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Corresponding author:
Sinlapachai Senarat,
E-mail: sinlapachai.s@rmutsv.ac.th

Research article

The Use of Hematological and Histopathological Biomarkers to Assess the Health of Aquatic Ecosystems in Koh Sichang, Thailand

Anek Sopon[1], Jes kettratad[1, 2], Ajcharaporn Piumsomboon[1], Gen Kaneko[3], and Sinlapachai Senarat[4,*]

1 Marine Ecology and Marine Resources Utilization Research Unit, Aquatic Resources Research Institute, Chulalongkorn University, Bangkok 10330, Thailand
2 Department of Marine sciences, Faculty of Science, Chulalongkorn University, Bangkok, 10330 Thailand
3 School of Arts and Sciences, University of Houston-Victoria, Victoria, Texas 77901, USA
4 Department of Marine Science and Environment, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang 92150, Thailand

Abstract Koh Sichang area in Thailand is a sink for a wide variety of contaminants such as heavily polluted water from industry and oil spills. This situation may affect the health status of fish living in the area, but such information remains scarce. In this study, we evaluated the health status of java rabbitfish *Siganus javus*, an important marine fish in Koh Sichang, using hematological and histopathological biomarkers. All fish samples were collected from the Koh Sichang area during December 2017 and January 2018. Although the salinity, pH, and dissolved oxygen levels at sampling points were all within the normal range, abnormal nuclei were observed in erythrocytes (up to ~6% of all erythrocytes) and in some leucocytes (neutrophil, lymphocyte and monocyte). Visceral organs (gill, kidney and liver) were apparently normal in terms of gross morphology, but a wide variety of the histopathological alterations were found at the microscopic level: epithelial hyperplasia and aneurysm in gills; blood congestion and melanomacrophage centers (MMCs) in the liver; renal degeneration, granuloma and MMCs together with unidentified parasites in kidney. Calculation of semi-quantitative parameters [histological alteration index (HAI) and the average value of alteration (AVA)] demonstrated the highest frequency of histopathological alterations in kidney, suggesting that kidney is a sensitive organ. Overall, our observations suggest that *S. javus* in Koh Sichang is under the pathological state and warrants conservation efforts.

Keywords: Erythrocytes, Fish Health, Hematology, Histopathology, Kidney

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INTRODUCTION

Koh Sichang is a small island well known as a popular tourist destination, which is located in an economically important area of Thailand. Because of the location, this area receives wastewater from public transport, industry, agriculture and urban runoff (Wattayakorn and Rungsupa, 2012). The Koh Sichang area has also received catastrophic oil spills, resulting in chemical contaminations that might exert harmful effects on aquatic animals (Wattayakorn and Rungsupa, 2012; Senarat et al., 2018). It is therefore important to monitor the health status of aquatic animals in this area. Fish would be a good sentinel organism for this purpose because they are dominant in many aquatic environments including the Koh Sichang area, and they are very sensitive to environmental changes/problems (National Research Council, 1991; Beeby, 2001; Frame and Dickerson, 2006).

Hematology is one of the important tools to monitor the health status and has been extensively used for aquatic organisms (Hayashi et al., 1998; Hrubec et al., 2000; Rodriguez-Cea et al. 2003; Thrall et al., 2007). Especially, abnormal blood composition has been well associated with the impaired fish health caused by environmental problems (Castro et al., 2019). Increased numbers of erythrocytes and leucocytes as well as the increase in the packed erythrocyte volume have been recorded in rohu, *Labeo rohita* (Hamilton 1822), from the polluted Lakes of Bangalore, India (Zutshi et al., 2010). Frequent erythrocyte alterations were reported from *Colossoma macropomum* living in the anthropic areas (Castro et al., 2019). The formation of chromatin-containing bodies in erythrocyte cytoplasm has often been used to monitor the water quality and the health of the aquatic organisms (Carins et al., 1975; Brugs et al., 1977; Rodriguez-Cea et al., 2003).

Histopathology is also a valuable yardstick to evaluate fish health and ecosystem status (Dalzochio et al., 2016) that identifies histological abnormalities at cellular, tissue, and organ levels (Teh et al., 1997; Senarat et al., 2015; Mansouri et al., 2016; Barbieri et al., 2016; Senarat et al., 2018a; Senarat et al., 2018b; Senarat et al., 2019; Senarat et al., 2020). For example, Louiz (2018) has evaluated the effect of pollution on the liver in black goby *Gobius niger* Linnaeus 1758 and grass goby *Zosterisessor ophiocephalus* (Pallas, 1814) in Tunisian lagoons. Liver lesions were found in specimens from polluted areas, suggesting the reduced liver function caused by environmental problems. Hepatic histopathological alterations were also found in *Micropterus salmoides* (Lacépède, 1802) from the Pigeon River, USA, which include hyperplastic basophilic hepatocytes, severe lipidosis, vacuolated and basophilic foci (Teh et al., 1997). In addition to the liver, gill is an important target of histopathological analysis. For example, abnormal gill structures including aneurysm, edema, hyperplasia and fusion were found in Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864), Iberian nase *Pseudochon drostoma* (Coelho, 1985) and rainbow trout *Oncorhynchus* mykiss (Walbaum, 1792), living in Northern Portuguese river, Portugal, contaminated with heavy metals (Fonseca et al., 2017). Along with the liver and gill, kidney has also been used in histopathological analysis in many fish species (Puttipong et al., 2021; Mangang et al., 2021).

