Songklanakarin J. Sci. Technol.
38 (1), 67-72, Jan. - Feb. 2016

http://www.sjst.psu.ac.th

Original Article

Histological organization of the female queen *Devario regina* (Fowler, 1934) during its juvenile stage

Piyakorn Boonyoung[1]*, Sinlapachai Senarat[2], Jes Kettratad[2], Pisit Poolprasert[3], Watiporn Yenchum[4], and Waristha Angsirijinda[5]

[1] *Department of Anatomy, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110 Thailand.*

[2] *Department of Marine Science, Faculty of Science, Chulalongkorn University, Pathum Wan, Bangkok, 10330 Thailand.*

[3] *Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Mueang, Phitsanulok, 65000 Thailand.*

[4] *Bio-Analysis Laboratory, Department of Chemical Metrology and Biometry, National Institute of Metrology (Thailand), Pathum Thani, 10120 Thailand.*

[5] *Faculty of Veterinary Medicine, Mahanakorn University of Technology, Nong Chok, Bangkok, 10530 Thailand.*

Received: 12 September 2014; Accepted: 23 August 2015

Abstract

Limited research has been reported in the basic information about the structural organizations of fish organs in their juvenile state that could be used as histopathological biomarkers. Thus, the histological structures of important organs of the female fish *Devario regina* (Fowler, 1934) during its juvenile stage were exclusively examined using histological and histochemical approaches. Specimens were collected during the fishing season (July and October 2010) from the Tapee river, Thailand. Using histological analysis, the digestive system was distinctly composed of two parts; the digestive tract and accessory organs (liver and pancreas). Based on their histological structure, the epithelial organization of the oral cavity and pharynx was lined by stratified epithelium whereas the intestine was covered by a simple columnar epithelium and contained several goblet cells. The goblet cells were negatively stained with hematoxylin and eosin (H&E) and Masson's Trichrome (MT). In contrast, they were positively stained with Periodic Acid Schiff reaction (PAS) and aniline blue (AB). The liver tissue in this fish was composed of polyhedral hepatocytes, with their sinusoids being distinctly located between the hepatocytes. The sinusoids were lined by a simple squamous epithelium. The pancreatic parenchyma mainly consisted of pyramidal cells that rested on a basal lamina in the acinar. Moreover, the pancreatic cells had a basophilic cytoplasm, a distinct basal nucleus and contained large eosinophilic zymogen granules. The excretory system especially referred to the kidney, and was composed of renal tubules and hematopoietic tissue. The female reproductive system was the ovary that was surrounded by a tunica albuginea. The ovary contained oocytes at differential stages of development including oogonia and the previtellogenic stage. Finally, the integument of this species consisted mainly of three layers of epidermis, dermis and hypodermis, respectively.

Keywords: *Devario regina* (Fowler, 1934), histochemistry, histology, systems, Thailand

* Corresponding author.
Email address: piyakorn.b@psu.ac.th

1. Introduction

Characterizations of the histological tissues of teleosts are important for an understanding of the structure and function of their organs. This knowledge can be used as histopathological biomarkers, a diagnosis for assessment of the health of aquatic organisms as a result of their physiology and fine structure. As a consequence the histology of many systems has been exclusively investigated in various fish species, including the structure and functions of the digestive system (Buddington *et al*., 1997; Caballero *et al.,* 2003) in *Gadus morhua* (Morrison, 1987), *Seriola dumerili* (Grau *et al*., 1992), *Puntius stoliczkanus* (Day, 1871) (Senarat *et al*., 2013a) and *Hemibagrus filamentus* (Senarat *et al*., 2013b), and. In addition, the histology and histochemical investigation of the excretory system have been reported for *Scyliorhinus canicula, Acipenser gueldenstaedi* (Genten *et al*., 2008), and *Hemibagrus filamentus* (Senarat *et al*., 2013b) as well as the histology of their reproductive systems (Crim and Glebe, 1990; West, 1990; Senarat, 2011c). However, these organs of the fish mentioned above have been examined at the adult stage rather than the juvenile stage. Despite the information about the histological and histochemical observations during the juvenile fish stage, this new information could provide crucial knowledge for further studies.

