

## มิถุนวิทยาและมิถุนเคมีของไตปลาตะกรับระยะวัยรุ่นจากปากแม่น้ำปราณบุรี ประเทศไทย Kidney Histology and Histochemistry in the spotted Scat, *Scatophagus argus*, during its Juvenile Stage from Paknam Pranburi Estuary, Thailand

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Received: 30 May 2017 ; Accepted: 5 October 2016

### บทคัดย่อ

พวกเราศึกษาโครงสร้างไตของปลาตะกรับ *Scatophagus argus* ช่วงระยะวัยรุ่น ปลาทุกตัวถูกประเมินด้วยเทคนิคทางด้านมิถุนวิทยาและมิถุนเคมี เนื้อเยื่อไตถูกแบ่งเป็นส่วนต้นและปลายภายใต้กล้องจุลทรรศน์แบบใช้แสง จากความแตกต่างด้านโครงสร้างพบว่าเนื้อเยื่อฮีมาโทโพอิติกและรีนัลคอร์ปัสเคิล (โกลเมอรูลัสและโบว์แมนส์แคปซูล) พบได้ในไตทุกส่วน ไตส่วนต้นประกอบด้วยเนื้อเยื่อฮีมาโทโพอิติกและท่อไตฝอยเล็กน้อย ขณะที่ท่อไตฝอยและรีนัลคอร์ปัสเคิลพบเพิ่มขึ้นในไตส่วนปลายเนื้อเยื่อไตของปลาตะกรับระยะวัยรุ่นจึงประกอบด้วยไตส่วนต้นและปลาย การศึกษาครั้งนี้เป็นข้อมูลพื้นฐานสำหรับการวิจัยในอนาคต เช่น โครงสร้างละเอียด จุลกายพยาธิวิทยา สรีรวิทยา สำหรับปลาตะกรับในบริเวณชะวากทะเลของประเทศไทย

**คำสำคัญ:** เนื้อเยื่อฮีมาโทโพอิติก สเคโทโพฟาจีตี โครงสร้างท่อไตฝอย

### Abstract

We observed the kidney structure of the spotted scat, *Scatophagus argus*, during the juvenile stage. All fish were carried out by using histological and histochemical techniques. The kidney tissue was divisible into the anterior and the posterior regions at the light microscopic level. Based upon different structure observations, the haemopoietic tissue and the renal corpuscle (a glomerulus and a Bowman's capsule) were found in all regions. The anterior kidney was comprised exclusively of hematopoietic tissues and a few renal tubules, whereas the renal tubule and renal corpuscle were gradually detected in the posterior kidney. The kidney of this fish was composed of two regions including anterior and posterior regions. This study provides baseline data for future planned/in-progress/anticipated work on the ultrastructure, histopathology and/or physiology of this economically/commercially/ecological fish species in estuaries of Thailand.

**Keywords:** Haematopoietic tissue, Scatophagidae, Renal structure

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## Introduction

Many studies have been published on histological observations of kidney tissues in fish<sup>1,2</sup>. Histologically, fish kidneys are composed of two distinct regions, the anterior and the posterior, according to the basic tissue and cell types present in each region<sup>1,2,3</sup>. Several components are found in the anterior kidney such as a majority of the hematopoietic tissue, interrenal tissue, chromaffin and adrenocortical endocrine cell types; examples include *Devario regina*<sup>1</sup> and *Hemibagrus filamentus*<sup>3,4</sup>. The posterior kidney contains the renal tubules and a few interstitial hematopoietic and lymphoid tissues; the posterior region is well known for its role as an osmoregulatory and excretory organ in teleost fishes<sup>2</sup>. One of the main reasons fish kidney histology has received renewed interest is because these studies have proven important in applied research related to fish physiology and histopathology. Histological alterations of the kidney may be good indicators of environmental stress<sup>3,4</sup>.

Although detailed description of the histological structure of the kidney tissue has been widely reported in teleost fishes, there are no reports on spotted scat, *Scatophagus argus*, with belonging to Scatophagidae. It is an euryhaline teleost and widely distributed in the near shore waters of the Indo-West Pacific Ocean such as India, Sri Lanka and Thailand<sup>5,6</sup>. *S. argus* is also considered as an commercially/ecologically important and dominant fish especially Paknam Pranburi Estuary, Thailand. Therefore, we report here results of studies carried out on kidney histology and histochemistry of the *S. argus* during its juvenile stage,

## Materials and Methods

Juvenile fish, *Scatophagus argus* (n = 20, standard length 3.04±0.21 cm) were field-obtained, there were by catch during fishing season (November to December 2015) from Pranburi Estuary, Prachuap Khiri Khan Province (N 12°24'08.5", E 099°59'00.2"). The tubular structure of kidney tissues of these fishes were collected from two areas (anterior and posterior kidneys) and were suddenly fixed in Davidson's fixative (about 48 h) for histological

observations.

