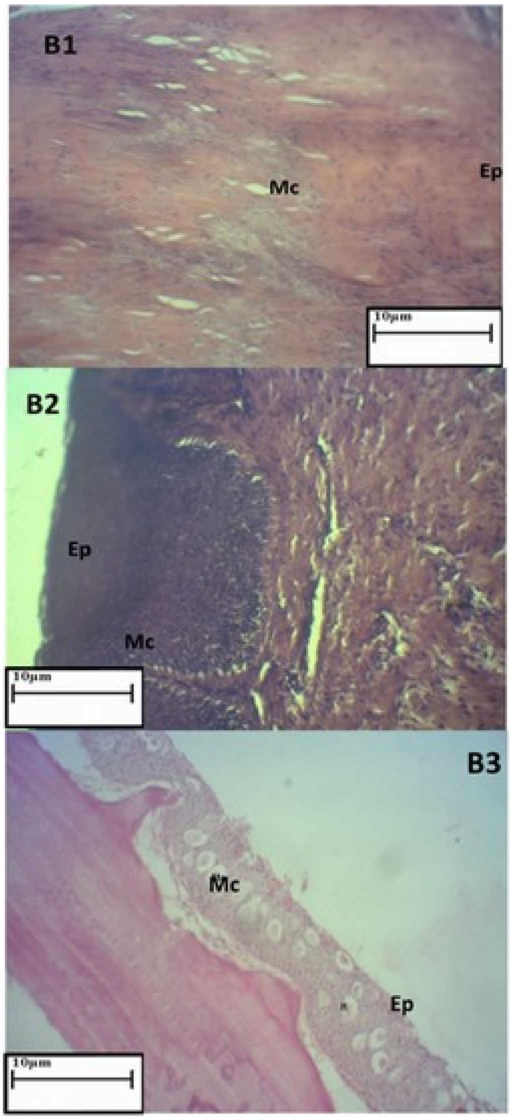

Fig. B1-B3: Photomicrograph of the skin of lateral surface of *Otolithes ruber*, *Huso huso* and *Pangasius hypophthalmus* showing epidermis (Ep), goblet cell (Mc) and club cell (Cc) (H&E).

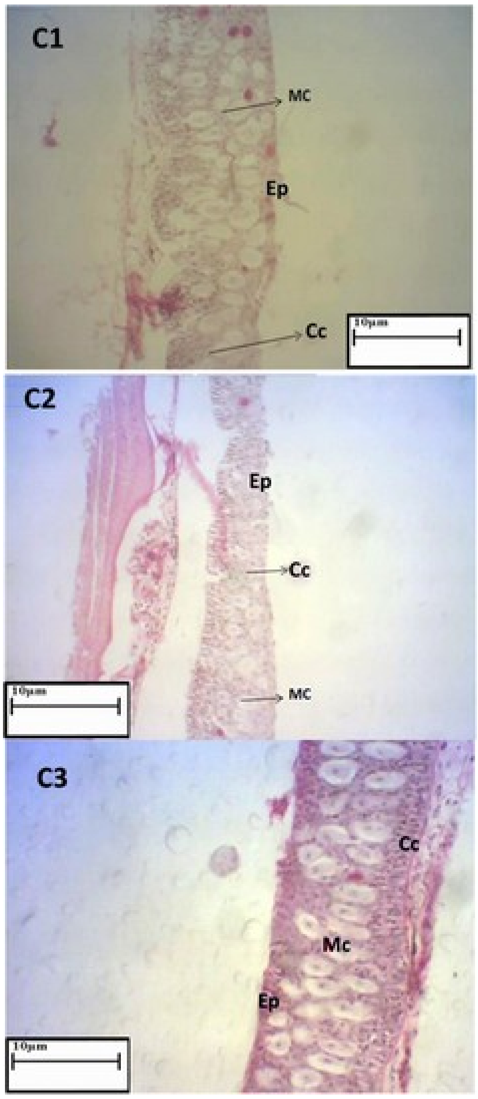

Fig. C1-C3: Photomicrograph of the skin of ventral surface of *Otolithes ruber*, *Huso huso* and *Pangasius hypophthalmus* showing epidermis (Ep), goblet cells (Mc) and club cell (Cc) (H&E).

Table 1. (mean ± SEM) of epidermal thickness, goblet cells, club cells and taste buds number (μm^2) in different areas of skin in *Otolithes ruber* analyzed by Hematoxylin-Eosin staining method

Region	Epidermal thickness	Goblet cells	Club cells	Taste buds
Dorsal	10.8 ± 0.83^a	13.6 ± 1.67^a	12.4 ± 1.14^a	1.6 ± 0.54^a
Lateral	9.8 ± 1.48^b	7.2 ± 0.83^b	Not seen	Not seen
Ventral	9.4 ± 1.14^b	8.8 ± 0.83^c	17.6 ± 0.89^b	1.4 ± 0.54^b

Different letters each column indicate a significant difference ($p<0.05$).

Table 2. (mean ± SEM) of epidermal thickness, goblet cells, club cells and taste buds' number (μm^2) in different areas of skin in *Huso huso* analyzed by Hematoxylin-Eosin staining method

Region	Epidermal thickness	Goblet cells	Club cells	Taste buds
Dorsal	11.2 ± 1.78^a	14 ± 1.87^a	9.8 ± 1.30^a	Not seen
Lateral	9.4 ± 0.89^b	7.6 ± 1.67^b	Not seen	Not seen
Ventral	8.4 ± 0.54^c	8 ± 1.22^c	17.4 ± 1.1^b	0.6 ± 0.54^a

Different letters each column indicate a significant difference ($p<0.05$).

