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Original Article

Health status in wild and captive short mackerel, *Rastrelliger brachysoma* from Thailand: Histopathology

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Abstract

Information regarding the health status of wild and captive *Rastrelliger brachysoma* (Bleeker, 1851) is scanty. This is an economically/ecologically important fish species in Thailand which has experienced recent dramatic declines in population numbers. Therefore, the aim of this research was to assess the health status of this fish using histopathology as biomarker. The results revealed previously the gill lesions including lamellae disorganization, fusion and edema with lifting of lamellar epithelium in the secondary lamellae. The skin histopathology included epidermal hyperplasia and the proliferation of the Malpighian cells. Pyknotic nuclei and vacuolar degeneration of exocrine acinar cells were detected in the pancreas tissue. Degeneration of the renal parenchyma and tubules as well as the melanomacrophage center (MMC) was observed in kidney tissue. In captive fish, the most common histopathology of gill tissue, primary lamellae disorganization and degeneration of secondary gills, was observed in all samples. In some areas of the kidney tissues, renal degeneration and damage to the glomeruli throughout the majority of the macrophage aggregates (MMC) were also found. Important histopathological observations of liver tissue were hepatic degeneration, focal coagulation necrosis and increased frequency of MMC. Interestingly, a gastric tumor was observed in only one male specimen. Additionally, it must be emphasized that histopathological alterations are an effective biomarker used to evaluate potential effects of the environmental pollution on fish health and marine biota.

Keywords: fish health, Gulf of Thailand, histopathology, short mackerel

1. Introduction

Short mackerel, *Rastrelliger brachysoma*, an epi-pelagic fish, is considered an important fishery resource of the Gulf of Thailand. In 2009, the population of *R. brachysoma* dramatically decreased as compared with previous years (2005-2008) (Department of Fisheries, 2009). *R. brachysoma* in the Gulf of Thailand is potentially exposed to a large number of pollutants, especially heavy metals (e.g., cadmium, iron, mercury, and lead) and petroleum hydrocarbons in sediment and water, which potentially play a role in the diminishing fish populations (Cheevaporn & Menasveta, 2003; Wattayakorn, 2012).

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Histopathology is a frequent diagnostic and health assessment protocol for study of the cells and tissues of plants and animals. It has been intensively recommended for use in wild fishes under stress conditions and/or in polluted environments (Meyers & Hendricks, 1985; Senarat *et al.*, 2015a). This biomarker has been used to predict the vital cellular alterations in sensitive organs such as gills, kidneys and liver tissues (Adams, 2002; Hinton *et al.*, 2001). Our ongoing investigations have shown that the histopathology of the brain, gonadal and hepatic tissues is correlated/associated to/with reduced health of *R. brachysoma* in the Upper Gulf of Thailand (Senarat *et al.,* 2015b, 2017a, 2017b), but there has not yet been any evidence about histological changes in other selected organs.

R. brachysoma is a potential marine fish species for aquaculture and has been under developmental studies by the Samut Sakhon Fisheries, Sumut Sakhon province, Thailand, since 2011. Several problems, including limited larval quantities and endocrinological dysfunction that prevent completion of the reproductive system, have emerged in rearing this fish under hatchery conditions (Senarat *et al.,* 2018.). Limited, but still not adequate success has been achieved by techniques such as induced breeding and increased reproductive capacity in captivity using various aquacultural techniques such as endocrine manipulative strategies, hormone priming, artificial fertilization and production of recombinant fish gonadotropins. Given the limited results with *R. brachysoma* aquaculture efforts, information about the health status of this fish in captivity is likely to prove beneficial in resolving outstanding problems. For this current study, the health status of wild and captive *R. brachysoma* was evaluated using histopathological biomarker.

2. Materials and Methods

Sexually mature, *Rastrelliger brachysoma* (approx. >14 cm in total length) were collected during the rainy (October to December 2013) and dry (January to February 2014) seasons. Thirty fish samples were captured in each season by using bamboo stake traps in Samut Songkhram Province, the Upper Gulf of Thailand (13°16’18.4”N, 100°02’13.4” E), in close proximity to industrial areas and human activities.