Under histological observation, fixed kidneys were processed via standard histological techniques<sup>8,9</sup>. All kidney blocks were cut with a rotary microtome at 5 - 6 µm thick and stained with Harris's haematoxylin and eosin (H&E), Periodic Acid Schiff (PAS) and Alcian blue (AB)<sup>9</sup>. Observations of the histological structures of the kidney tissues were observed under a light microscope (LM).

## Results and Discussions

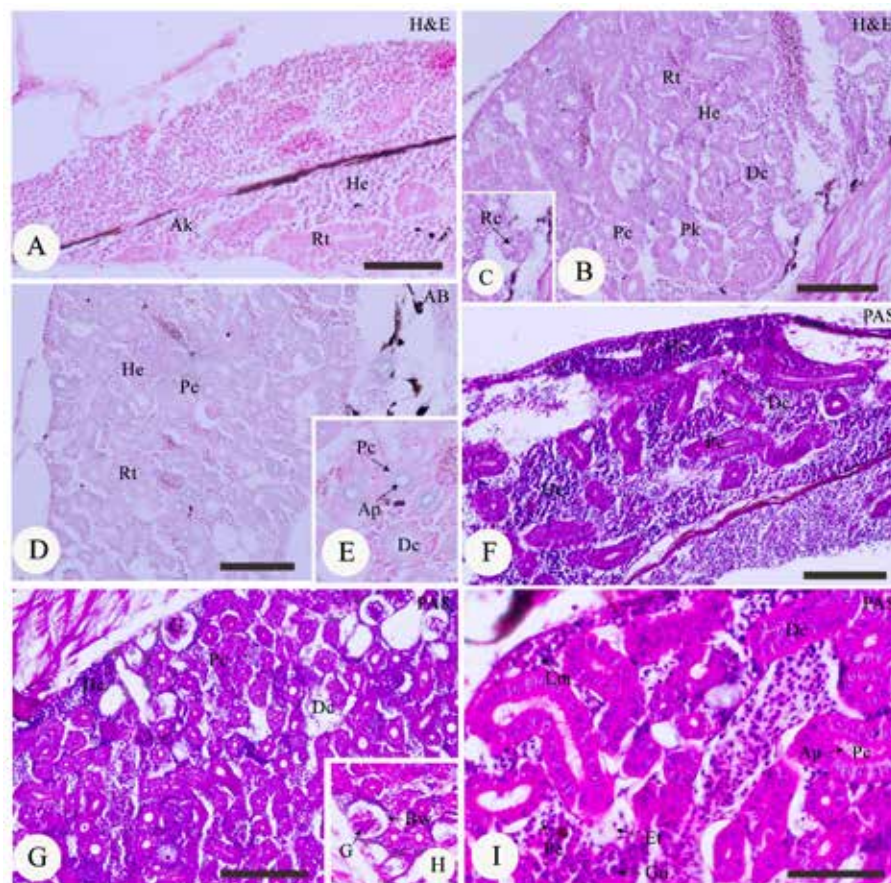
A histological confirmation of the *S. argus* kidney revealed that into it composed of two regions including anterior and posterior regions (Figures 1A-1I). Overall, the kidney of *S. argus* had numerous similarities to the teleost kidney<sup>2,10</sup>.

Longitudinal plans of the anterior kidney were covered by a capsule comprised thick of loose connective tissue layer with inserting smooth muscle. The haematopoietic-lymphoid tissue and a few excretory tubules were clearly observed. Note that the haematopoietic-lymphoid tissue of the anterior region was exclusively observed with abundant cell types (Figures 1A, 1F), similar to that reported in *Cyprinus carpio* and *Poecilia reticulata*<sup>2</sup>. The functions of haematopoietic-lymphoid tissue are associated with haematopoiesis and immunity in fish. Early in fish development, the entire kidney is involved in production of immune cells and the early immune response<sup>11</sup>. Note that a few renal corpuscles and renal tubules also were found in the anterior kidney.

