Songklanakarin J. Sci. Technol.
37 (1), 1-5, Jan. - Feb. 2015

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

Original Article

Histopathological finding of liver and kidney tissues of the yellow mystus, *Hemibagrus filamentus* (Fang and Chaux, 1949), from the Tapee River, Thailand

Sinlapachai Senarat[1], Jes Kettratad[1], Pisit Poolprasert[2], Watiporn Yenchum[3], and Wannee Jiraungkoorskul[4]*

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

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

[3] *Bio-Analysis Laboratory, Department of Chemical Metrology and Biometry, National Institute of Metrology (Thailand), Khlong Luang, Pathum Thani, 12120 Thailand.*

[4] *Department of Pathobiology, Faculty of Science, Mahidol University, Ratchathewi, Bangkok, 10400 Thailand.*

Received: 24 June 2014; Accepted: 7 November 2014

Abstract

A histological finding of liver and kidney tissues of yellow mystus, *Hemibagrus filamentus* (Fang and Chaux, 1949), collected from the Tapee River (Chawang District, Nakhon Si Thammarat Province, Thailand) was conducted to examine the pathological lesions. Lesions, including cellular swelling, hydropic degeneration, or vacuolation of hepatocytes were found. From this, coagulative necrosis with pyknotic nuclei, infiltration of macrophage, constriction of sinusoidal capillaries and damage of endothelial cells of blood vessels were observed in liver tissues. The dilation of both Bowman's space and the glomerulus were observed from kidney lesions. Moreover, necrosis in renal tubular cells was also observed in some areas. Although preliminary, the results from this study clearly show the need for further investigation into stress factors and vectors affecting the health of *H. filamentus* and other fish species within the Tapee River.

Keywords: *Hemibagrus filamentus*, fish, histopathology, kidney, liver, Tapee River

1. Introduction

The Tapee River is the longest river in southern Thailand, and it is recognized as the most ecologically and economically important freshwater body of water in the region. Its headwaters start from Khao Luang Mountain (Nakhon Si Thammarat Province), where it supplies water for people who live along of this river, and it eventually flows to the Gulf of Thailand. The Tapee River has a total length of 230 km, and drains 13,454 km^2 that includes a forested area of 4,072 km^2 and an agricultural area of 7,086 km^2 covering parts of Surat Thani, Nakon Si Thammarat, and Krabi Province (Laudee and Prommi, 2011). The water quality and trophic status of the Tapee River has been classified as having a clean-moderate and oligotrophic-mesotrophic status (Leela-hakriengkrai and Peerapornpisal, 2011). Despite its water quality and trophic status, the Tapee River is vulnerable to pesticides pollution, as it receives agricultural runoff from paddy fields, Indian rubber plantations, and vegetable and fruit crops. Common agricultural practices within Nakhon Si

* Corresponding author.
Email address: wannee.jir@mahidol.ac.th

Thammarat Province involve the application of herbicides such as glyphosate, paraquat, and atrazine and, these agricultural chemicals have been extensively used over a longer period of time. Hence, it is likely that the Tapee River receives substantial inputs from herbicide residue residing in surrounding soils and sediments as well as directly from surface water runoff. Even though low to high concentration levels of herbicides remain in the river, the chronic input and presence of these chemicals coupled with the potential for biomagnification through the food web is of great concern. Similarity, the chronic presence of agricultural chemicals can have a direct negative effect on both river systems and on the health of specific species or groups, such as fishes that live in proximity to such areas (McGlashan and Hughies, 2001).

One of the economically important freshwater fish in the Tapee River is the yellow mystus, freshwater catfish or green catfish, *Hemibagrus filamentus* (Fang and Chaux, 1949) belonging to the Order Siluriformes and Family Bagridae. This species is found in the basin and also in a variety of lotic and lentic ecosystems with substrates of sand and mud, feeding mainly on fish, crustaceans, larval aquatic insects, and plant matter (Hee and Rainboth, 1999). According to the International Union for Conservation of Nature (IUCN), the yellow mystus is also listed as a threatened species (IUCN, 2013). Moreover, as the most widely consumed fish with a high economic value for Thai people, the yellow mystus has been selected as a sentinel species for pesticides contamination. As mentioned above, it is probable that the environmental contamination in the Tapee River may negatively impact fish. Sub-lethal affects may include histological alterations that can provide an early warning of stress and potential overall impact to the population. Currently, the histopathological biomarker is one useful tool that can indicate environmental pollutants, especially for sub-lethal and chronic effects (Fatma, 2009; Nikalje *et al.*, 2012). This histotechnique can also diagnose and predict vital changes in histological structure of both tissues and organs (Hinton *et al.*, 2001; Adams, 2002; Dietrich and Krieger, 2009).