The java rabbitfish *Siganus javus* (Linnaeus, 1766) is one of the most important economical marine fish in Southeast Asia, particularly in Thailand. This fish is widely found around Koh Sichang, but to our knowledge the current study is the first detailed record of hematological and histopathological features of *S. javus* from the area. Our study indicates potential health risks of *S. javus* in the Koh Sichang area, which warrants the environmental protection practices around the island.

MATERIALS AND METHODS

Materials

Fish samples and environmental factors

Thirty-eight samples of adult *Siganus javus* with 7.16 ± 2.41 (mean ± standard deviation) inches of total length were collected by using swing and fishing hooks from two main stations (station 1: 13° 8'9.41"N, 100°47'58.75"E and station 2: 13° 8'47.98"N, 100°47'42.26"E) around Koh Sichang, Thailand (Figure 1), during December 2017 and January 2018. Physical and chemical parameters, such as water depth, water temperature, salinity, pH and dissolved oxygen (DO) during the time of sampling were measured using an EC900 AMTAST Waterproof DO Kit 9-in-1 Meter (AMTAST, Lakeland, FL, USA). The experimental protocol was approved by the Institutional Animal Care and Use Committee, Aquatic Resources Research Institute (IACUC ARRI) [1931001].

Figure 1. Sampling localities of *Siganus javus* from Koh Sichang, Thailand, including station 1 and station 2.

Hematological observation

All fish specimens were euthanized by a rapid cooling shock (Wilson et al., 2009). The bloods were collected by venipuncture of the caudal vertebral vein using a 21G × 1″ needle and a 1- or 2-mL syringe (NIPRO, Japan), following the standard method of Watson et al. (1989). Blood smears were prepared on glass slides using the manual wedge technique, fixed in methanol for 1 min, air-dried, and stained with the Wright-Giemsa solution for 25 min. The slides were washed three times with phosphate-buffered saline (PBS, pH 7.4) and mounted by the mounting medium. The blood cells were characterized by visual examination of the peripheral blood smear according to the guideline of standard hematology (Theml et al., 2004) and photographed using a Leica DM 1000 light microscope equipped with a 100× oil-immersion lens (Leica, Wetzlar, Germany). Additionally, the number of abnormal erythrocytes (30 cells/fish per slide) and leucocytes (20 cells/fish per slide) were quantified.

Histopathological observation

All fish samples were abdominally opened and assessed for the external morphology of visceral organs. Gill, kidney and liver were fixed with the 10% neural buffer formalin (NBF). The anterior, middle, and posterior parts of each organ were randomly selected, cut into small pieces (1 × 1 cm), and then processed by the permanent histological preparation method (Presnell and Schreibman, 1997; Suvarna et al., 2013). The paraffin-embedded tissues were sectioned at 4 μm thickness and stained by Harris's hematoxylin and eosin (H&E) (Presnell and Schreibman, 1997; Suvarna et al., 2013). Histopathological alterations of the organs were photographed using a Leica digital 750 light microscope using a digital camera (Leica, Wetzlar, Germany).

All tissues (gill, liver and kidney) were semi-quantitatively analyzed for their histopathological alterations and classified into several degrees of damage based on the histological alteration index (HAI) (Poleksic and Mitrovic-Tutundzic, 1994) and average value of alteration (AVA) (Poleksic and Mitrovic-Tutundzic, 1994; Schwaiger et al., 1997).

To calculate the HAI, degrees of alterations in each tissue were evaluated by the standard criteria of progressive tissue damage stage (Table 1) following previous publications (Poleksic and Mitrovic-Tutundzic, 1994; Paulo et al., 2012; Dos Santos et al., 2018; Barbierl et al., 2019). The HAI was calculated using the equation: $HAI = 1 \times \sum I + 10 \times \sum II + 100 \times \sum III$, where I, II, and III corresponded to alterations of stage I, II and III (Table 1), respectively. Based on the HAI, tissue damage was classified into five categories including 0 to 10 (normal organ/tissue functioning), 11 to 20 (slight alteration in the organ/tissue), 21 to 50 (moderate alteration in the organ/tissue), 51 to 100 (severe alteration in the organ/tissue) and values above 100 (irreparable alteration in the organ/tissue) [Poleksic and Mitrovic-Tutundzic, 1994].

The AVA was calculated according to the frequency of occurrence and severity of lesions, which can be classified into three categories; score 1 (no pathological alteration of organs, score 2 (slight or mild pathological alterations of organs), and score 3 (severe and extensive pathological alterations of organs), following Poleksic and Mitrovic-Tutundzic (1994).

Table 1. Classification of the severity of histopathological alterations in adult *Siganus javus*. Adapted from Poleksic and Mitrovic-Tutundzic (1994); Paulo et al. (2012); Dos Santos et al., (2018); Barbierl et al., (2019)

Histopathological alterations	stage
Gill	
Disorganization of secondary lamellae	I
Blood congestion	I
Epithelial hyperplasia	I
Lamellar aneurysm	II
Liver	
Vacuolar degeneration of hepatocyte	I
Sinusoidal dilatation	I
Blood congestion	I
Melanomacrophage centers (MMCs)	I
Degeneration of hepatic cytoplasm	II
Kidney	
Unidentified parasite	I
MMCs	I
Renal degeneration	II
Necrotic tissue	III
Granuloma	III

RESULTS

Environmental factors

Environmental factors were recorded to compare the station 1 and station 2 (Table 2). In both stations, temperature, pH, and DO were within the standard range for marine environmental resources (Pollution Control Department, 2017). We found no major differences in environmental factors and biological characteristics of *S. javus* (see below). We therefore described the results of fish from both stations together.

