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Original Article

Liver histopathology of selected estuarine fishes from the Pranburi River estuary of Thailand

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Abstract

Informative reviews on the environmental problems and pollutions in the estuarine are documented, and they found that aquatic organism has been radically affected. In this study, we thus assessed to establish baseline data on the liver histopathology collected from ten estuarine fishes living in Pranburi River estuary (PRE), Thailand, during 2016-2017. The fishes were divided into two distinct groups, including pelagic fishes (*Ambassis vachellii*, *Ambassis nalua*, *Auriglobus nefastus*, *Chelon subviridis*, *Eubleekeria splendens*, *Gerres filamentous*, *Lutjanus russellii*, and *Nuchequula gerreoides*) and demersal fishes (*Butis butis* and *Upeneus tragula*). Livers of all fishes were morphologically observed and then processed by standard histological techniques. This study revealed that the hepatic vacuolar degeneration occurred in all fish species and indicative of hepatocellular lipidosis. However, we noted that this lesion exclusively occurred in demersal fishes. Some similar reports on the small sizes of the melanomagcrophage centers (MMCs) were mainly scattered in the liver tissue of demersal fishes. Interestingly, our study showed that the blood congestion and proteinogenous plate in the central vein (30% prevalence in 2017) were first-detected in *C. subviridis*. All abnormalities seen in these liver samples indicated that all estuarine fishes, especially demersal fishes, might associate with the reduced functionality of liver as well as health status. Consequently, the environmental quality monitoring in PRE of Thailand should be additionally investigated in further studies.

Keywords: estuarine, histopathology, liver, teleost, Thailand

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1. Introduction

Pranburi River estuary (PRE), Thailand is one of the most critical areas and contains recreational fisheries, commercial fisheries, and mangrove plant communities. Furthermore, this area is a significant sink and remains free from pollution and habitat modification (Wattayakorn, 2012). Previous reports regard that there had been a variety of enriched pollutants, especially heavy metals (cadmium, iron, lead, and mercury) while the petroleum hydrocarbon exhibited occasional acute pollution events in both sediment and water (Cheevaporn & Menasveta, 2003; Hungspreugs & Yuangthong, 1983; Wattayakorn, 2012). As far as possible, these pollutants are likely to elicit a potential threat to the health of the aquatic organisms (Dietrich & Krieger, 2009; Senarat *et al.*, 2018). Therefore, an understanding of estuarine fish health becomes critically important to ensure the effective management policy and strategies to implement rules of environments.

A histopathological change is an accurate bio-monitoring and biomarker for predicting the health of a fish population (Meyers & Hendricks, 1985). It is commonly used as an indicator of early warning signs on ecological risk assessment and diseases (Meyers & Hendricks, 1985). This biomarker provides valuable information from the histological alterations of vital organs and tissue under chronic and sub-lethal effects (Adams, 2002; Dietrich & Krieger, 2009; Hinton, Segner & Braunback, 2001). The fishes hepatic changes are highly sensitive tools in impact assessments to indicate the effects of pollution (Hinton *et al.*, 2001; Louiz, Palluel, Ben-Attia, Aït-Aïssa & Hassine, 2018; Senarat, Kettratad, Poolprasert, Jiraungkoorskul & Yenchum, 2015). Similarly, several reports suggested that the liver is particularly susceptible to damages from a variety of toxicants (Louiz *et al.*, 2018) and chemicals xenobiotic exposure (Arellano, Ortiz, Gonzalez de Canales & Sarasquete, 2001; Fanta, Rios, Romao, Vianna & Freiberger, 2003).

To emphasize the monitoring of estuarine ecosystems, we evaluated some changes in the liver histopathology in two fish groups (pelagic and benthic fishes) living in PRE of Thailand as a biomarker. All selected fish species are both economic and ecological important estuarine juvenile stages, which used the estuary as major nurseries and feeding grounds.