Three *R. brachysoma* broodstock undergoing sexual maturation in standard culture systems were provided from the Samut Songkhram Coastal Fisheries Research and Development Center, Samut Songkhram, Thailand. All fish (approx. 18 cm in total length) during October to December 2013 were kept. Afterwards, they were acclimatized and maintained in mechanically circulated filtered seawater with constant aeration at 26–28 ˚C, a salinity of 30–32 ppt, and a photo-period of 12:12 hrs light: dark. *R. brachysoma* were fed on fresh squid and polychaetes twice daily to maintain excellent health.

All fish samples were quickly euthanized by the rapid cooling method (Wilson *et al.,* 2009). Both external and internal anatomies first were inspected via stereomicroscopy. Gill, skin, pancreas, kidney tissues, and especially testis in captive fish were dissected and fixed in Davidson's fixative and processed using standard histological protocols (Bancroft & Gamble, 2002). The paraffin blocks were cut at 5 µm and stained with Harris’ hematoxylin and eosin (H&E) (Bancroft & Gamble, 2002). For histochemical investigations to identify the chemical composition and special cells sections were stained with specific stains, including Masson's Trichrome (MT), periodic acid Schiff (PAS), aniline blue (AB), gram positive (GP), and reticulin (RT) (Bancroft and Gamble, 2002). Each histopathological alteration in the selected organs was calculated for the two seasons in wild fish; prevalence alterations were recorded as percent prevalence.

3. Results and Discussion

3.1 Histology and histopathology of wild *R. brachysoma*

Light micrographs of anatomical features and histopathology of *R. brachysoma* organs from the upper Gulf of Thailand are shown in Figures 1–4. Histological alterations and prevalence are provided in Table 1.

3.1.1 Histology and histopathology of gills

The gill anatomy of *R. brachysoma* consisted of four parts including the gill arch, gill racker, filament and lamellae (Figure 1A). The gill arch was supported by a cartilaginous tissue coupled with skeleton muscles; each gill arch was typically composed of primary lamellae and made up of double rows, called secondary lamellae. The primary lamellae were lined by stratified epithelium and other cells including mucous and pillar cells (Figures 1B–D). Among these cells, the mucous cells were positively stained with PAS, indicating glycoprotein (Figure 1D). The prevalence of gill abnormalities, especially lamellae disorganization, was found at 70–90% prevalence (Figure 1E, Table 1); dilation and congestion of blood vessels in the interlamellar spaces were found with approximately 10–30% prevalence. Hyperplasia of epithelial cells of the primary lamellae were found (Figure 1G), as similarly occurred in *Perca fluviatilis* and *Rutilus rutilus* in mining effluents of the subarctic lake system in Russia (Tkatcheya & Hyma, 2004). Moreover, the noticeable architectural loss was observed in secondary lamellae, where there was fusion with the edema with the lifting of lamellar epithelium (Figures 1E–1I). These lesions (Figure 1G) were similar to the field reports in *Scardinius erythrophthalmus* and *P. flavescens* in Dame Lake, Bulgaria, and associated with toxic heavy metals (Velcheva *et al.*, 2010). Also, these types of lesions (Figure 1G) were previously associated with exposure to copper in *Solea senegalensis* (Arellano *et al.*, 1999), *Oreochromis niloticus* (Figueiredo-Fernandes *et al.*, 2006) and *Poronotus triacanthus* (Jiraungkoorskul *et al.*, 2007). Interestingly, edema with lifting of the lamella epithelium (Figure 1G-1I) is suggested as being a defense mechanism of cells to stress conditions; separation of epithelial lamellae increases the distance across the membrane/cell/tissue, creating a greater distance from water-borne pollutants (Arellano *et al.*, 1999). Overall, histopathology in gill tissues of *R. brachysoma*, is indicative of respiratory problems as well as disruption of the functional role of countercurrent exchange between blood and water.