External and internal characteristics

No abnormalities were observed in the external and internal characteristics of all fish examined (Figures 2A-2B), but the pronounced visceral adipose accumulation was found in 19 out of the 38 samples (Figure 3, 50% prevalence).

Table 2. Environmental parameters collected from Koh Sichang, Thailand, during December 2017 and January 2018-2019.

Environmental parameters	Stations		Permissible limits and reference
	1	2	
Water depth (m)	14.3	15.6	-
Water temperature (°C)	28.3	28.3	28 – 32 (Pollution Control Department, 2017)
Salinity (psu)	28.56	28.8	-
pH	8.16	8.13	7.0 – 8.5 (Pollution Control Department, 2017)
Dissolved oxygen (DO) (mg/L)	4.31	4.50	≥ 4 (Pollution Control Department, 2017)

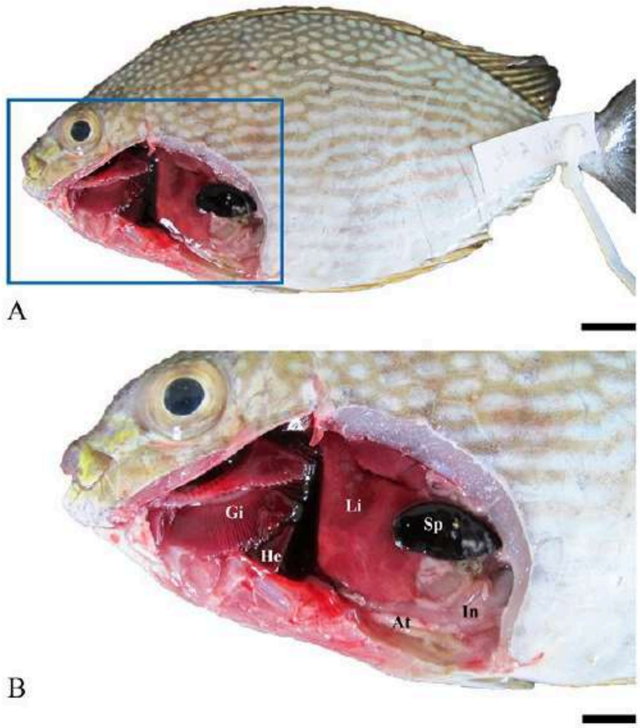

Figure 2. External morphology (A) and visceral organs (B) of adult *Siganus javus* from Koh Sichang. Abbreviations: At = adipose tissue, Gi = gill, In = intestine, Li = liver, He = heart, Sp = spleen. Scale bar A = 0.5 mm, B = 0.2 mm.

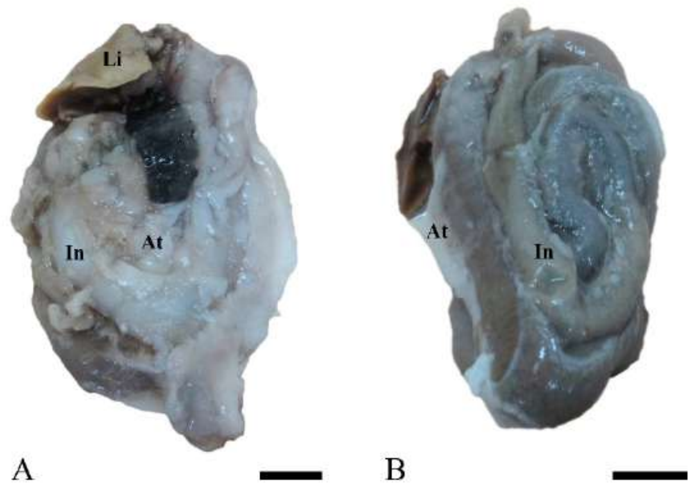

Figure 3. The prominent accumulation of adipose tissue (At) in the visceral organ of adult *Siganus javus* observed in 50% of individuals from Koh Sichang (A). The remaining individuals had normal adipose morphology (B). Abbreviation: In = intestine Scale bar A-B = 0.3 mm.

Hematological parameters

By the peripheral blood smear analysis, different types of blood cells including erythrocytes, leucocytes and platelets were identified (Figure 4).

Erythrocytes of the elliptical shape were the prominent blood cell in this fish (Figure 4A). Their oval, dark-purple nucleus was centrally located in the cell, being surrounded by the light pink cytoplasm (Figure 4A). We found several erythrocytic nuclear abnormalities. Some nuclei showed notched (20.17% proportion) and blebbed (9.21% proportion) shapes (Figures 4B-4D, Table 3). Interestingly, the present study also identified potentially apoptotic cells in the blood smear (Figures 4E, 4G). On the other hand, thrombocytes occupied 8.02% of total blood cells in unhealthy fish (Table 3). These cells had a spindle-shape and contained a purple-stained nucleus (Figure 4F).

