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Research article

Pathological microscopy in liver parenchyma of gray-eel catfish, *Plotosus canius*, from Ang-Sila area, Chonburi Province, Thailand

Sinlapachai Senarat[1], Jes Kettratad[1,2], F. Gerald Plumley[2], Sansareeya Wangkulangkul[3], Wannee Jiraungkoorskul[4], Piyakorn Boonyoung[5] and Pisit Poolprasert[6,*]

[1]*Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Aquatic Resources Research Institute, Chulalongkorn University, Bangkok 10330, Thailand*
[3]*Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand*
[4]*Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[5]*Department of Anatomy, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand*
[6]*Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand*

Abstract

This is the first discovered abnormal liver of gray-eel catfish (*Plotosus canius*), which was collected from the Ang-Sila area, Chonburi Province, Thailand. Anatomically, the liver tissue was observed to be both shrunken in overall appearance and with almost white colorization in all lobules. Based on histological and histochemical observations, the liver parenchyma in this species was clearly altered. A large number of vacuolar structures in the hepatocyte were shown as empty spaces (H&E stained), but these structures appeared after the ORO reaction as orange color, indicative of hepatocellular lipidosis. The loss of glycogen in hepatocytes was also observed by using PAS reaction. In addition, necrosis with poor sinusoids structure was occasionally seen in some liver areas. All abnormalities seen in these liver samples might be related to reduced functionality as well as health status of *P. canius*; further, the altered health of these fish might interfere with other animals/ humans within ecological dynamic., Overall, results shown here demonstrate the importance of anatomical and histological studies and we suggest such studies should be incorporated into water quality monitoring in the Ang-Sila zone of Thailand and represent an important consideration for other locations around the world.

Keywords: Histopathology, Liver, *Plotosus canius*, Thailand

***Corresponding author:** Pisit Poolprasert. Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, 65000, Thailand. Email: poolprasert_p@psru.ac.th

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INTRODUCTION

Histopathology is recommended to monitor fish health and environmental problems (Fatma, 2009; Nikalje et al., 2012) and is widely used to identify long-term changes of tissues and organs (Hinton et al., 2001; Adams, 2002; Dietrich and Krieger, 2009). Liver tissue in fish plays a central role in several key processes, for example assimilation of nutrients, detoxification and biotransformation of pollutants (Hinton et al., 2001; Genten et al., 2008). Hence, the alteration of this organ is a reflection on fish health and an entire aquatic ecosystem (Hinton et al., 2001; Adams, 2002). For instance previous observations have demonstrated that induced liver histopathology, is apparent after long-term exposure to organic compounds and heavy metals in teleost fish (Hinton et al., 1992; Senarat et al., 2015; Senarat et al., 2018).

During the recent research on the stomach content and digestive system of *Plotosus canius*, an important economical species that is widely distributed and likely plays a significant role in the food web/food chain of the Ang-Sila coastal region, Chonburi Province, Thailand (Keawjam, 2014), we found liver pathology as one of the most interesting features, which needs to be clarified. Therefore, the objective of this study was to describe the pathological features of liver tissues in *P. canius*. These results provide information required to better understand liver lesion in these fish and is part of a larger project investigating fish health and, by default, habitat health.

MATERIALS and METHODS

All voucher species of *Plotosus canius* in this study were mainly obtained from former work of Keawjam (2014). Twenty necropsy specimens of the liver from *P. canius* with approximately 45±0.41 cm were randomly collected from Ang-Sila area, Chonburi Province, the Upper Gulf of Thailand during October to November 2013. Only dead specimens were chosen for this research. Therefore, the Animal Use Protocol Review and Approval Process was not required.

Liver tissue was removed from each fish and then roughly dissected into three fragments about 5 × 5 mm. All fragmented tissues were observed under a stereomicroscope and then divided into two groups for either histological or frozen analyses.

For histological analysis, liver tissues were fixed in Davidson's fixative about 48 hours and processed by standard histological techniques (Bancroft and Gamble, 2002; Humason, 1979). Paraffin sections with 4 µm thickness were cut and stained by Harris's hematoxylin and eosin (H&E) to classify the liver structure and periodic acid-Schiff (PAS) was used to clarify glycoprotein in liver tissues (Bancroft and Gamble, 2002).

For frozen analysis, liver tissues were stored at –20ºC in aqueous medium. Then, they were frozen sectioned using a cryostat, with 10 mm thickness, and stained with oil red O (ORO) for general lipid determination (Culling, 1963).

All histological features of the liver tissues were analyzed under a light microscope and digital images were generated with a Canon EOS 1100D.

RESULTS

Pathological morphology of liver tissue

A total of 20 liver samples were examined for this study. Three out of the livers exhibited clinically evident pathology as observed by gross examination. Three livers with pathology were chosen for detailed study. It was found that they were shrunken and almost white colorization in all lobules, when compared with normal liver anatomy (Figures 1A–1B).