Devario regina is an important ornamental fish in commercial aquaculture in Thailand, yet there have been no reports about the structural histology of their system during their juvenile stage. The goal of this research was to study the histological and histochemical organizations in detail of several systems including the digestive system, kidney, ovarian tissue and the integument of the juvenile *D. regina.* Because these organs have extremely important functions, they could be used assess if they were sensitive to change that depended on environmental problems and aquatic pollution (Blazer, 2002; Frame and Dickerson, 2006; Raskovic *et al*., 2010; Yenchum, 2010; Reddy and Rawat, 2013).

2. Materials and Methods

During the fishing season (July and October 2010), juvenile female of *Devario regina* (n=30, standard length approx. 2-4 cm) was obtained from the Tapee river, Chawang District, Nakhon Si Thammarat Province, Thailand (8°28'10" N, 99°29'45" E).

In the laboratory, the fish was euthanized by a rapid cooling shock (Wilson *et al*., 2009). The fish organs were then dissected and fixed in Davidson's fixative and exclusively processed under standard histological techniques as well as histological analysis (Humason, 1979). The tissue blocks of specimens were sectioned at 6-7 μm thickness using the rotary microtome and floated on a 0.5% gelatin solution. Finally, these sections were progressively stained with Hematoxylin and Eosin (H&E). Some sections from histological analysis were histochemically stained with Masson's trichrome (MT), Periodic Acid Schiff (PAS) and Aniline blue (AB) pH 2.5 (Humuson, 1979; Bancroft and Gamble, 2002). The three sets of stained tissue were examined for their histological structures using a light microscope (LM).

3. Results and Discussion

In the present study, we examined the detail of various systems from *Devario regina* obtained from a natural environment. Details are as follows:

3.1 Histological organizations of the digestive system

3.1.1 Oral cavity and pharynx

The first part of the digestive tract of *D. regina* was the buccal cavity and pharynx. Histologically, the digestive tract was comprised three layers including mucosa, submucosa and muscularis, respectively. In the buccal cavity, the mucosal layer was lined by a stratified polygonal squamous epithelium, which was similar to other teleosts (Albrecht *et al.,* 2001) i.e. *Cyprinus carpio* and *Gnathonemus petersii* (Genten *et al.,* 2008). However, it was different from some species including *Squalus acanthias* in that the mucosal layer was lined by a stratified cuboidal epithelium (Andrew and Hickman, 1974). Moreover, there were numerous taste buds and mucous cells. The taste buds were the mucous-secreting cells that were also observed between the stratified squamous epithelium (Figures 1A and C). The taste buds themselves had an intensely positive reaction to PAS and were a typical pear-shape located in the epidermal hillock. Each taste bud consisted of sensory cells that had an elongated nucleus and was supported by connective tissue. In addition, sustentacular cells were located at the periphery of the taste cells. Beneath the mucosa was a submucosal layer that was contained by loose connective tissue. Muscularis showed a layer of smooth muscle tissue. The mucous cells reacted positively with PAS, to indicate that the mucous cells produced glycoprotein substances (Figure 1C). The pharynx of the *D. regina* showed two parts comprising the upper and lower pharynx. The upper pharynx or pharyngeal pad was lined by stratified squamous epithelium with a covering from the honey pad, just like the zebra fish (Menke *et al.,* 2011). In the teleost, the honey pad is seen to be stomachless such as in the zebra fish and some cyprinids (Genten *et al.,* 2008). A submucosal and muscularis layers was present in the pharynx. The lower pharynx was covered by stratified squamous epithelium with numerous mucous cells. Their mucous cells were positively stained with PAS. Other layers were similar to those seen in previous part (Figure 1C).