3.1.2 Histopathology of skin

An ectoparasite infection of the skin was observed in the present study; this is probably a parasitic isopod sp. That was about 2–3 cm in total length (Figure 2A). The prevalence of infestation with parasitic diseases in *R. brachysoma* was greater/higher in the rainy season (50% prevalence) than in the

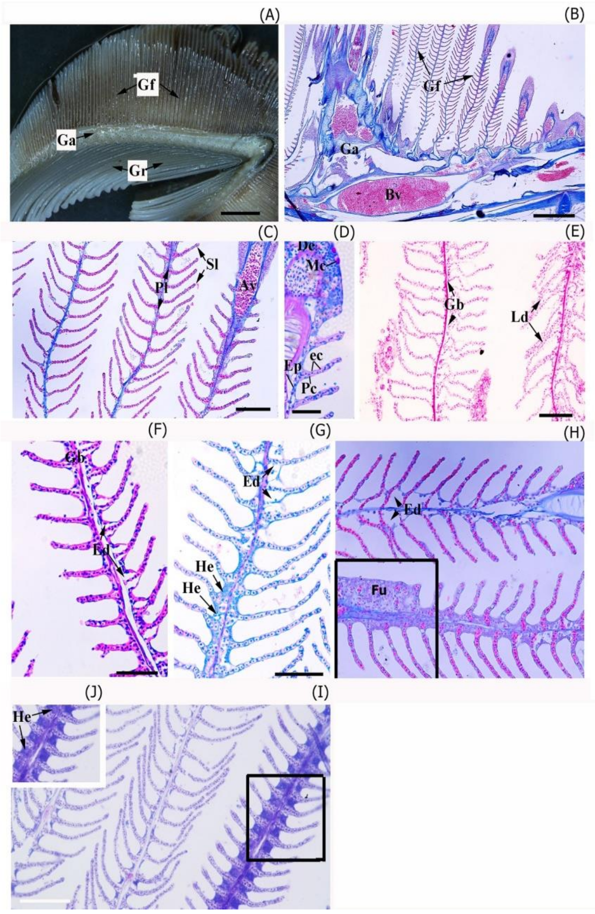

Figure 1. Anatomy (A), light photomicrograph of histology (B-D) and histopathology (E-I) in gill tissues of *Rastrelliger brachysoma*. Av = arterial vessel, Bv = blood vessel, ec = erythrocytes, Ed = edema, Ep = epithelium of primary lamellae, De = distal end of primary lamellae, Fu = fusion of secondary lamellae, Ga = gill arch, Gb = gill abnormality, Gf = gill fillament, Gr = gill raker, He = epithelial hyperplasia of primary lameallae, Ld = lamellar disorganization, Mc = mucous cells, Pc = pillar cell, Pl = primary lamellae, Sl = secondary lamellae. Scale bar A = 3 mm, B = 1 mm, C, E-G, H, J = 50 µm, D = 20 µm. E, F = Harris's hematoxylin and eosin (H&E); B, C, H, I = Masson's Trichrome (MT); J, K = gram positive (GP); D, G = Periodic Acid Schiff (PAS).

dry season (10% prevalence) (Figure 2B, Table 1). The infected area showed ulceration with a white halo or epidermal plaques; the area also showed cuticle disorganization and epidermal hyperplasia together with the proliferation of Malpighian cells accompanied by spongiosis. Several lymphocytes were also infiltrated in both, the hypodermis and dermis, a condition known as chronic dermatitis (Figures 2C–2F). Regarding the accumulation of some white blood cells, it is related to the immune response of the tegument system (Pronina *et al.*, 2014). Another characterization, proliferation of mucous-secreting cells were slightly stained with PAS, demonstrating the presence of low levels of glycoproteins (Figure 2G). The increased number of mucous-secreting cells may be a reasonable protection against infection (Genten *et al.*, 2008; Robert, 2000).

Table 1. Prevalence alteration (%) of histopathological observations in *Rastrelliger brachysoma* caught from the Upper Gulf of Thailand.