Table 3. (mean ± SEM) of epidermal thickness, goblet cells, club cells and taste buds' number (μm^2) in different areas of skin in *Pangasius hypophthalmus* analyzed by Hematoxylin-Eosin staining method

Region	Epidermal thickness	Goblet cells	Club cells	Taste buds
Dorsal	10 ± 1.41^a	10 ± 1.58^a	14 ± 2.82^a	Not seen
Lateral	9 ± 1.22^b	5.2 ± 1.30^b	Not seen	Not seen
Ventral	7.4 ± 1.81^c	5.8 ± 1.30^b	20.6 ± 1.94^b	1.6 ± 0.58^a

Different letters each column indicate a significant difference ($p<0.05$).

Discussion

The epidermis is the outermost layer in skin tissue, which in fish consists of four cell types including epidermal, mucosal, granular and alarm cells (Sire and Akimenko 2004; Pickering 2006). However, in the morphological study of catfish, two cell types were detected. In club cells chromatin was compact, and protein/carbohydrates proportion was high (Dvir et al. 2014). In sturgeon and catfish, the size and number of goblet cells were low due to the young age sample. Since the mucous discharge protects the fish and covers the surface of the body. Mucous secreting cells are important. Main mucous secreting cells are goblet or alarm cells, neutral cells of glycogen and glycoprotein, and reacts positively with Schiff reagent in PAS staining (Engelmann et al. 2000). The number of goblet cells in *Otolithes ruber* was the most and the number of goblet cells in the catfish was the least. There was also a significant difference between the goblet cells in the ventral and caudal regions between sturgeon and catfish. In a survey done by Hidel and Smith showed the dispersed mucus cells on the surface as a protection center against bacteria, fungi, and parasites (Hidel and Smith 2007). In reproduction season, most mucus cells in the *Euryglossa orientalis* has more abundant dispersion and secretion of mucin in the ventral area to facilitate movement. The fish skin mucus was also analyzed and it became clear that the mucosal proteins differ in molecular weight in two seasons. Mucosal cells are common in the epidermis at the front of the European eel body but less common in tail and fins (Mastrodonato et al. 2005). The neutral mucous cells of the European eel have also been positively reacted with AB/PAS specific staining. These cells contain sialomucin and sulfomucin. These cells are different in the germline layer, and when they evacuate their content, they form a rod and die (Fast 2002). Histological studies about the tissue structure of the dorsal region of the skin in *Argyrosomus hololepidotus* showed that the epidermis contains goblet cells, superficial cells, squamous cells, and epithelial cells, and the histochemistry results showed that mucin-containing goblet cells have neutral glycoprotein (Fuczyska et al. 2008). Mucin containing glycoprotein sulfate and carboxylic acid were stained with PAS and AB (PH = 2.5) at the same time and showed a positive reaction, both red and blue, and finally purple. In that study, club cells were not observed, but in the present study, club cells were observed in all three species of fish and this number was the highest in catfish. Mucous secreting cells have not been observed in the epithelium lamprey, mudskipper, and polydon (Park 2002). Also, in neotropical catfish, mucosal goblet cells were small or even not seen, but two types of epidermis and club cells were detected and club cells had two nuclei with a clear nucleus and very compact chromatin and a high protein/carbohydrates proportion (Murray et al. 2012). In the present study, the number of goblet cells in different areas of pangasius catfish is less than sturgeon species. Mucus secretion from goblet cells increased when the fish are exposed to environmental stressors (Hiroi 2004; Selki et al. 2005). The highest number of mucosal cells in a study on silver carp was reported in the ventral region and the lowest number of mucosal cells was reported in the tail region (Park and Kim 2000). In the present study, the number of club cells (alarm cells) is mainly in the ventral and tail regions of all three species, and especially the caudal peduncle of catfish was more observed. The reason behind the name of the club in club cells is because they resemble the shape of the club (Pinky and et al. 2008). In the present study, the number of club cells in the dorsal, abdomen, and peduncle was more than the other parts, and the number of club cells in catfish was higher than *Otolithes ruber* and sturgeon. Catfish reacted to the stress conditions before the sampling, and according to the performance of club cells in the production of club cells in terms of safety and stress conditions, this observation can be justified. Also, the size of the neutral mucus cells is smaller than the mucus alarm cells, and the size difference in the studied species is exactly the opposite (Palaksha et al. 2008; Saw et al. 2010). Obvious differences have been observed in a recent study on the skin of the studied species in different parts. The researchers believe that the function of any part of the fish's skin is justified by the diversity of cells and the thickness of the epidermis of each area (Dvir et al. 2014). In the present study in three species, there was a significant difference between dorsal and ventral regions in club cells number. But in catfish the greatest number of club cells found in ventral region. Overall, there are differences and similarities between the three species studied, such as the absence of club cells and taste buds in the lateral region, which has been discussed by other researchers compared to other species.

Acknowledgement

The authors of the paper thank all who cooperated with us in collecting samples and other laboratory steps.

Conflict of interest

We need to declare no conflict of interest.

Funding

This paper is sponsored by Khorramshahr University of Marine Science and Technology.

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