In this study, three important types of leucocytes including monocytes (Figures 4G, 4I), neutrophils (Figure 4H) and lymphocytes (Figure 4J) were observed. Monocytes were larger than lymphocytes. They had a large nucleus with a horseshoe or bean shape (Figure 4I) and the basophilic nucleoplasm. Monocytes occupied a low proportion in blood cells (9.60% proportion) and were rarely found in the blood smear. Neutrophils were found at a higher proportion (21.57%) (Table 3). Neutrophils had an oval shape and contained a multi-lobulated nucleus (Figure 4H). Lymphocytes were small agranulocytes of approximately 12-15 µm diameter (Figure 4J). Lymphocytes occupied the highest proportion in leucocytes in this study (35.65%) (Table 3). This cell had a large nucleus with dark purple color surrounded by a narrow layer of lightly basophilic cytoplasm without granules.

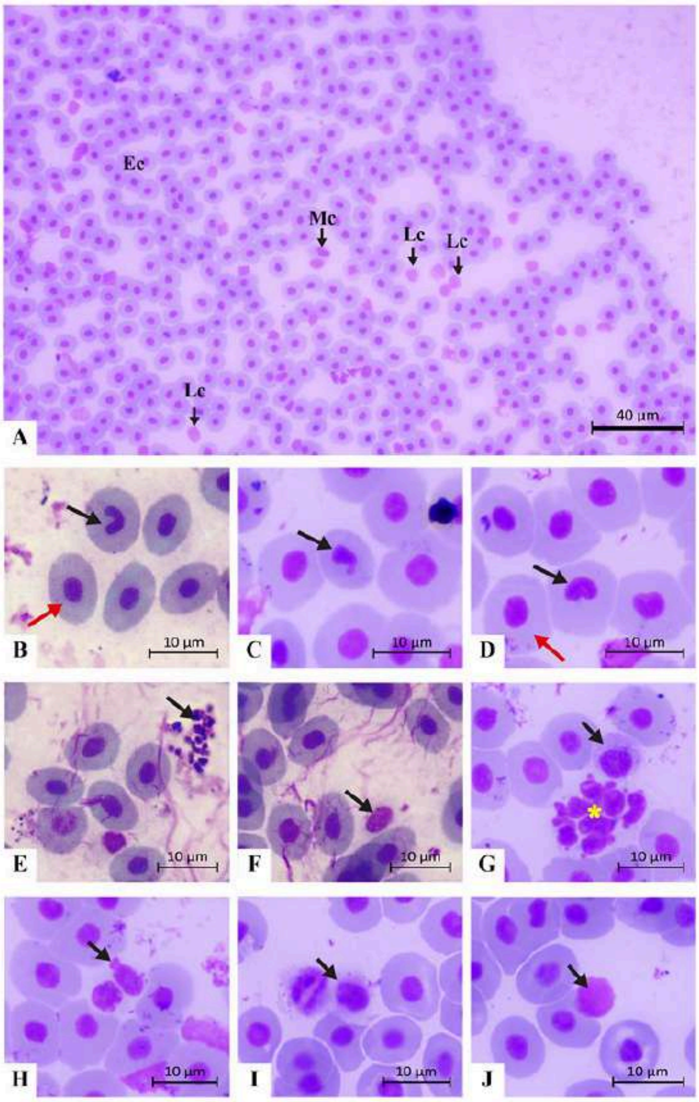

Figure 4. Blood cells of adult *Siganus javus* from Koh Sichang. A: An overview of a blood smear slide containing erythrocytes (Ec), monocytes (Mc) and lymphocytes (Lc). B: An erythrocyte containing a notched nucleus (arrow) with normal erythrocytes (red arrow indicates one of them). C: An erythrocyte containing a blebbed nucleus (arrow). D: An erythrocyte containing a large, notched nucleus (arrow) with normal erythrocytes (red arrow indicates one of them). E: Potentially apoptotic cells (arrow). F: A thrombocyte. G: A monocyte (arrow) located close to a large apoptotic cell (yellow asterisk). H: A neutrophil with two lobes of nucleus (arrow). I: A small monocyte (arrow). J: A lymphocyte (arrow).

Table 3. Proportion of blood cell types of adult *Siganus javus* from Koh Sichang during December 2017 and January 2018.

Blood cell types	Unhealthy fish (Percentage proportion, %) N=38 (Mean ± SD)
Notched nuclei of erythrocyte	20.17 (mean = 6.05 ± 2.74 from total erythrocyte [n=30])
Blebbed nuclei of erythrocyte	9.2 (mean = 2.76 ± 0.84 from total erythrocyte [n=30])
Thrombocytes	8.02 (mean = 1.60 ± 0.71 from total blood cell [n=20])
Monocytes	9.60 (mean = 1.92 ± 0.66 from total blood cell [n=20])
Neutrophils	21.57 (mean = 4.31 ± 1.20 from total blood cell [n=20])
Lymphocytes	35.65 (mean = 7.13 ± 2.24 from total blood cell [n=20])

Histological and histopathological examination of gill

The gill showed a comb-like structure consisted of the gill raker, gill arch and gill filaments. Many histopathological alterations were observed. The disorganization of secondary lamella was the most frequent gill lesion found (Figures 5A-5B). The lamellar aneurysm, which is the hypertrophic walls and blood congestion, were also identified from all individuals tested (Figures 5C-5D). The epithelial hyperplasia was also observed (Figure 5B).