Tissues	Lesions	Alteration prevalence (%)	
		Rainy season	Dry season
Gills (n = 10)	Lamellae disorganization	90	70
	Dilation and congestion of the blood vessels in the interlamellar spaces	30	10
	Hyperplasia of the epithelial cells of primary lamellae	20	10
Skin (n = 20)	Isopod sp.	50	10
	Ulceration with white halo or epidermal plaques	10	0
Pancreas (n = 10)	Pyknotic nuclei of acinar cell	100	60
	Vacuolar degeneration of acinar cells	40	10
	Melanomacrophage accumulation	50	30
	White blood cell infiltration	100	70
Kidneys (n = 20)	Renal tubule degeneration	100	95
	Glomerular degeneration	100	95
	MMC	100	95
	Pyknotic nuclei of renal tubule	100	85
	Karyolysis of renal tubule	100	80
	Lipid accumulation	25	15
	Hyaline degeneration	10	0

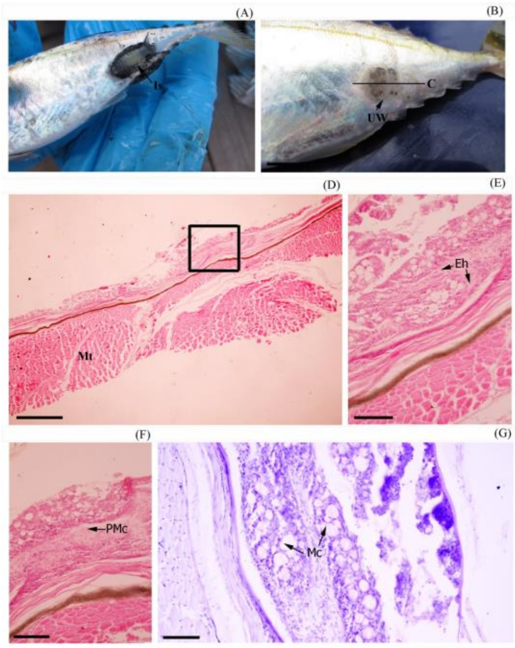

Figure 2. Anatomy (A-B) and light photomicrograph of histopathology (C-G) in skin tissue of *Rastrelliger brachysoma*. C = cross section in figure C, Eh = epidermal hyperplasia, IS = isopod sp., Mc = mucous cells, Mt = muscular tissue, PMc = proliferation of malphigian cells, UW = ulceration with white halo. Scale bar C = 200 µm, D-E = 50 µm, F = 20 µm. C, E = Harris's hematoxylin and eosin (H&E); D = Periodic Acid Schiff (PAS).

3.1.3 Histology and histopathology of the pancreas

The exocrine pancreas of *R. brachysoma* is composed of clusters of pyramidal acinar cells that are arranged around blood vessels. Each acinar cell contained an oval nucleus surrounded by a dark basophilic cytoplasm (Figure 3A). The cytoplasm also contained several eosinophilic zymogen granules, which were positively stained with MT and PAS staining methods (Figures 3B, 3D), but were negatively stained with AB reaction (Figure 3C). Together, these stains confirmed that the acinar cells in *R. brachysoma* produced glycoproteins. Additionally, the islets of Langerhans, an endocrine component, were seen among the acinar structures, as has been similarly observed in several fish (Genten *et al.*, 2008). Pancreas histopathological changes included exocrine acinar cell replacement by pancreatic adipose cells (Figures 3E–H). According to Robert (2000), this lesion may be associated with acinar atrophy before cells are replaced by fat tissues. Accumulations of melanization and white blood cell infiltrations were also seen in the adipose tissue (Figure 3G). Furthermore, acinar cells showed pyknotic nuclei and vacuolar degeneration, which was more pronounced in the rainy season than in the dry season (Table 1). This lesion may disrupt the mechanism that produces digestive enzymes (Buddington & Kuzmina, 2000). Also, damage of the islet of Langerhans was observed, but only rarely (Figure3H); decreased levels of these cells, as observed in this study, likely results in reduced levels of, several hormones, including insulin, glucagon and somatostatin.