2. Material and Methods

2.1 Fish species and study area

A total of 100 individuals of the ten estuarine fish species were collected in February-April during 2016-2017 from PRE, Thailand (12° 24.314’ N, 99° 58.597’ E). There were two distinct groups: first, pelagic fishes (*Ambassis vachellii*, *Ambassis nalua*, *Auriglobus nefastus*, *Chelon subviridis*, *Eubleekeria splendens*, *Gerres filamentous*, *Lutjanus russellii*, and *Nuchequula gerreoides*) and second demersal fishes (*Butis butis* and *Upeneus tragula*). All these fishes have lived near industrial, residential, and aquacultural estuarine areas; all places here are primarily contaminated. This echoed to the chemical analyses on water and sediment reports studies (Cheevaporn & Menasveta, 2003; Hungspreugs & Yuangthong, 1983; Wattayakorn, 2012). Physical and chemical parameters including dissolved oxygen (DO), salinity, pH, and water temperature were recorded during the time of fish sampling using an EC900 AMTAST Waterproof DO Kit 9-in-1 Meter (AMTAST, Lakeland, FL, USA).

Ten fish in each group were collected and then preserved in Davidson's fixative. All fish samples were maintained as voucher specimens at the Fish Biology and Aquatic Health Assessment Laboratory (FBA-LAB), Department of Marine Science, Faculty of Science, Chulalongkorn University, Thailand.

2.2 Observation and histology of livers

Dissected liver samples were removed from all fishes and then they were morphologically documented using a Leica M50 stereomicroscope (Germany). Tissue fragments of the liver were processed by the routine histological techniques (Presnell, Schreibman, & Humason, 1997; Suvarna, Layton, & Bancroft, 2018). Paraffin blocks were cut at 4 µm thickness by a rotary microtome. All sections were histologically stained with a counterstain to Harris’s hematoxylin and eosin (H&E) (to observe the basic structure), periodic acid-Schiff (PAS) (to detect glycoproteins) (Presnell *et al.*, 1997; Suvarna *et al.*, 2018) and Grimelius staining (GS) (to detect the reticular fiber) (Grimelius & Wilander, 1980). Histopathological alterations of liver tissue were viewed using a light microscope (LM) and pictures were taken with a Leica 750 digital camera (Germany). Each lesion was examined under 10x and 40x objective lens light microscope and recorded as a percent prevalence.

Furthermore, the relative amounts of vacuolar hepatocyte degeneration in the liver were visually scored according to Velmurugan, Selvanayagam, Cengiz & Unlu (2009) with minor modifications as follows: − no observation; + weak observation; ++ moderate observation; and +++ strong observation, respectively.

3. Results and Discussion

3.1 Environmental factors

Observations on environment factors were measured to compare between 2016 and 2017 (Table 1). All factors

Table 1. Environmental parameters from the Pranburi River estuary of Thailand between 2016 and 2017

Environmental parameters	Year		Permissible limits and references
	2016	2017	
Dissolved oxygen (DO) (mg/L)	5.29±0.48	4.77±0.23	≥ 3 (Mackenthun, 2004)
Salinity (ppt)	29.36±2.66	19.49±3.48	≤ 1.00 (Mackenthun, 2004)
pH	7.67±0.09	7.88±0.31	7.0 – 8.5 (PCD, 2010)
Water temperature (°C)	26.63±1.37	30.06±1.09	28 – 32 (Duangsawasdi, 1987 and PCD, 2010)

showed that the latter being more than the standard values (Mackenthun, 2004), but the value of salinity was quite-differed between year. Results indicate in PRE that the water quality criteria adequate for fish life were noted.

3.2 Liver histology

The fixed liver morphology of fish was shared with the large organ and anteriorly located in the peritoneal cavity (Figures 1a-b). No abnormality of the cream colored liver was morphologically found in all fish groups. Parenchymal livers of the fishes were histologically shared and they were not easily distinguished to be a lobular architecture (Figure 2a). However, two important compartments, including hepatic sinusoids composing of capillary and hepatic cord were radially arranged (Figure 2a). The hepatocyte in the hepatic cord was a large spherical cell with a centrally situated nucleus. A prominent basophilic of this cell was identified (Figure 2a). The hepatic central was evident, which associated with the sinusoids (Figure 2a). In agreement with previous documents, it showed several teleosts, including *Gnathonemus petersii*, *Pangasius micronemus,* and *Rutilus rutilus* (Genten, Terwinghe & Danguy, 2009) and *Rastrelliger brachysoma* (Senarat *et al.*, 2015, 2018). The accumulation of glycogen was prevailingly found in the hepatocytes, which was the feature that gave a positive reaction to the PAS reaction (Figure 2b).