Although field surveys can provide presence-absence, histological methods are a more effective precursor measure of fish health. Within the Tapee River, this may best provide measures of impact to fish relative to agriculture zones. The histopathology involving liver and kidney tissue of the yellow mystus is still not well known and warrants further investigation. Therefore, the aim of this study was to investigate the histopathological alteration in liver and kidney as a biomarker of the yellow mystus, *H. filamentus,* obtained from the Tapee River, Thailand.

2. Materials and Methods

2.1 Animal collection and study site

Mature fish samples (n=20, 16-20 cm standard length) were collected from the Tapee River, Chawang District, Nakhon Si Thammarat Province, Thailand (8°28'10" N, 99°29'45" E). The representative study site was selected during the rainy season (June-July 2013) and divided into three sampling sites. The length of each sampling site was about six kilometers and each collecting site was two kilometers apart. All fish was euthanized by rapid cooling technique (Wilson *et al.*, 2009).

2.2 Histological study

Upon euthanasia, each fish had both the liver and kidney immediately removed and fixed in Davidson's fixative for 48 hrs at room temperature. All specimens were processed using standard histological techniques by being dehydrated through a graded series of ethanol, cleared with xylene, embedded with melted paraffin (Bancroft and Gamble, 2002; Humason, 1979). Sections of 5-6 µm thickness were cut by rotary microtome and stained with Harris's hematoxylin and eosin (H&E). Finally, the histological structure and histopathological lesion recorded as a mean prevalence in liver and kidney was investigated by using a light microscope.

3. Results

3.1 Histopathology of liver

The liver was encapsulated with a thin layer of connective tissues. The polygonal hepatocytes arranged along the sinusoid arraying from the hepatic central vein. The hepatocytes typically contained central nuclei with densely stained chromatin. Cytoplasm of hepatocyte was slightly eosinophilic stained. Histopathological alterations of the liver in *H. filamentus* from the Tapee River consisted of cellular swelling, eosinophilic cytoplasm of hepatocytes, constriction of sinusoidal capillaries, and damage of endothelial cell of blood vessels. Some liver areas showed focal necrosis and contained severe infiltration of macrophage, pyknotic nuclei and large lipid vacuoles in the cytoplasm of hepatocytes (Figure 1A). The detected prevalence in liver tissues of this fish is shown in Table 1.

3.2 Histopathology of kidney

Our research team has previously reported the histology of *H. filamentus* kidney (Senarat *et al.*, 2013). Histopathological alterations of kidney in *H. filamentus* from the Tapee River showed kidney degeneration, glomerular alterations, Bowman's capsule rupture, hydropic swelling, and cellular debris accumulation in lumen. In some cases, the tubular cells showed the pyknosis and hyaline droplet accumulation, dilation of the Bowman's capsule and glomerulus. The dilation of the lumen in the tubules, degeneration in the hemopoitic tissue, rupture in the collecting tubules and necrosis are presented (Figure 1B-D). The prevalence of several lesions was observed in kidney tissues of this fish. Details of its prevalence are given in Table 1.