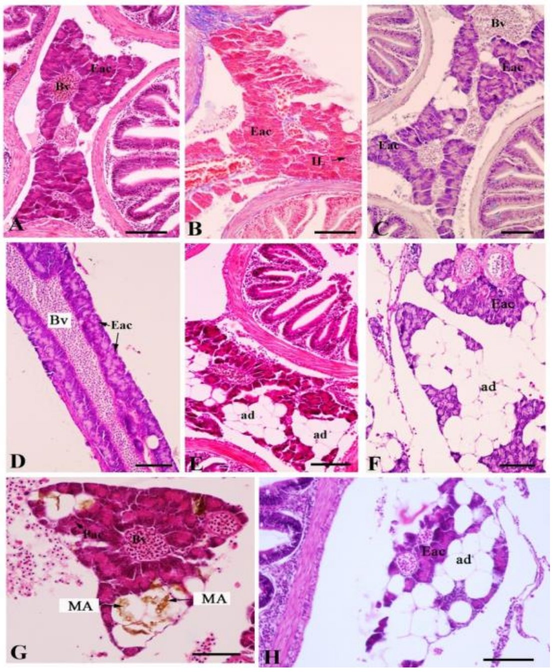

Figure 3. Light photomicrographs of histology (A-D) and histopathology (E-H) in pancreas tissues of *Rastrelliger brachysoma*. ad = adipose tissue, Bv = blood vessel, Eac = exocrine acinar cell, Exd = exocrine duct, IL = islet of Langerhans, MMC =melanomacrophage center, MA = melanization, Pac = pynotic nuclei of acinar cells. Scale bar A-H = 20 µm. A, E, G, H = Harris's hematoxylin and eosin (H&E); B = Masson's Trichrome (MT); D, F =Periodic Acid Schiff (PAS); C = aniline blue (AB).

3.1.4 Histology and histopathology of kidneys

The kidneys of *R. brachysoma*, based upon the hematopoietic and renal tissues were clearly composed of two parts: anterior and posterior regions (Figures 4A–4B). The anterior part mostly contained a complex of hematopoietic and chromaffin tissues (Figure 4A). The posterior part consisted of the renal corpuscle (glomerulus and Bowman's capsule), and the first and secondary proximal convoluted tubule segments (Figure 4E). According to PAS reaction, the first segment of the proximal tubules was a high simple cuboidal epithelium, which was covered by the well-brush border. The secondary proximal tubules were low cuboidal epithelia with basally round nuclei and eosinophilic cytoplasm. Less apical brush border was found in this segment (Figure 4F). Kidney histopathology was found during the rainy season more than in the dry season, for example, glomerular and renal tubule degenerations, and lipid accumulation (Figures 4G–I). These lesions have been reported in many fish species living in environments with natural petroleum contamination (Pacheco & Santos, 2002). Focal melanomacrophage centers (MMC) were observed during rainy (100% prevalence) and dry (95% prevalence) seasons in the kidney tissues (Figure 4H, Table 1).