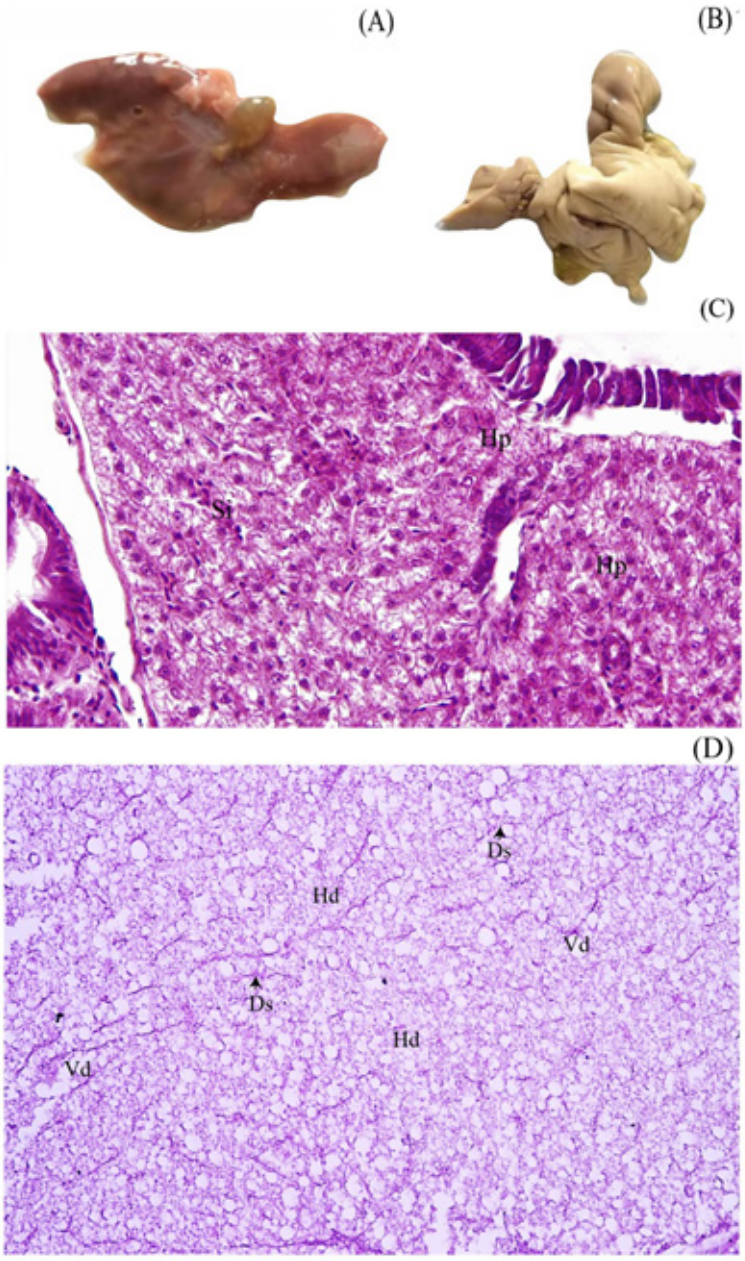

Figure 1 Anatomies (A-B) and light micrographs (C-D) of liver tissue of *Plotosus canius*; A,C: normal tissue and B,D = abnormal tissue. Abbreviations: Hp = hepatocyte, Hd = hepatic parenchyma density, Ds = degeneration of sinusoids, Si = sinusoids Vd = vacuolar degeneration. Note: Scale bar 1 cm (A-B), 100 µm (C) (H&E = Harris's hematoxylin and eosin).

Histopathological features of liver tissue

Histological observations revealed that the liver parenchyma was arranged in hepatocyte plates, surrounded by sinusoids (Figure 1C). The hepatocytes had a polyhedral shape with spherical and central nucleus; granular eosinophilic cytoplasm was also observed (Figure 1C).

Liver architecture of the three *P. canius* tissues was clearly altered. Degenerative changes, including necrosis with poor sinusoid structure, were observed (Figure 1D). These changes were caused by disintegration of the hepatic structure and a decrease in the hepatic parenchyma density (Figure 1D). Histochemical techniques also revealed a loss of glycogen in hepatocytes, as demonstrated by using PAS reaction (Figures 2A–2C). These three livers with obvious pathology also contained substantial vacuolization, which presented as empty spaces with H&E staining (Figure 1D) and as orange when submitted to ORO staining (Figures 2D–2E). These features are considered to be features of hepatocellular lipidosis.