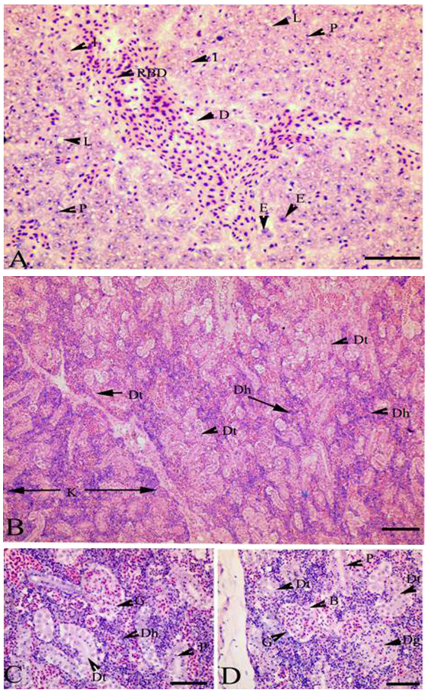

Figure 1. Micrographs of liver (A) and kidney (B-D) of *H. filamentus*; B = Bowman's capsule rupture, D = damage of endothelial cell, Dg = dilation of glomerulus, Dh = degeneration in the hematopoietic tissue, Dt = degeneration of renal tubules, E = eosinophilic cytoplasm of hepatocytes, G = degeneration of glomerulus, I = infiltration of macrophage, K = kidney disorganization, L = lipid vacuoles, P = pyknosis, RBD = red blood cells congestion. Note: Scale bar 50 µm (A); 100 µm (B); 20 µm (C-D) (H&E stain).

Table 1. Alteration prevalence (%) of dominant histopathological lesions in the liver and kidney of *Hemibagrus filamentus* caught from Tapee River during rainy season.

Liver (n = 20)	Alteration Prevalence (%)
Cellular swelling	100
Eosinophilic cytoplasm in hepatocyte	100
Infiltration of macrophage	70
Pyknotic nuclei	100
Large lipid vacuoles	90
Kidney (n=20)	**Alteration Prevalence (%)**
Bowman's capsule rupture	100
Glomerular alterations	100
Kidney degeneration	100
Hydropic swelling of renal tubules	90
Cellular debris	90
Pyknosis of renal tubules	100
Hyaline droplet of renal tubules	90

4. Discussion

Freshwater fishes are critical as a protein-rich food source but they are also susceptible to the acute and chronic presence of pollutants (Thingran, 1974). The release of pollutants to the aquatic environment is particularly toxic to aquatic organisms including fish. Fish mortalities have been associated with the river receiving various pollutants (Prashanth *et al.*, 2005; Marina and Martinez, 2007), and the results of our study show that fish from the Tapee River express histopathological alterations in liver and kidney during rainy season. It is plausible that a lot of water during rainy reason could dilute pesticides and other pollutants. In the same manner, the atrazine herbicide group has also been reported from rainy season in both sediment and fish tissue (*Puntioplites proctozyston*) from Nan river, northern Thailand (Senarat, 2011). However, our observation is not unreasonable that the lesions on the target organs might have been a direct outcome of pollutants including pesticides, metals and fertilizers, which enter the river with runoff. Fish are susceptible to change in their surrounding environments as well as an increase in pollution, and fish health provides a good precursory measure of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may be evident at the cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Histological analysis therefore appears to be a very sensitive parameter and crucial in determining cellular changes that may occur in target organs, such as liver and kidney (Dutta, 1996). Histology may prove to be a cost-effective tool to determine the health of fish populations, therefore reflecting the health of an entire aquatic ecosystem in the bio-monitoring process.

The liver is the organ most associated with processes of detoxification and biotransformation, and due to its function, position, and blood supply, it is the most affected by contaminants in the water. The livers of *H. filamentus* have shown vacuolar degeneration in the hepatic cells, necrosis, dilation and congestion in the sinusoid (Monsefi *et al.*, 2010). Dilation of the sinusoid occurred whenever the efflux of hepatic blood was impeded. A consequence of endothelial cell injury was the loss of its barrier function with extensive blood accumulation in the liver. These disruptions of the sinusoid were considered the early structural features of the vascular disorder (Gregus, 2008; Jaeschke, 2008). Vacuolation of hepatocytes has been shown as a common response to exposure of fish to a variety of different pollutants (Meyers and Hendricks, 1985). The vacuolization of hepatocytes may show an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system. In the case of vacuolization, the nuclei and hepatocytes were unusually compressed towards the sinusoidal spaces. These histological changes may signify the imbalance biochemical synthesis, inhibition of protein synthesis, energy depletion, desegregation of microtubules, and shifts in substance utilization (Meyers and

Hendricks, 1985).