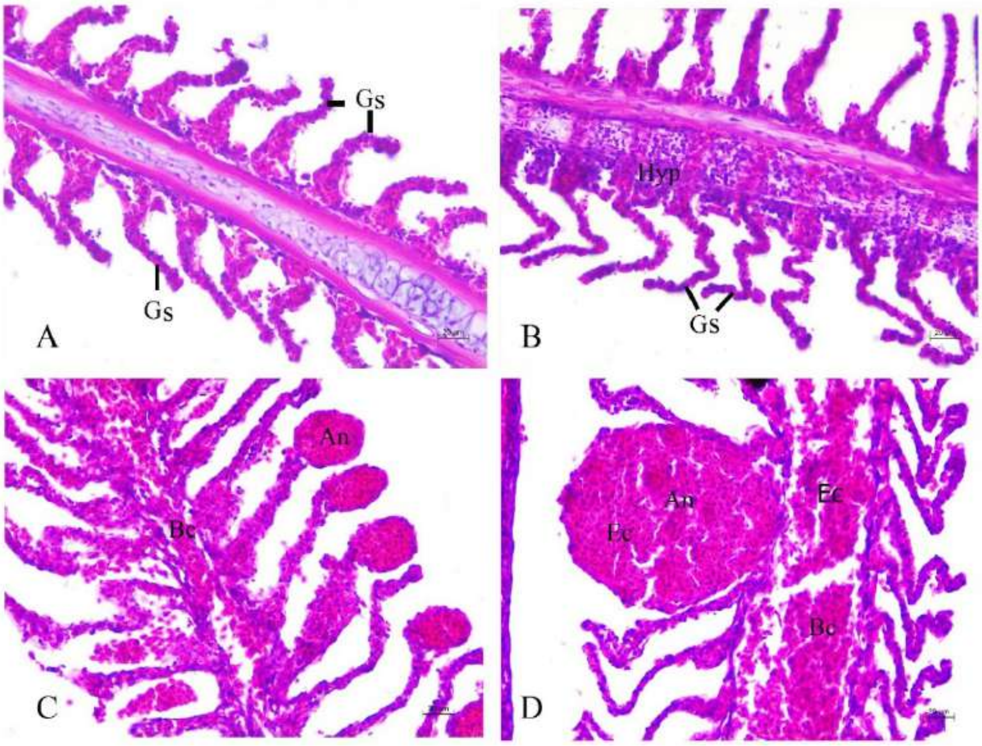

Figure 5. **Histopathology of gill in adult *Siganus javus* from Koh Sichang.** A: Disorganization of secondary lamellae (Gs). B: Disorganization of secondary lamellae (Gs) together with hyperplasia of gill epithelium (Hyp). C: Lamellar aneurysm (An) and blood congestion (Bc). D: High magnification showing lamellar aneurysm (An) with an erythrocyte (Ec).

Histological and histopathological examination of liver

The liver tissue consisted of numerous hepatocytes and hepatic sinusoids (Figure 6A). Degeneration of hepatocytic cytoplasm and the progressive loss of sinusoidal structure (or dilation of venous sinus) were observed (Figure 6B) throughout the liver with blood congestion (Figure 6C). The vacuolar degeneration (or vacuolated cytoplasm) associated with the foamy appearance was observed (hepatocellular lipidosis, Figure 6B). Clusters of melanomacrophage centers (MMCs) containing yellowish-brown pigments were found in the liver (Figures 6C-6D).

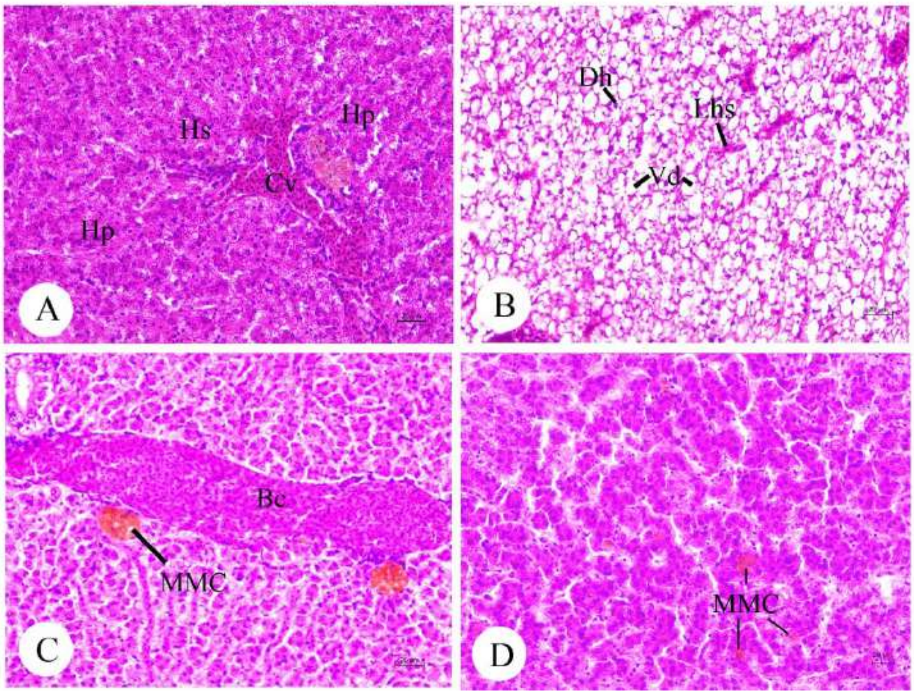

Figure 6. Histology and histopathology of liver (A-D) in adult *Siganus javus* from Koh Sichang. A: A lobular liver was composed of hepatocytes (Hp), hepatic sinusoid (Hs) and the central vein (Cv). B: High magnification showing the hepatic degeneration (Hd), loss of hepatic sinusoids (Lhs) and vacuolar degeneration (Vd). C-D: Blood congestion (Bc) and the clusters of melanomacrophage centers (MMCs) among the hepatocytes.