3.3 Liver histopathology

The histolopathological observation in parenchymatous hepatic tissue between fish groups was diagnosed and presented in both figures (Figure 2 and 3), percent prevalence (Table 2), and semi-quantitative scoring (Table 3). Our observation found that histopathological alteration shared a presentation to a liver degeneration and the substantial vacuolization of hepatocyte as empty spaces with H&E staining (Figures 2c-f), especially demersal fishes (Figures 2g-h, Table 3). These features explained that they were typically chartered of hepatocellular lipidosis. At the same time, a lack of hepatic glycogen storage was shown (PAS reaction; Figure 2g) together with the degeneration of reticular tissue (Figure 2h).

It has been noticeable that the potential pathway of hepatocellular lipidosis involved in the specific alterations in both lipid and protein metabolisms (lipidosis) throughout an abnormality of the triglyceride accumulation in hepatocytes (Hinton & Lauren, 1990). An overview of the potential abnormalities of hepatocellular lipidosis are related to various pollutants after being exposed to chlorinated hydrocarbon contamination and other important pollutants (Hendricks, Meyers, & Shelton, 1984; Hinton *et al.*, 1992; Robertson & Bradley 1992; Schrank, Cormier & Blazer, 1997), including polychlorinated biphenyl (Anderson *et al.*, 2003; Teh, Adams

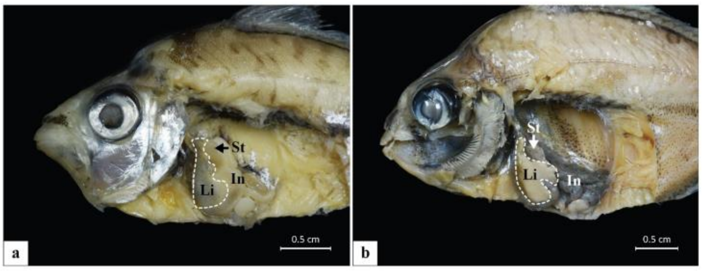

Figure 1. Representative figures of the liver morphological characteristics in *Nuchequula gerreoides* (a) and *Eubleekeria splendens* (b) Abbreviations: Intestine (In), liver (Li) and stomach (St)