3.1.2 Intestine

In general, the intestinal organ has a crucial function for digesting the diet, with an alkaline digestion and nutrient absorption. In this study, many parts of the digestive tract were seen and differently separated, excepted no stomach

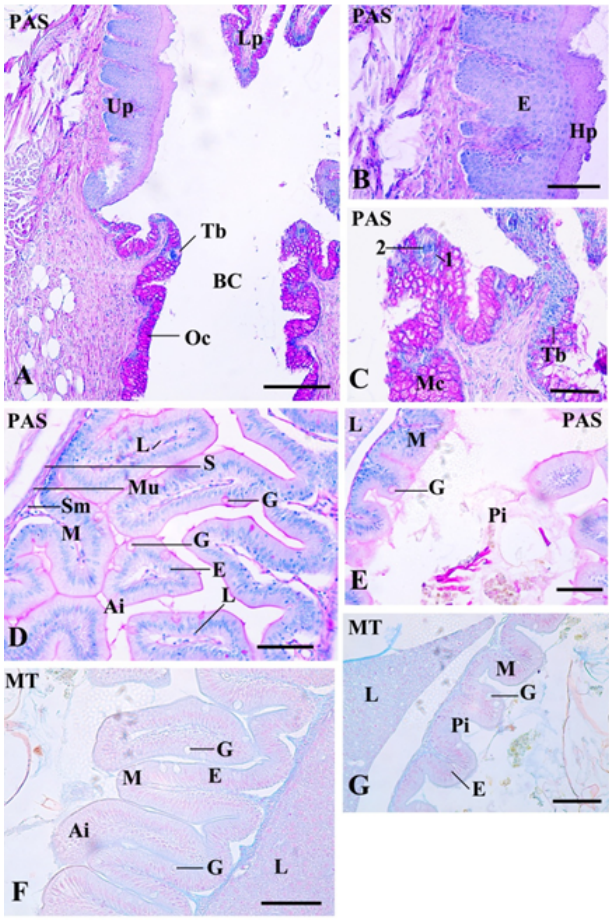

Figure 1. Micrographs showing tissues of the oral cavity (Oc) (A-C); Ai = anterior intestine (D, F); Pi = posterior intestine (F, G); (A) = 50 µm; (B – F) = 20 µm. Tb = taste buds (1), epithelial hillock (2), BC = buccal cavity, E = epithelium, Lp = lower pharynx, G = goblet cells, Hp = horny pad, L = liver, Lc = lymphocytes, M = mucosa, Mc = mucous cells, Mu = muscularis, S = serosa, Up = upper pharynx. (Masson's trichrome (MT), Periodic Acid Schiff (PAS) and Aniline blue (AB)).

was seen. However, we tried to separate the intestine structure based on the types and composition of the cells. Therefore, the long section of the intestine was separated into the anterior and posterior intestines. The anterior intestine had numerous mucosal folds that were covered by a simple columnar epithelium. The characteristics of the epithelium agreed with that of *S. acanthias* (Leake, 1975) and *P. stoliczkanus* (Senarat *et al.*, 2013a). Each epithelial cell contained an elongated nucleus and an acidophilic cytoplasm (H&E stained). Some parts of the epithelial layer had lymphocytes inserted. As for the detailed histochemical techniques, the upper part of epithelium cell was stained positively with PAS and aniline blue (AB). This clearly indicated that this cell produced glycoproteins and mucopolysaccharine (Figure 1). Moreover, between the epithelial of the mucosal layer, goblet cells (mucus-secreting cells) were found, and these were not stained with H&E and MT. In contrast, goblet cells had a positive reaction with PAS and AB as in several other fish (Cinar and Senol, 2006; Senarat *et al.*, 2013a). In addition, we have also observed that the number of goblet cells in the posterior part was greater than the anterior part of the intestine, and again this was similar to other fish (Canan *et al.*, 2012; Senarat *et al.*, 2013a). The functions of the goblet cells are for mucosal protection and lubrication for fecal expulsion (Murray *et al.*, 1994). Beneath the epithelial layer was the laminal propria containing connective tissue that stained positively with MT (greenish). No muscularis mucosae were observed in this part. The submucosal, muscularis and serosa layer were slightly formed and were rarely separated. The structure of the posterior intestine was similar to that of the anterior intestine.