Histological and histopathological examination of kidney

The kidney parenchyma of *S. javus* was composed of the renal corpuscle (Bowman´s capsule and glomerulus) and renal tubule in the large area of hematopoietic tissue (Figure 7A). Histopathological alterations including the renal tubular degeneration and necrotic tissue were seen (Figures 7B-7C). The cluster of MMCs was observed in kidneys (Figure 7D). In addition to the histopathological alterations, unidentified parasites and granuloma were found in kidneys (Figures 7E-7F).

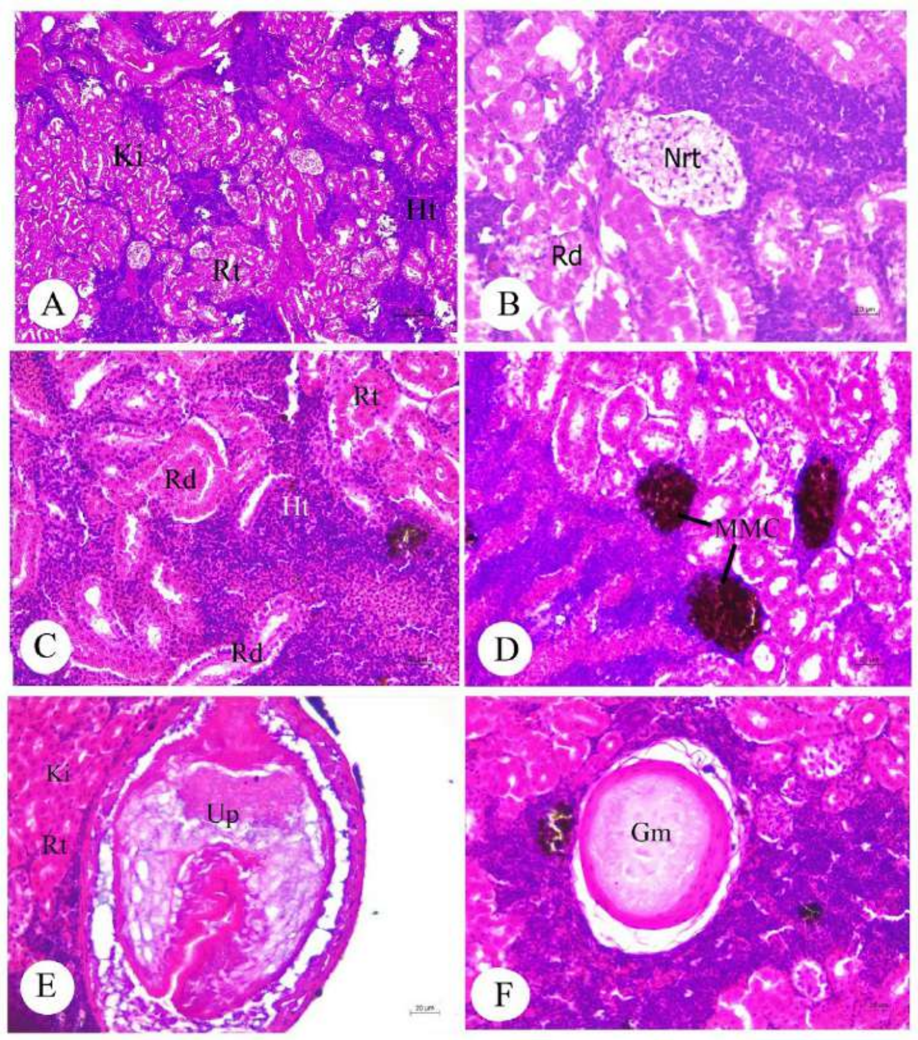

Figure 7. Histology and histopathology of kidney (A-F) in adult *Siganus javus* from Koh Sichang. A: The kidney parenchyma (Ki) contained the renal tubule (Rt) and hematopoietic tissue (Ht). B-C: A large parenchyma of renal degeneration (Rd) and necrotic tissue (Nrt). D: Large clusters of melanomacrophage center (MMCs). E: Unidentified parasite (Up) found close to the renal tubule (Rt) of kidney (Ki). F: Granuloma (Gm). Abbreviations: Ht = hematopoietic tissue, Rt = renal tubule

Semi-quantitative analysis on histopathological alterations

Finally, we calculated the HAI and AVA for the accurate ecotoxicological assessments. Although many histopathological changes were observed, based on the HAI, the gill was classified as a normal organ/tissue functioning (9.42, Figure 8A). The AVA showed that the most individuals had no pathological alteration in the gill (76.31 %, n = 29), and 23.68 % of individuals (n = 9) had slight or mild pathological alterations (Figure 8B). The liver HAI was also classified this organ as a normal organ/tissue functioning (10.15, Figure 8A), and the liver AVA was found to be no pathological alteration (56.63%, n = 20) in many individuals. Individuals of slight or mild pathological alterations (42.10%) and severe and extensive pathological alterations (5.26%) were also found (Figure 8B). However, the kidney HAI was classified as a severe alteration (54.94, Figure 8A). The major kidney AVA was the slight or mild pathological alteration (55.26%, n = 21), 21.05% was classified as severe and extensive pathological alterations (Figure 8B).