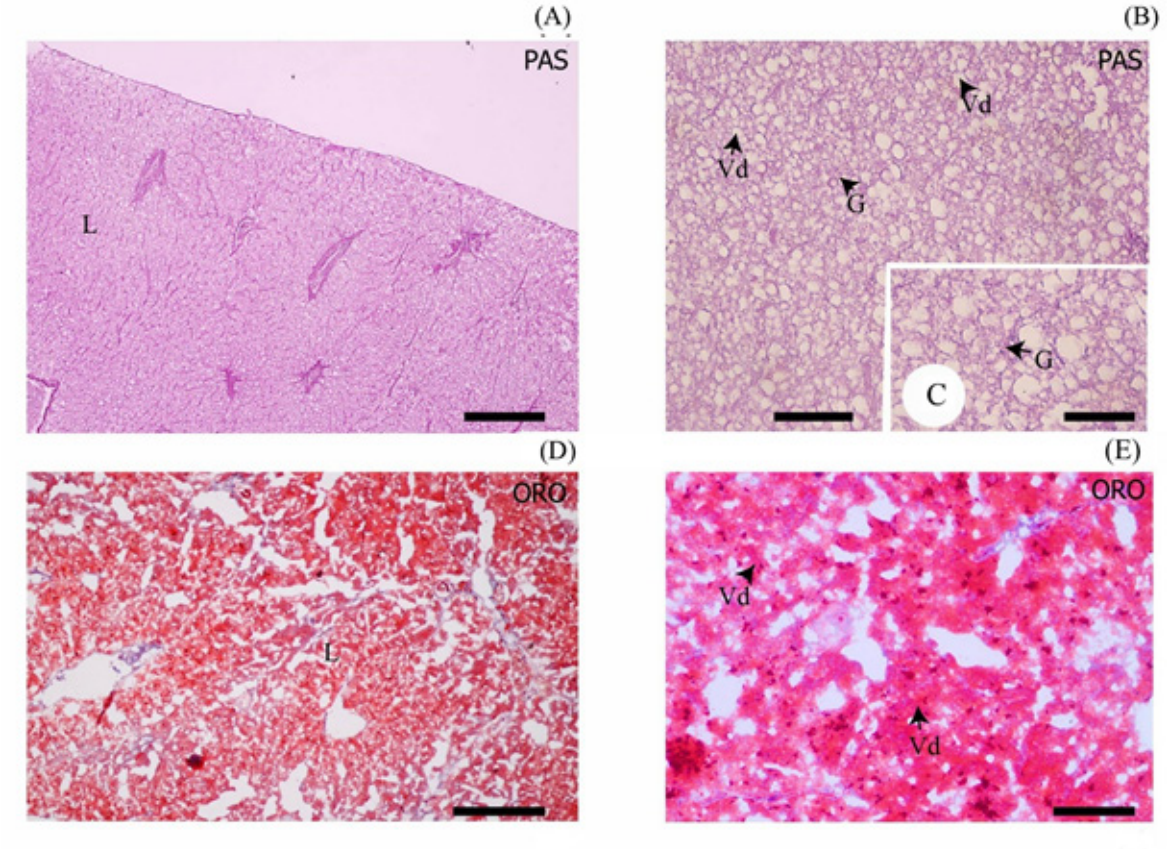

Figure 2 Light micrographs of liver tissue (L) of *Plotosus canius*. Abbreviations: G = glycogen, Vd = vacuolar degeneration. Note: Scale bar 200 µm (A, D), 50 µm (B, E), 10 µm (C) (PAS = periodic acid-Schiff, ORO = oil red O).

DISCUSSION

The normal anatomy and histology from liver of *P. canius* was newly explored in this time. Interestingly, we found that major degenerative changes were present in three of 20 fish examined and included hepatocellular lipidosis and cellular which is thought to be related to signs of liver damage, as suggested by similar conclusions in other fish (Greenfield et al., 2008). It is unclear if these lesions result from environmental factors (e.g., pollutants) and/or are age-related. These two possibilities are discussed below.

It has been suggested that the pathway of these types of lesions involve alterations in lipid and protein metabolism (lipidosis) and these are probably the most specific abnormal accumulating triglycerides in hepatocytes (Hinton and Laure´n, 1990). It was possible that the pollutants-related hepatocellular lipidosis was suggested with exposure to chlorinated hydrocarbon contamination and other pollutants (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 TiO2 nanoparticle (Diniz et al., 2013). Combined with the previous findings and our observation is possible that had a polluted environment (Hinton and Laure´n 1990).

It was postulated that the occurrence of hepatocellular lipidosis was also probably associated with old age and nutritional value of food supplies (Hinton et al., 1992; Robertson and Bradley, 1992; Yilmaz and Genc, 2006; Yilmaz and Akyurt, 2005; Sanad et al., 2015). Similarly, Genc et al. (2005) showed that the improper formulation of dietary soy-acid oil mixed with yellow grease induced the appearance of hepatic lipidosis in the hybrind tilapia *Orechromis niloties*.. Lipidosis of *Lutjanus guttatus* also was detected after exposed to nutritional stressors (Ruiz-Ramírez et al., 2019).

As described above, a comprehensive review of liver pathology in *P. canisus* is still deficient and this work represents only our first attempt to understand the problem. What is clear is that liver histopathology, especially hepatocellular lipidosis, is a cause for concern in *P. canisus* as this pathology could result in the total loss of liver function. To completely understand lipid histopathology, it is recommended that water quality monitoring in the Ang-Sila zone of Thailand could be enhanced, and similar conclusions are likely valid for other localizations in the Gulf of Thailand and many other parts of the world.

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