3.1.3 Accessory organs

The accessory organs associated with the digestive system were the liver and pancreas (Figure 1G) as follows: Liver: the liver functions include storage of nutrients, production of bile and detoxification of xenobiotic substances. Moreover, the liver maintains metabolic homeostasis that includes the processing of carbohydrate, protein, lipid and vitamins. The liver also plays an important role in the synthesis of plasma proteins, such as albumin, fibrinogen, and the complement factors (Genten *et al.*, 2008). The parenchyma of liver in this species was surrounded by a fibroconnective capsule. This was composed of polyhedral hepatocytes and a portal tract. As to the detail, their hepatocyte cords were separated by sinusoids that contained several red blood cells, but a lobular shape was not found in this fish. Each hepatocyte was a large cell with an oval nucleus containing one prominent nucleolus (Figure 3E). The vacuolization and small granules in hepatocytes were glycogen and fat storage areas that stained positively with PAS (Figure 3F). In addition, bile ducts were present among the liver parenchyma. It was covered by a simple cuboidal epithelium and surrounded by connective tissue that was similar to other fish (Unal *et al.*, 2001). Pancreas: the parenchyma of the pancreas was present within the liver, and called the hepatopancreas. It was composed of many clusters of pyramidal cells in the acinar and portal afferent vein. The characteristic of the pyramidal pancreatic cell was the presence of a prominent nucleus, surrounded by a basophilic cytoplasm and contained various eosinophilic zygomogen granules (Figure 3E).

3.2 Histological organizations of the excretory system

The *D. regina* kidney was lined in a retroperitoneal location beneath the vertebral column and was a complex organ. Overall the kidney parenchyma consisted of hematopoietic renal corpuscle and tubules. In a longitudinal section, the parenchyma of this organ was present above the ovarian tissue. Generally, the renal corpuscle was composed of a

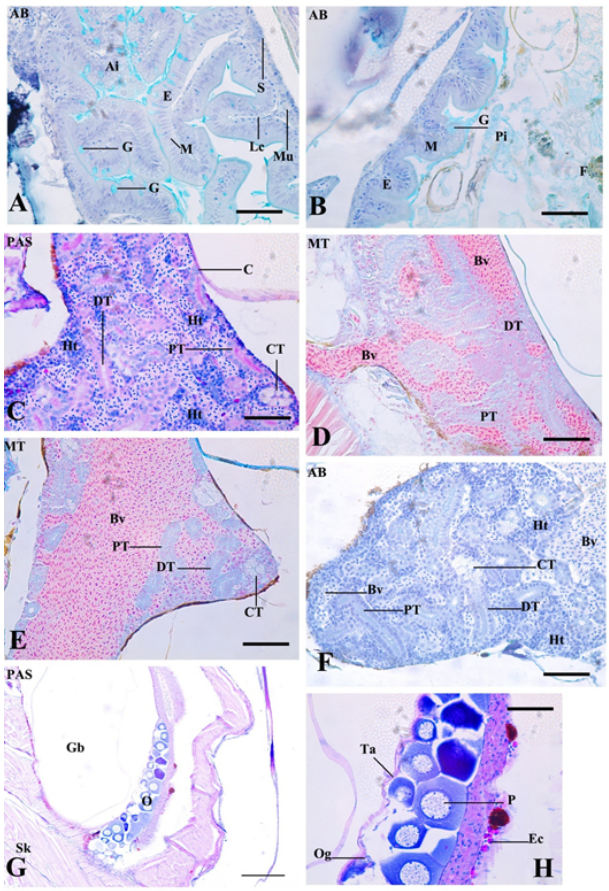

Figure 2. Micrograph showing tissues of the anterior intestine (Ai) (A); posterior intestine (Pi) (B); Kidney (B-F) and Ovaries (G-H); (A – F, H) = 20 µm; (G) = 100 µm. Bv = blood vessels, C = capsule, CT = collecting duct, DT = distal tubules, E = epithelium, Ec = eosinophilic cells, Gb = gas bladder, Ht = hematopoietic tissue, Lm = lamina propria, M = mucosa, Mu = muscularis, Og = oogonia, P = previtellogenic stage, PT = proximal tubules, S = serosa, Sk = sekeletal muscle, Ta = tunica albuginea. (Masson's trichrome (MT), Periodic Acid Schiff (PAS) and Aniline blue (AB).