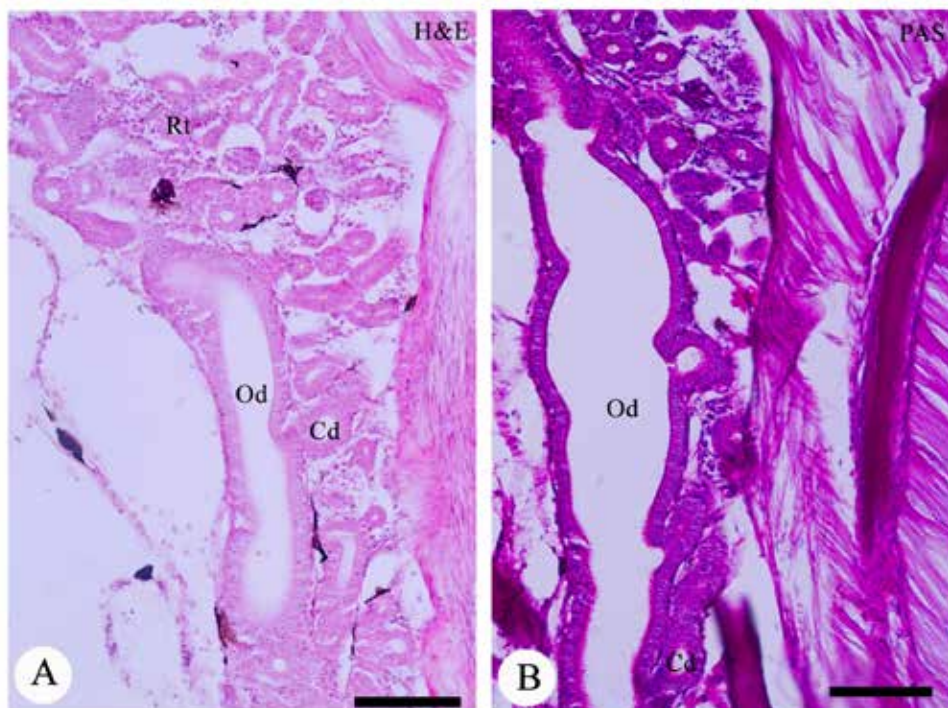
The posterior kidney was mainly composed of renal tubules and renal corpuscle; on the other hand, a lesser amount of interstitial hematopoietic tissue was present in the posterior region (Figure 1B-1C, 1G-1H). According to PAS reaction, cells detected in the hematopoietic tissue included proerythroblasts and erythrocytes of the erythropoiesis (Figure 1I). This study was similarly observed in *C. carpio* and *P. reticulata*<sup>2</sup> and *Sarotherodon mossambicus*<sup>11</sup>. The granulopoiesis and lymphoplas-mopoietic series were also present (Figure 1I), as similarly reported in *Clarias gariepinus* and *S. mossambicus*<sup>11</sup> and *Oreochromis niloticus*<sup>12</sup>. The renal corpuscle of *S.*

*argus* was formed by a glomerulus, which was surrounded by Bowman's capsule (Figure 1H). This capsule also had a double wall, forming visceral and parietal layers. The internal or visceral layer included anastomosing glomerular capillaries and podocytes, also called glomerulus (Figure 1I). The epithelium of this layer was enclosed by a single layer of simple squamous epithelium, as also called the parietal layer. The capsular space occurred between the two layers of the renal corpuscle. Light microscopically, the histological structure of the renal tubule revealed a connection to the renal corpuscles. Each tubule was composed of two proximal convoluted tubule segments (Figures 1B, 1G, 1I), as similar to previous report<sup>2,13</sup>. The first segment of proximal convoluted tubules had high simple columnar epithelium; a well-developed apical brush

border was also detected. The epithelium contained a spherical nucleus, which was basally located with a slightly eosinophilic cytoplasm (H&E staining method). On the other hand in histochemical results, the characterization of the apical brush border was positively reacted in both AB as bluish and PAS as pinkness (Figures 1E, 1I). The second distal convoluted tubule segment was lined by a low simple columnar epithelium. The nucleus of this epithelium was oval in shape with less obvious/abundant/developed apical brush border. Anderson and Mitchum<sup>14</sup> who reported that the functional structure in this segment might be related to divalent-ions. The collecting duct also had columnar epithelium; the collecting duct transformed into a larger duct before entering the opisthonephric duct (Figures 2A-2B).