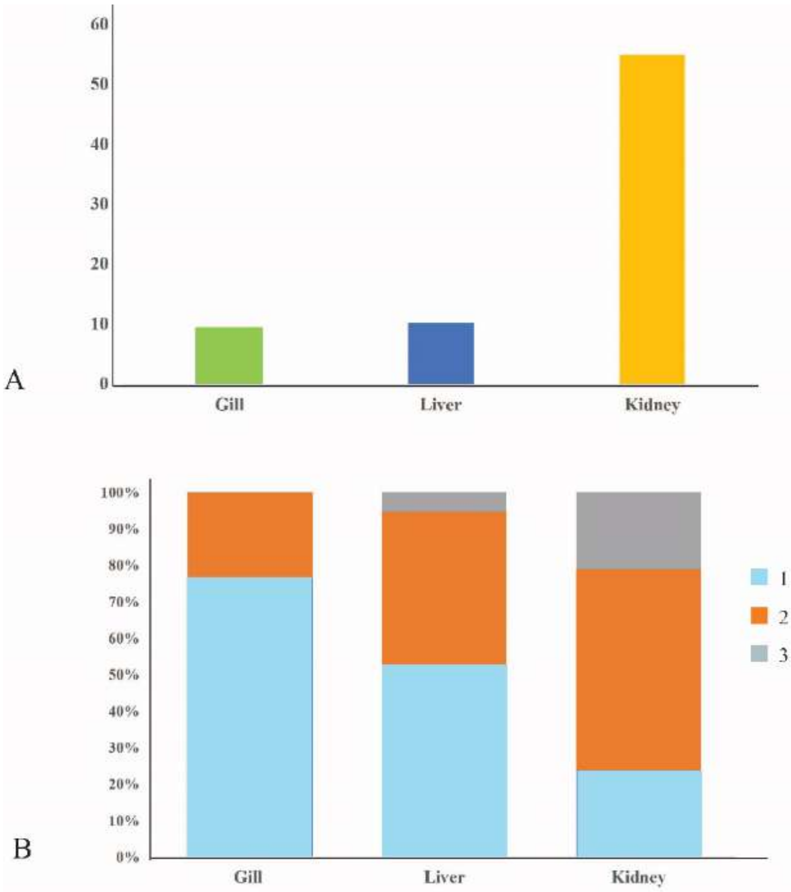

Figure 8. The mean of histological alteration index (HAI) [A] and relative frequency of average value of alteration (AVA) [B] of the selected organs (gill, liver and kidney) of *Siganus javus* from Koh Sichang, Thailand.

Note: 1 = no pathological alteration of organs, 2 = slight or mild pathological alterations of organs), and 3 = severe and extensive pathological alterations of organs)

DISCUSSION

Our observation showed pronounced visceral adipose tissue accumulation in some *S. javus* individuals. This phenotype is observed under a relatively favorable nutritional condition (Hillestad et al., 1998; Xiong et al., 2018). Because the Koh Sichang area receive water from urban area, this area may be rich in nutrients. Since nutritional status also causes hematological and histopathological alterations, it is important to investigate the nutritional status of *S. javus* in the Koh Sichang area. Proximate analysis will be a crucial next step in further studies.

Hematological parameters have become a promising biomarker for environmental stressors (Borges et al., 2007; Sudova et al., 2009; Li et al., 2011), although these parameters are also influenced by nutrition and disease (Adams et al., 1996). The features of *S. javus* erythrocytes were found to resemble those previously reported in other teleosts (Kousar and Javed, 2015; Okomoda et al., 2018; Singkhanan et al., 2019). However, the erythrocytic nuclear abnormalities in both notched and blebbed shapes were recorded in this study. Such nuclear abnormalities are known to be increased by copper and cadmium toxicity (Jiraungkoorskul et al., 2007; Summak et al., 2010; Guner et al. 2011; Kousar and Javed, 2015) and genotoxic damage (Kousar and Javed, 2015; Ali et al., 2008; Summak et al., 2010), which might reduce the respirational functions. The detailed monitoring of water quality will be required for the Koh Sichang area.

In this study, we found thrombocytes and leucocytes from the blood smear slide. Thrombocytes play an important role in clotting (Stosik et al., 2019; Singkhanan et al., 2019), inflammatory exudates and phagocytic activity (Tavares-Dias et al., 1999), whereas leucocytes are involved in the innate and acquired immune defense (Ballarin et al., 2004). The leucocytes found in *S. javus* contain monocytes, neutrophils and lymphocytes. The characteristic of monocytes is similar to those in a series of previous studies on *C. chanos, C. subviridis, D. pusilla* and in *C. subviridis* (Singkhanan et al., 2019), but the proportion of monocytes was relatively low in this study (9.60%). Because the monocyte is a marker of phagocytosis (Secombes and Fletcher, 1992), antigen presentation (Vallejo et al, 1992) and the production of cytokines (Secombes, 1991; Secombes and Fletcher, 1992), the low occurrence of monocyte of *S. javus* may indicate the normal inflammation condition. Neutrophils of *S. javus* were visually similar to those in other fish (Rowley et al., 1988; Ikeda, 1986; Ellis, 1977). It is well known that neutrophils are the first line of innate immune against infectious diseases and play a major role in the resolution of antimicrobial molecules (Ellis, 1977; Ranzani-Paiva et al., 2004). Neutrophils are also involved the T-cell immune response to protect the host against the attack of various pathogens (Ranzani-Paiva et al., 2004; Garcia et al., 2007). The small agranular lymphocytes showed the highest proportion in of *S. javus* as reported in other species (Modra et al., 1998). This blood cell plays an important role in the immune response and the biological defense system (Galagarza et al., 2017; Singh and Tandon, 2009) by producing antibodies and chemical substances that serve as a defense against the pathogen and parasite infections (Jalali et al. 2009; Musa et al., 2013). However, little data has been available for lymphocytes used for a monitoring purpose. An increase in lymphocyte numbers was found in fish collected from polluted river (Singh et al., 2009) and in fish after injected with *Corynebacterium* sp., a gram-positive bacterial species (Silveira-Coffigny et al., 2004).