The kidney is one of the major organs that express toxic effects. The histopathological changes in the kidney at the level of the glomerulus and tubule in fish after exposure to pollutants such as pesticides and herbicides have been reported by many researchers (Melaa *et al.*, 2007; Aysel *et al.*, 2008; Yenchum, 2010). Hydropic swelling, hyaline droplet deposition, glomerular degeneration, and necrosis of renal tubules were shown after exposure to carbofuran (Yenchum, 2010). According to Aysel *et al.* (2008), after exposure to ammonia the kidney tissues of the hyperemia and glomerulonephritis showed necrosis, phagocytic areas, intercellular space among parenquimal cells, and atypical cells similar to the histological study by Melaa *et al.* (2007), who made these observations after methyl mercury exposure. Furthermore, fish exposed to deltamethrin showed degeneration in the epithelial cells of the renal tubule, pyknosis in the hematopoietic tissue, dilation of the glomerulus, and degeneration of the glomerulus and hypertrophied cells (Cengiz and Unlü, 2006). The hyaline droplets likely represented protein reabsorbed from the glomerular filtrate after being contaminated by glyphosate (Jiraungkoorskul *et al.*, 2003). Other fish species exposed to natural petroleum also displayed dilation of blood capillaries in the glomerulus and inflammation (Pacheco and Santos, 2002).

In brief, the histological changes in the current study were observed in the liver and kidney of the yellow mystus, *Hemibagrus filamentus,* occupying in the Tapee River. It was indicated that the fish were responding to the direct effects of the contaminants. The pathological changes were induced in the kidneys of different fish by different pollutants but the extent of damage varies depending upon the dose of pollutants, duration of exposure and toxicity of pollutants, and susceptibility of fish. Nevertheless, the concentration of pesticides and other pollutants in the natural environment (water and sediment) need to be investigated. Overall, this information confirms that histopathological alterations are good biomarkers for field assessment, in particular in tropical areas that are naturally subject to a multiplicity of environmental variations. Here we emphasize that histopathology is able to evaluate the early effects and the responses to acute exposure to chemical stressors. The data from this study will enhance our understanding of ecosystems and organism health, which can then be conveyed to local people and communities to effective manage and thus decrease the use of pesticides, herbicides, and other contaminants.

Acknowledgments

The authors would like to thank the members of the Fish Research Unit, Department of Pathobiology, Faculty of Science, Mahidol University for technical assistant throughout the study. We are also grateful to Dr. T.W. Miller for improving the English language.

References

Adams, S.M. 2002. Biological indicators of aquatic ecosystem stress. American Fisheries Society. 3, 104-112.

Aysel, C.K.B., Gulten, K. and Ayhan, O. 2008. Sublethal ammonia exposure of nile tilapia (*Oreochromis niloticus* L.): Effects on gill, liver and kidney histology. Chemosphere. 72, 1355-1358.

Bancroft, J.D. and Gamble, M. 2002. Theory and practice of histological techniques. Churchill Livingstone, London, UK.

Cengiz, E.I. and Unlü, E. 2006. Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. Environmental Toxicology and Pharmacology. 21, 246-253.

Dietrich, D.R. and Krieger, H.O. 2009. Histological analysis of endocrine disruptive effects in small laboratory fish. John Wiley and Sons, New Jersey, U.S.A.

Dutta, H.M. 1996. A composite approach for evaluation of the effects of pesticides on fish. In Fish morphology, J.S.D. Munshi and H.M. Dutta, editors. Lebanon, Science Publisher Inc., pp 249-254.

Fatma, A.S.M. 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World Journal of Fish and Marine Science. 1, 29-39.

Gregus, Z. 2008. Mechanisms of toxicity. In Toxicology the basic science of poisons, C.D. Klaassen, editor. Casarett and Doull's. 7th edition, McGraw-Hill, New York, U.S.A., pp. 1-128.

Hee, N.H. and Rainboth, W.J. 1999. The bagrid catfish genus Hemibagrus (Teleostei: Siluriformes) in central Indochina with a new species from the Mekong river. Raffles Bulletin of Zoology. 47, 555-576.

Hinton, D.E., Segner, H. and Braunback, T. 2001. Toxic response of liver. In Target organ toxicity in marine and fresh water teleosts, D. Shlenk and W.H. Benson, editors. Taylor and Francis, London, UK., pp 224-268.