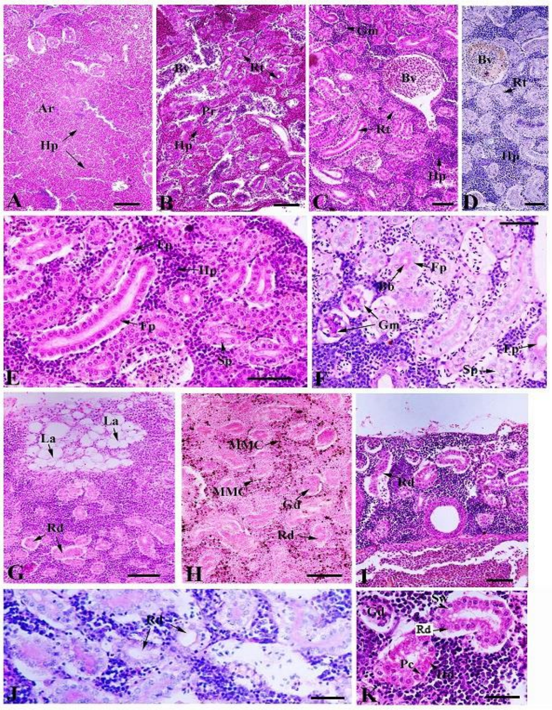

Figure 4. Light photomicrographs of histology (A-E) and histopathology (G-K) in kidney tissues of *Rastrelliger brachysoma*. Ar = anterior kidney, Bb = brush border, Bv = blood vessel, Fp = first segment of proximal tubule, Gd = glomeruli degeneration, Gm = glomeruli, Hd = hyaline degeneration, Hp = hematopoietic tissue, Kl = karyolysis of renal epithelium, La = lipid accumulation, MMC = melanomacrophage center, Pc = pycnotic nuclei of renal epithelium, Pr = posterior kidney, Rd = renal degeneration, Rt = renal tubule, Sp = secondary segment of proximal tubule, Sw = cloudy swelling. Scale bar A-D, G-I = 50 µm, E-F, J-K = 20 µm. A, B = Reticulin (RT); C, E, G, H, I, K Harris's hematoxylin and eosin (H&E); F, J = Periodic Acid Schiff (PAS); D = aniline blue (AB).

This lesion is involved with host responses to foreign materials (Coillard & Hodson, 1996). The prevalence and intensity of the MMC are also a potentially useful biomarker of environmental degradation and pollution (Coillard & Hodson, 1996). The presence of other histopathological alterations was rarely found including the impairment of renal tubules with pyknotic nuclei, karyolysis and glomerular degeneration. Moreover, each epithelium of the renal tubules exhibited cloudy swelling and hyaline droplets which represented protein in the cytoplasm (Figure 4K). Because the important function of the renal epithelium is the excretion of divalent ions (Genten *et al.*, 2008), the occurrence of renal histopathology was likely to reduce this function. Previous reports also highlighted that heavy metals, particular mercury and cadmium, were likely associated with effects on the renal epithelium (Robert, 2000). This result has been observed repeatedly by many researchers who studied fish subjected to pollutant contamination such as pesticides and herbicides (Aysel *et al.*, 2008).

3.2 Histology and histopathology of captive *R. brachysoma*

3.2.1 Histopathology of gill tissue

Histopathology features such as irregular thickness of primary lamellae and disorganization throughout degeneration of secondary gills were observed in both sexes of *R. brachysoma* (Figures 5A–C). Histopathology of the secondary gills included edema and fusion in a majority of the fish examined.

3.2.2 Histopathology of kidneys

Anatomically, the kidney tissue was a pair of organs located in the upper abdominal zone. Histopathologically, some areas of the kidney tissue showed renal degeneration and damaged structural integrity of the glomeruli with collapse and distortion in all fish (100% prevalence, n=3). Highly pigmented phagocytic cells, namely the macrophage aggregate, was observed in all kidney tissues (100% prevalence, n=3). Unusual epithelial cells, with densely eosinophillic cytoplasm and exfoliated with small dilated nuclei, pynotic nuclei and small vacuolar of the renal tubules were observed. Additionally, an infectious agent was identified according to the H&E method and putatively described as *Myxospore* spp. (Figures 5D–G).

3.2.3 Histopathology of liver tissues

Important histolopathological observations of the liver tissues included hepatic degeneration and focal areas of coagulation necrosis (100% prevalence, n=3). Hepatic tubular vacuolation, nuclear pyknosis and karyolysis of the hepatocytes were also intensively found**.** Numerous lesions in the liver tissue of this fish were also detected including blood congestion and small infiltration of the melanomacrophage center (MMC) (100% prevalence, n=3). Additionally, the granuloma and parasitic cysts were revealed under the H&E and RT methods (Figures 6A–E).