**Figure 1** Micrograph of anterior (Ak) and posterior (Pk) kidneys of *Scatophagus argus* during its juvenile stage; (A-B), Ap = apical brush border, Bw = Bowman's capsule, Et = Erythrocytes, Dc = second distal convoluted tubule segments, G = glomerulus, Gn = granulopoiesis, He = haematopoietic tissue, Lm = lymphoplasmapoietic series, Pc = proximal convoluted tubule segments, Pe = proerythroblast, Rt = renal tubules. Harris's haematoxylin and eosin (H&E), Periodic Acid Schiff (PAS) and Alcian blue (AB). Scale bar A, B, D, F, G = 100  $\mu$ m, I = 50  $\mu$ m.



**Figure 2** Micrograph of the collecting duct (Cd) and the opisthonephric duct (Od) of the kidney of *Scatophagus argus* (A-B); Rt = renal tubules, Harris's haematoxylin and eosin (H&E), Periodic Acid Schiff (PAS). Scale bar A-B = 100 µm

### Conclusion

The current study revealed that the kidney of *S. argus* juveniles is the first described of this species and consisted of two distinct regions; anterior and posterior kidneys. The anterior kidney was composed primarily of hematopoietic tissue and few renal tubules. The posterior kidney contained mainly renal tubules with a lesser amount of interstitial hematopoietic tissues, where the renal tubules and glomerulus were gradually increased.

### Acknowledgement

The authors are thankful to the Microtechnique laboratory, Department of Biology, Faculty of Science, Prince of Songkhla University, and the members of the Fish Research Unit, Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, for technical support in their laboratories and Ms. Jirapa Kumrak, for her suggestion about the method of Alcian blue.

### References

- Boonyoung P, Senarat S, Kettratad J, Poolprasert P, Yenchum W, Angsirijinda W. Histological organization of the female queen *Devario regina* (Fowler, 1934) during its juvenile stage. *Songklanakarin Journal of Science and Technology* 2016; 38: 67-72.
- Genten F, Terwinghe E, Danguy, A. *Atlas of Fish Histology*. Science Publishers Enfield, NH: USA; 2008.
- Senarat S, Kettratad J, Poolprasert P, Yenchum W. Anterior kidney of the yellow mystus, *Hemibagrus filamentus* (Fang and Chauv, 1949). *Walailak Journal of Science & Technology* 2013; 10: 597-600.
- Senarat S, Kettratad J, Poolprasert P, Jiraungkoo-skul W, Yenchum W. Histopathological findings of liver and kidney tissues of the yellow mystus, *Hemibagrus filamentus* (Fang and Chauv, 1949), from the Tapee River, Thailand. *Songklanakarin Journal of Science and Technology* 2015; 37: 1-5.
- Pinto L, Punchihewa NN. Utilisation of mangroves and seagrasses by fishes in the Negombo Estuary, Sri Lanka. *Marine Biology* 1996; 126: 333-345.
- Kottelat M. Scatophagidae. In: Carpenter K.E., Niem V.H. (Eds). *FAO species identification field guide for fishery purposes. The living marine resources of the*

- Western Central Pacific. Vol. 6. FAO, Rome. 2001.  
P. 3623-3626.
7. Presnell JK, Schreibman MP. Humason's Animal Tissue Techniques. 5th ed. US: Johns Hopkins University Press; 2013.
  8. Suvarna KS, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. 7th ed. Canada, Elsevier; 2013.
  9. Bancroft JD, Gamble M. Theory and practice of histological techniques. Churchill Livingstone, London, UK; 2002.
  10. Roberts JR. Fish Pathology. 4th ed. Bailliere Tindall, London; 2000.
  11. Boomker J. The haemocytology and histology of the haemopoietic organs of *Clarias gariepinus* and *Sarotherodon mossambicus*. Journal of Veterinarian Research 1979; 46: 217-222.
  12. Abdel-Aziz EH, Abdu BBS, Ali TE, Fouad HF. Haemopoiesis in the head kidney of tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae): a morphological (optical and ultrastructural) study. Fish Physiology and Biochemistry 2010; 36: 323-336.
  13. Mumford S, Heidel J, Smith J, Morrison, J, Mac Conbell C, Blazer V. Fish Histology and Histopathology. National Conservation Training; 2007.
  14. Anderson BG, Mitchum DL. Atlas of Trout Histology. Wyoming Game and Fish Commission Bulletin; 1974.