Humason, G.L. 1979. Animal tissue techniques. 4th ed. San Francisco: W.H. Freeman and Company.

IUCN 2013. *Hemibagrus filamentus*. In Red List of Threatened Species, International Union for Conservation of Nature (IUCN), Version 2013.2.

Jaeschke, H. 2008. Toxic responses of the liver. In Toxicology the basic science of poisons, C.D. Klaassen, editor. Casarett and Doull's. 7th edition, McGraw-Hill, New York, U.S.A. pp 557-582.

Jiraungkoorskul, W., Upatham, E.S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S. and Pokethitiyook, P. 2003. Biochemical and histopathological effects of glyphosate herbicide on nile tilapia (*Oreochromis niloticus*). Environmental Toxicology. 18, 260-267.

Laudee, P. and Prommi, T.O. 2011. Biodiversity and distribution of Trichoptera species along the Tapee River, Surat Thani Province, Southern Thailand. Zoosymposia. 5, 279-287.

Leelahakriengkrai, P. and Peerapornpisal, Y. 2011. Water quality and trophic status in main rivers of Thailand. Chiang Mai Journal of Science. 38, 280-294.

Marina, M.P.C. and Martinez, C.B.R. 2007. Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. Neotropical Ichthyology. 5, 327-336.

McGlashan, D.J. and Hughies, J.M. 2001. Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Diving Range. Journal of Fish Biology. 59, 55-67.

Melaa, M., Randi, M.A.F., Ventura, D.F., Carvalho, C.E.V., Pelletier, E., Oliveira, C. and Ribeiro, C.A. 2007. Effects of dietary methyl mercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicology and Environmental Safety. 68, 426-435.

Meyers, T.R. and Hendricks, J.D. 1985. Histopathology. In Fundamentals of aquatic toxicology, G.M Rand and S.R. Petrocelli, editors. Hemisphere Publishing Corp, New York, U.S.A. pp 283-331.

Monsef, M., Gholami, Z. and Esmaeili, H.R. 2010. Histological and morphological studies of digestive tube and liver of the Persian tooth-carp, *Aphanius persicus* (Actinopterygii: Cyprinodontidae). Istanbul University Faculty of Science (IUFS) Journal of Biology. 69, 57-64.

Nikalje, S.B., Muley, D.V. and Angadi, S.M. 2012. Histopathological changes in gills of a freshwater major carp, *Labeo rohita* after acute and chronic exposure to textile mill effluent (tme). International Journal of Environmental Science. 3, 108-118.

Pacheco, M. and Santos, M.A. 2002. Biotransformation, genotoxic, and histopathological effect of environmental contaminants in European eel (*Anguilla anguilla* L.). Ecotoxicology and Environmental Safety. 53, 331-347.

Prashanth, M.S., David, M. and Mathad, S.G. 2005. Behavioural changes in freshwater fish, *Cirrhinus Mrigala* (Hamilton) exposed to cypermethrin. Journal of Environmental Biology. 26, 141-144.

Senarat, S., Kettratad, J., Poolprasert, P. and Yenchum, W. 2013. Anterior kidney of the yellow mystus, *Hemibagrus filamentus* (Fang and Chaux, 1949). Walailak Journal of Science and Technology. 10, 597-600.

Senarat, S. 2011. Correlation between herbicide contamination and effects on reproductive system of cyprinid fish, *Puntioplites proctozysron* in Nan River, Wiang Sa Dist, Nan Province. M.Sc. Thesis in Zoology, Chulalongkorn University, Bangkok, Thailand.

Thingran, V.G. 1974 Fisheries in India in the context of aquatic pollution. International Journal of Ecology and Environmental Science. 1, 15-18.

Wilson, J.M., Bunte, R.M. and Carty, A.J. 2009. Evaluation of rapid cooling and tricaine methanesulfonate (MS 222) as methods of euthanasia in zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science. 48, 785-789.

Yenchum, W. 2010. Histological effects of carbofuran on guppy *Poecilia reticulata* Peters. Ph.D Thesis in Biological Science, Chulalongkorn University, Bangkok, Thailand.