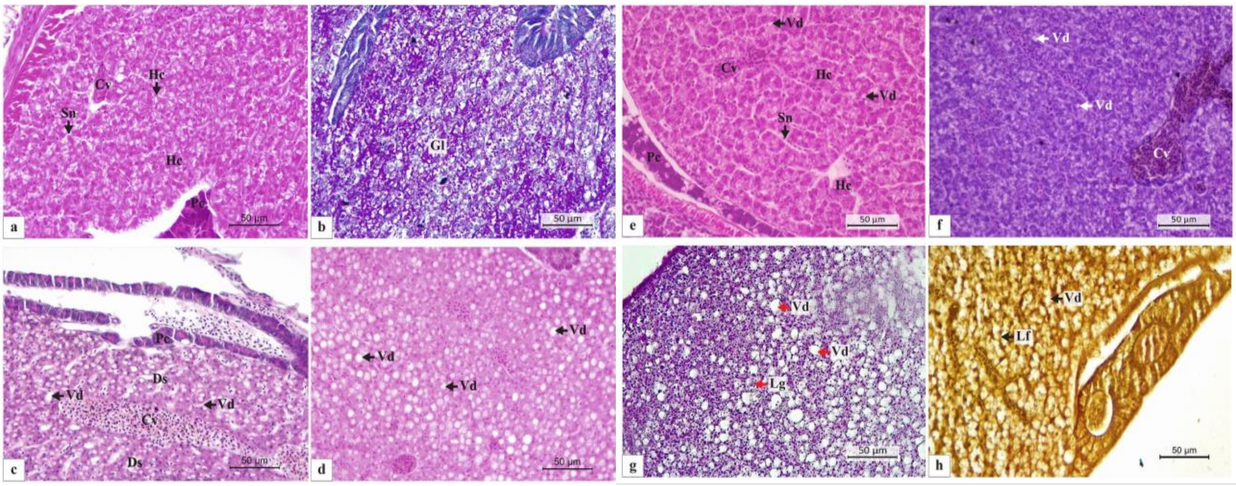

Figure 2. Light photomicrographs of liver histology and histopathology from representative estuarine fishes including *Ambassis vachellii* (a), *Butis butis* (b), *Gerres filamentous* (c), *Ambassis nalua* (d), *Nuchequula gerreoides* (e), *Eubleekeria splendens* (f), *Auriglobus nefastus* (g) and *Upeneus tragula* (h). Abbreviations: central vein (Cv), dilatation in the sinusoids (Ds), glycogen (Gl), hepatocytes (Hc), loss of fiber structure (Lf), loss of glycogen (Lg), pancreas (Pc), sinusoids (Sn) and vascular degeneration (Vd). Staining methods: H&E (a, c, d, e, f, and g), PAS (b) and GS (h)

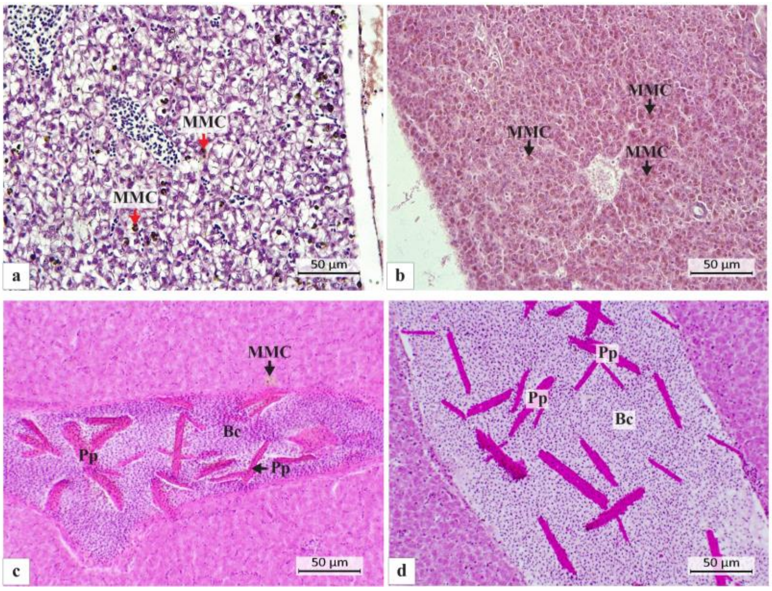

Figure 3. Light photomicrographs of liver histopathology of selected fishes, a-b: Melanomagcrophage centers (MMC) in *Butis butis* (a) and *Upeneus tragula* (b); c-d: Blood congestion (Bc) and proteinogenous plate (Pp) in the liver of *Chelon subviridis.* Staining method: H&E (a-d)

Table 2. Alteration prevalence (%) of dominant histopathological observations in the liver tissue of selected estuarine fishes

Fish types	Fish species	Histopathological alterations				
		Vacuolar degeneration		Melano-macrophage centers		Blood congestion and proteinogenous plate
		2016	2017	2016	2017	2017
Pelagic fish	*Auriglobus nefastus*	80	70	0	0	0
	Lutjanus russellii	50	80	0	0	0
	Ambassis vachellii	50	50	0	0	0
	Ambassis nalua	50	60	0	0	0
	Gerres filamentous	60	60	0	0	0
	Nuchequula gerreoides	60	60	0	0	0
	Eubleekeria splendens	60	60	0	0	0
	Chelon subviridis	50	60	0	10	30
Demeral fish	*Butis butis*	90	90	60	50	0
	Upeneus tragula	90	90	40	60	0

Table 3. Semiquantitative scoring of the vacuolar hepatocyte degeneration of selected estuarine fishes