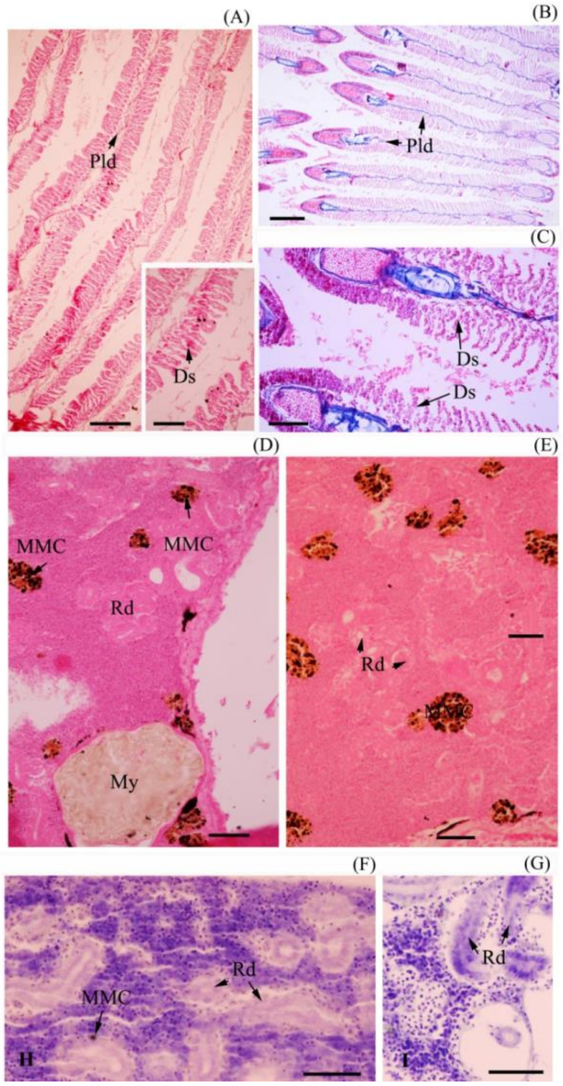

Figure 5. Light photomicrograph of histopathology in gill (A-C) and kidney (D-G) tissues of *Rastrelliger brachysoma*. Ds = degeneration of secondary gills, Dg = damaged structure of the glomeruli with collapse and distortion, Eoc = eosinophillic cytoplasm of renal epitheliums, MMC = macrophage aggregate, My = Myxospore spp, Pld = primary lamellae disorganization, Rd = renal degeneration. Scale bar A, C = 200 µm, B, E, F = 50 µm, G, H, J = 20 µm.

3.2.4 Histopathology of the digestive tract

A normal gross anatomy of the digestive tract was observed in captive fish. Details of histopathology of the digestive canal are given. Briefly, the stomach of one male specimen revealed a tumor that, based on histological observations, as a possible fibromas (33.3% prevalence, n=1 from three samples) (Figures 6F-G). The incidence of a gastric tumor in one male *R. brachysoma* is considered as a new report in Thailand. However, the occurrence of this lesion from captive fish is difficult to explain in simple terms. For instance,