glomerulus and the Bowman's capsules among the renal tubules. However, this structure in *D. regina* was slightly different in the juvenile stage (Figures 2B-F). In general, the renal tubules can be classified into three types based on their localization and histochemical reactions: the proximal tubule, distal tubule and collecting duct. The first segment of the renal tubule was the proximal tubule, and this was lined by a simple cuboidal epithelium. The PAS reaction gave a positive stain with a pinkish brush border (Figure 2C). In contrast, the distal tubule was jointed and similar to the previous segment, but it reacted only slightly with PAS in the brush border. These segments within the kidney were joined and became the collecting duct. This was lined by a simple columnar epithelium with a surrounding thin connective tissue.

3.3 Histological organizations of the female reproductive system

Histologically, the reproductive system of the female *D. regina* showed a pair of ovaries that were elongated in shape and suspended by mesovariam from the peritoneal cavity. It could be classified into two parts; the germinal and stromal compartments. The germinal compartment was composed of epithelial cells and oogonia. The stromal compartment, showed a different stage of the oocyte. Only the nucleolar chromatin and a perinucleolar stage were shown in this juvenile fish (Figures 2G-H).

3.4 Histological organizations the body integument

The integument or skin covers the fish body. The function of this organ is to protect against mechanical injury and noxious agents. Generally, it was histologically composed of three layers: the epidermis, dermis and hypodermis in the adult fish *Pelvicachromis pulcher, Astronotus ocellatus* and *Acipenser gueldenstaedtii* (Genten *et al.*, 2008) but in the juvenile stage in this fish there was rarely any difference with respect to the PAS reaction of the three layers (Figures 3A-B). Based on the MT, we described that the epidermis was connective tissue with a bluish color. Above the dermis was chromatophores with a black color (Figures 3C-D).

Although, there were little differences in the characteristics among species as far as the histological structure

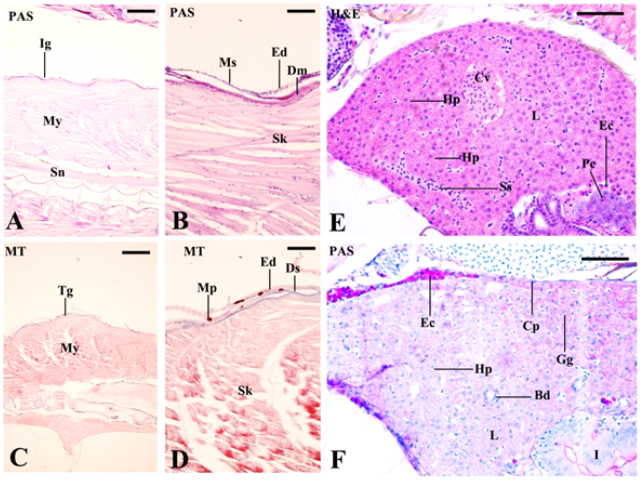

Figure 3. Micrograph showing tissues of the integument (Ig) (A-D) and liver (L) (E-F); (A-B, D) = 50 µm; (C) = 100 µm; (E-F) = 20 µm. Bd = bile duct, Cp = capsule, Cv = central vein, Dm = dermis, Ec = eosionophilic cell, Ed = endodermis, Gg = glycogen, Hp = hepatocytes, I = intestine, Mp = melanocyte, Ms = mucous cell, My = myomere, Pc = pancreas, Sk = skeletal muscle, Sn = spinal cord, Ss = sinusoids, Tg = integument. (Hematoxylin and Eosin (H&E), Masson's trichrome (MT), Periodic Acid Schiff (PAS)).

of these systems was concerned, in this report we have provided some new information about *D. regina* juvenile specimens. This fundamental data may result in a better understanding of many systems of this fish. Moreover, further studies in other fish species and other aspects such as the behavior, a histopathology and physiology will be studied.

Acknowledgements

We gratefully acknowledge the Department of Anatomy, Faculty of Science, and Prince of Songkla University for support throughout this study. Thanks also to Dr B. Hodgson for assistance with the English language.

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