Fish types	Fish species	Vacuolar hepatocyte degeneration	
		2016	2017
Pelagic fish	*Auriglobus nefastus*	++	++
	Lutjanus russellii	++	++
	Ambassis vachellii	+	++
	Ambassis nalua	+	+
	Gerres filamentous	++	+
	Nuchequula gerreoides	+	+
	Eubleekeria splendens	+	+
	Chelon subviridis	+	+
Demeral fish	*Butis butis*	+++	+++
	Upeneus tragula	+++	+++

Note: − no observation; + weak observation; ++ moderate observation; and +++ strong observation

& Hinton, 1997) and titanium dioxide nanoparticle (Diniz *et al.*, 2013). Moreover, the abnormal nutritional intake and age are associated with the appearance of hepatocellular lipidosis (Genc, Yilmaz & Akyurt, 2005; Hinton *et al.*, 1992; Robertson & Bradley, 1992; Sanad, Gamaal. & Hemmaid, 2015; Yilmaz & Genc, 2006). Similar to the previous report, it showed that nutritional imbalance and inadequate of dietary soy-acid oil mixed with yellow grease could probably induce the formation of hepatic lipidosis induced lesions in *Orechromis niloties* (Genc *et al.*, 2005). The occurrence of lipidosis was a response to nutritional stressors in *Lutjanus guttatus* (Ruiz-Ramírez *et al.*, 2019).

We observed that small sizes of the melanomagcrophage centers (MMCs) were mainly scattered among the liver tissue in the demersal fishes, *Butis butis* (60% prevalence in 2016 and 50% prevalence in 2017) and *U. tragula* (40% prevalence in 2016 and 60% prevalence in 2017) [Figures 3a-3b and Table 2]. However, small clusters of MMCs (10% prevalence in 2017) were only seen in *C.*

subviridis (Figure 3c). This feature was a mononuclear phagocytic cell and generally occurred together with the vacuolar hepatocyte degeneration (Figures 3a-3b). It hence suggests that this phenomenon may be governed as often in maintaining functional homeostatic responses and balance in response to the different adaptive physiology (Barni *et al.*, 2002). It is well known that the occurrence of MMCs plays a pivotal role in the inflammatory immune response (Agius & Roberts, 2003). Consequently, the increasing number and an area of MMCs are related to an increasingly stressful environment and potential marker of fish health (Blazer & Dethloff, 2000). Since the important reports recorded that the PRE has been becoming contaminated with various anthropogenic wastes, especially lead and petroleum hydrocarbon in sediment (Cheevaporn & Menasveta, 2003; Wattayakorn, 2012). The demersal fishes appeared to be more sensitive to sediment pollution. However, we argued that the occurrence of MMCs is mostly associated with a life history (i.e. sex, developmental stage and spawning seasons) and environmental changes (i.e., temperature and UV exposure) (Blazer, Fournie & Weeks-Perkins, 1997; Steinel & Bolnick, 2017). The continuous monitoring of the environmental pollution levels should offer new insights into the empirical evidence for the use of MMCs as the pollutant marker.

An interesting alteration demonstrated that the blood congestion and proteinogenous plate in *C. subviridis* only occurred with 30% prevalence in 2017 (Figures 3c-3d and Table 2). The proteinogenous plate was a fragmented feature with eosinophilic coagulation and only found in the central hepatic vein (Figure 3c). Unfortunately, this lesion of fish has never been reported in literature reviews. It was possible that this pathological lesion was the intramural fibrin deposition-like structure (or the excessive perivillous deposition of fibrinoid material) and found in subendothelial or intramuscular within the wall of large fetal vessels (Redline *et al.*, 2004; Khong *et al.*, 2016). Although the mechanism of this lesion is unknown, it involves the destruction of blood cells (Redline *et al.*, 2004; Khong *et al.*, 2016). The accurate question on the blood biochemistry/profiles should be further investigated.

4. Conclusions

The conclusive data from this present study showed that fish health becomes impaired because all lesions of the liver might be associated to reduce functional capacity and health status. The empirical evidence on the liver of demersal fishes was also underscored that it appears to be relatively affected in terms of the exclusive lesions concerning sediment pollution. This is probably the very first time to understand and debate this long-term problem clearly. Hopefully, more comprehensive water/sediment quality monitoring and pollutants in the PBR of Thailand could be enhanced.

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