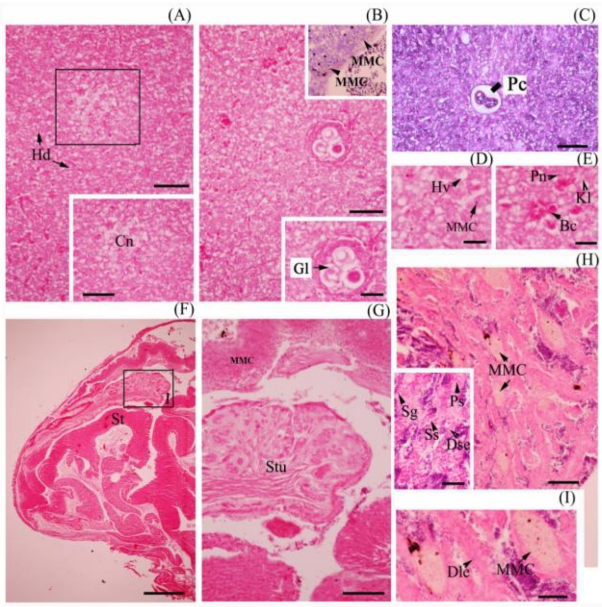

Figure 6. Light photomicrograph of histopathology in liver (A-E), stomach (F-G) and testis (H-I) of *Rastrelliger brachysoma*. Bc = Blood congestion, Cn = coagulation necrosis, Dle = degeneration of Leydig cells, Dse = degeneration of Sertoli cells, Eop = eosinophils, Gl = granuloma, Hd = hepatic degeneration, Hv = Hepatic tubular vacuolation, Kl = karyolysis, MMC = melanomacrophage center, Pc = parasitic cyst, Pn = nuclear pyknosis, Eosinophilic cytoplasm of the spermatogonia (Sg), primary spermatocytes (Ps), secondary spermatocytes (Ss), St = stomach, Stu = stomach or gastric tumors. Scale bar H = 200 µm, J2 = 100 µm, A, C, E, I, L, M, N = 50 µm, D_1, F, G, K = 10 µm.

the lesion may be due to important factors including genetic predisposition and/or viral infection (Marlins *et al.*, 1987), as with humans and other animals. Because of interest in this lesion, its etiology would require further study.

3.2.5 Histopathology of the testicular tissues

The testicular histopathology showed the eosinophilic cytoplasm of the spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Also, the degeneration of Sertoli and Leydig cells in all samples (100% prevalence, n=3) was noted. Moreover, the macrophage aggregate associating with the dilation of blood vessel and congestion were also detected (Figures 6H-I). Most histopathological analysis from the current study revealed that the infiltration of the MMC was mainly found in the kidneys, livers, stomachs and testis. These observations were similar and comparable with reports in kidney teleosts (Agius, 1979, 1980, 1981a) such as *Salmo gairdneri* (Agius, 1981b), *Morone saxatilis* (Harper & Wolf, 2009) and *Sparus aurata* (Mesguer *et al.*, 1994), testicular tissues (Blazer, 2002) and *R. brachysoma* liver (Senarat *et al.*, 2015b). The possible finding of MMC in these organs of wild and captive *R. brachysoma* may occur as a direct result of fish suffering either from living under stressful conditions (e.g., overcrowding, excessive noise and predator aggression), as suggested by others (Agius & Roberts, 2003; Alvarez-Pellitero *et al.*, 2007; Sitja-Bobadilla, 2008; Roberts, 2000), or caused by environment contaminants and poor water quality (Patiño *et al.*, 2003). A combination of biotic, environmental and contaminants causes is also possible. Another reason for the presence of MMC may be related to infected tissues from antibacterial phagocytic capacity (Agius & Roberts, 2003), nocardiosis (Chen, 1992) and/or tuberculosis (Chinabut, 1999). Many former investigators have explained that if the MMC had increased in tissues, it might be associated with infectious diseases through the change of environmental quality or stress response resulting from exposure to toxicants (Marty *et al.*, 2003; van Dyk *et al.*, 2010; Wolke, 1992). Therefore, it is noteworthy that the finding of the myxosporean infections in *R. brachysoma* kidney from the hatchery area may relate to the density of the MMC. However, the test of microbial organisms and identification of parasitic diseases in a cultural system can affect histological alterations in all tissues of *R. brachysoma*, which will be investigated in further work.

Overall, results shown here emphasize the serious health consequences revealed by anatomical and histopathological studies of wild and captive *R. brachysoma* in the Gulf of Thailand. It is difficult to know the etiology of the various histological lesions with great certainty, but it is much clearer that these lesions can be closely linked to declines in natural populations and, with less certainty, to issues associated with reproductive capacity and rearing this species in captivity. Further studies are required to understand the etiology as well as the consequences of these lesions in both wild and captive fish.

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