Histopathologically, the disorganization of gill was the most frequently observed lesion in *S. javus*. It is suggested that such lesions are the consequence of a variety of water pollution problems (Cantanhêde et al., 2014; Paruruckumani et al., 2015). The appearance of lamellar aneurysm was also identified from all individuals. These lesions have been related to the exposure to a high concentration of heavy metals (Fonseca et al., 2017), for example copper in *Solea senegalensis* Kaup, 1858 (Arellano et al., 1999) and *Poronotus triacanthus* (Peck, 1804) (Jiraungkoorskul et al., 2007), and particularly impair the cellular defense mechanisms and physiological response against biotic and abiotic stresses (Arellano et al., 1999). In addition, the epithelial hyperplasia was also noted. Previous observations suggest that the increased lamellar epithelium is a defense against water-borne pollutants that allows more distance from the environment across the membrane.

Histopathological changes observed in the liver of *S. javus* are also a common abnormality in fish under nutritional and environmental stresses (Greenfield et al., 2008; Senarat et al., 2018; Senarat et al., 2019). It is suggested that the underlying mechanism for the hepatic lipidosis is the disruption of carbohydrate, lipid and protein metabolism (Hinton and Laure´n, 1990). Previous observations showed that hepatocellular lipidosis can be induced by several pollutants, typically chlorinated hydrocarbon and anthropogenic contaminations (Hendricks et al., 1984; Hinton et al., 1992; Robertson and Bradley 1992; Schrank et al., 1997), including PCBs (Teh et al., 1997; Anderson et al., 2003) and TiO_2 nanoparticle (Diniz et al., 2013). Additionally, age, overnutrition and nutritional imbalance likely cause the hepatocellular lipidosis in fish (Hinton et al., 1992; Robertson and Bradley, 1992; Yilmaz and Akyurt, 2005; Yilmaz and Genc, 2006; Senarat et al., 2015; Ruiz-Ramírez et al., 2019). Thus, the above results indicate the presence of hepatic abnormalities associated with environmental or nutritional stress in *S. javus* from Koh Sichang.

Compared to the gill and liver, less information is available for histopathological alterations in kidney, although lesions of renal tubular degeneration and necrosis of *S. javus* have been associated with heavy metals, particular mercury and cadmium (Robert, 2000). An important function of the renal epithelium is to excrete metabolic wastes and the divalent ions (Genten et al., 2008). It is possible that the kidney function of *S. javus* in this study is impaired.

The occurrence of MMCs in *S. javus* were documented, which is well known to play crucial roles in responses against pathogen invasion (Agius and Roberts, 2003; Louiz et al., 2018). Previous observations suggested that the MMCs are more frequently observed in unhealthy fish, possibly in relation to parasitic infestation (Roganovic-Zafirova and Jordanova, 1998). The prevalence and intensity of MMCs might be a biomarker for stress, environmental degradation and pollution (Couillard and Hodson, 1996).

Although we found several histopathological changes in gill, liver and kidney, the semi-quantitative analysis indicated that *S. javus* from Koh Sichang is under normal conditions in terms of gill and liver HAI and AVA. However, the kidney data indicate that the specimens have severe alterations in consistent with our observation of severe and frequent pathological alterations compared to other organs. These results suggest that the kidney is the most sensitive organ to the environmental stressors, at least in *S. javus*. To further investigate this possibility, laboratory exposure experiments with cell area measurements should be useful (Campos-Garcia, 2016; Mansouri et al., 2017; Pirsaheb et al., 2019; Sayadi et al., 2020). It will also be useful to compare hematological and histopathological changes between sites of different pollution levels, which we could not do in this study because of the no major environmental difference in the two sampling sites.

CONCLUSION

The health status of java rabbitfish *Siganus javus* from Koh Sichang, Thailand has been diagnosed using a multi-biomarker approach. Abnormal erythrocytes and the presence of some leucocytes, along with the histopathological data, indicate that the fish live challenging natural environment. Investigations on the pollutants in fish and environment are being considered for future research.

AUTHOR CONTRIBUTIONS

Anek Sopon, Jes kettratad, Ajcharaporn Piumsomboon, Gen Kaneko and Sinlapachai Senarat assisted in conducting the experiments, performed the statistical analysis and data visualization and wrote the manuscript. Anek Sopon, Jes kettratad and Sinlapachai Senarat designed and conducted all of the experiments and wrote the manuscript. All authors have read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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