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**Research article**

# Histopathological observation and health status of the zebra-snout seahorse *Hippocampus barbouri* Jordan & Richardson, 1908 in captivity

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## Abstract

The health status of the zebra-snout seahorse, *Hippocampus barbouri* in captivity has been required for approval for aquaculture. In this study, we investigated the histopathological appearance of three vital organs including gill, kidney and liver in captive *H. barbouri* during its juvenile and adult stages, by using histological techniques. In juveniles from stage 14-days (100% prevalence) towards stage 30-days adults (100% prevalence), the gills exhibited intraepithelial edema and necrosis while hepatic tissue showed evidence of intracytoplasmic vacuoles. In addition, histological alteration to renal tissues was observed the degeneration of renal tubules, the presence of melanomacrophage, and the infection of trematode parasites. The parasites were found in stage 30-days adult fish in the kidney (33.3 % prevalence). Taken together, this study highlights the issue of health in captive rearing of *H. barbouri*, in particular histopathological alterations in gill, liver and kidney tissues, suggesting that aquaculture of this seahorse species requires improved methods and protocols for maintenance and preventing infection.

**Keywords:** Captive zebra-snout seahorse, Histopathology, Kidney, Liver, Gill

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## INTRODUCTION

The zebra-snout Seahorse, *Hippocampus barbouri* Jordan & Richardson, 1908, belongs to the family Syngnathidae, is one of the most important fishery resources of Thailand. This seahorse species can be found along tropical and temperate shallow shelter reefs, and in algae and seaglass beds (Foster and Vincent, 2004). In recent years, overexploitation, particularly for traditional Chinese medicine (TCM), overfishing and habitat deterioration have been the main causes of dramatic declines in *H. barbouri* (Vincent et al., 2011). Further indicative of its decline, *H. barbouri* is also listed in the Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Hutton and Dickson, 2000).

Due to population declines in *H. barbour*, initial and ongoing work by the Phuket Marine Biological Center (PMBC), Thailand under its Culture Project has developed a strategy involving research and development of captive breeding and aquaculture of this species in Thailand toward stock recovery and conservation. Numerous feeding studies involving live feed for aquaculture of *H. barbour* have been performed, with results showing increased growth rates (Kamnurdnin, 2017; Wung et al., 2020; Wilson and Vincent, 2000; Planas et al., 2008; Sheng et al., 2006); however, there are no known studies on the health status of this species of seahorse in captivity through microanatomical methods. This information is essential for understanding environmental factors influencing its reproductive capacity, growth rate, and survival under aquaculture. Hence, in the present study, we assessed the health of captive juvenile to adult *H. barbour* using histopathological methods.

## MATERIALS and METHODS

### Seahorse collection

Captive broodstock of *Hippocampus barbouri* were reared in a standard culture system from the Phuket Marine Biological Center (PMBC), Thailand. Juvenile stages (at 1-day, 5-days, 7-days, 14-days and 20-days) and adult stage (at 30-days) (Table 1) were received as voucher specimens from Kamnurdnin (2017). Previous observations showed that the adult stage of *H. barbouri* in captivity is considered as individuals over 30-days of age, from which secondary growth occurs (Kamnurdnin, 2017). The experimental protocol was approved by the Animal Care and Use Committee of Faculty of Science in accordance with the guide for the care and use of laboratory animal prepared by Chulalongkorn University (Protocol Review No. 1623004).

**Table 1** Summary of size and number of samples of *Hippocampus barbouri* in captivity.

| Seahorse stages | Days | Numbers | Total length (cm) |
|---|---|---|---|
| Juveniles | 1 | 3 | 15.60±0.78 |
| | 5 | 3 | 21.70±3.94 |
| | 7 | 3 | 29.00±3.22 |
| | 14 | 3 | 36.00±2.75 |
| | 20 | 3 | 42.90±2.96 |
| Adult | 30 | 3 | 57.30±3.75 |

### Histological techniques

All seahorse samples were quickly euthanized by a rapid cooling method (Wilson et al., 2009). The external anatomy was visually evaluated under stereomicroscope, then the whole fish were transferred to Davidson's fixative. Cross sections of all samples were dissected approximately 1 cm from head to tail and were then processed using standard histological protocols (Presnell and Schreibman, 1997; Senarat et al., 2020a; Senarat et al., 2020b). The paraffin blocks were sectioned at a thickness of 4 µm and stained with Harris's hematoxylin and eosin (H&E), Masson's Trichrome (MT) and periodic acid-Schiff (PAS) (Presnell and Schreibman, 1997; Suvarna et al., 2013). Histopathological abnormalities of the fish were examined and photographed under a light microscope (Leica digital 750). Each histopathological abnormality in the respective organs was calculated and presented as a percent prevalence (%). The occurrence of melanomacrophage centers and infection of trematode parasites was also documented from the stained tissue sections.

## RESULTS

We observed histopathological conditions in several vital organs (gill, liver, kidney) and the mesentary through histological techniques, as shown in Figures 1–3. The prevalence of histopathological lesions is also provided in Table 2.

**Table 2** Prevalence alteration (%) of histopathological observations in *Hippocampus barbouri* in captivity.

| Tissues (n = 3) | Days | Lesions | Percent prevalence |
|---|---|---|---|
| Gills | 14 | Lamellar epithelium lifting and intraepithelial edema of secondary gills | 100.0 |
| | 20 | | 33.3 |
| Livers | 14 | Lipidosis and cellular degeneration of hepatocyte | 100.0 |
| | 20 | | 100.0 |
| Kidneys | 14 | Renal degeneration | 100.0 |
| | 20 | | 100.0 |
| | 30 | MMC and trematode parasites | 33.3 |

## Histology and histopathology of gills

Histology of the *H. barbouri* gill in healthy individuals is shown in Figure 1A. The structure of the four-gill filament (or respiratory filament) arises from the gill arch in a perpendicular manner, where they are parallel to each other. Each gill filament is histologically observed as double rows, in which each filament is divided into the primary and secondary lamellae [respiratory lamellae (Figure 1A)]. Only an intense lifting of the lamellar epithelium and intraepithelial edema of the secondary gills clearly showed alterations in juveniles at 14-days (100% prevalence, Figure 1B), 20-days (33% prevalence) and adults at 30-days (100% prevalence, Figures 1C-1D).

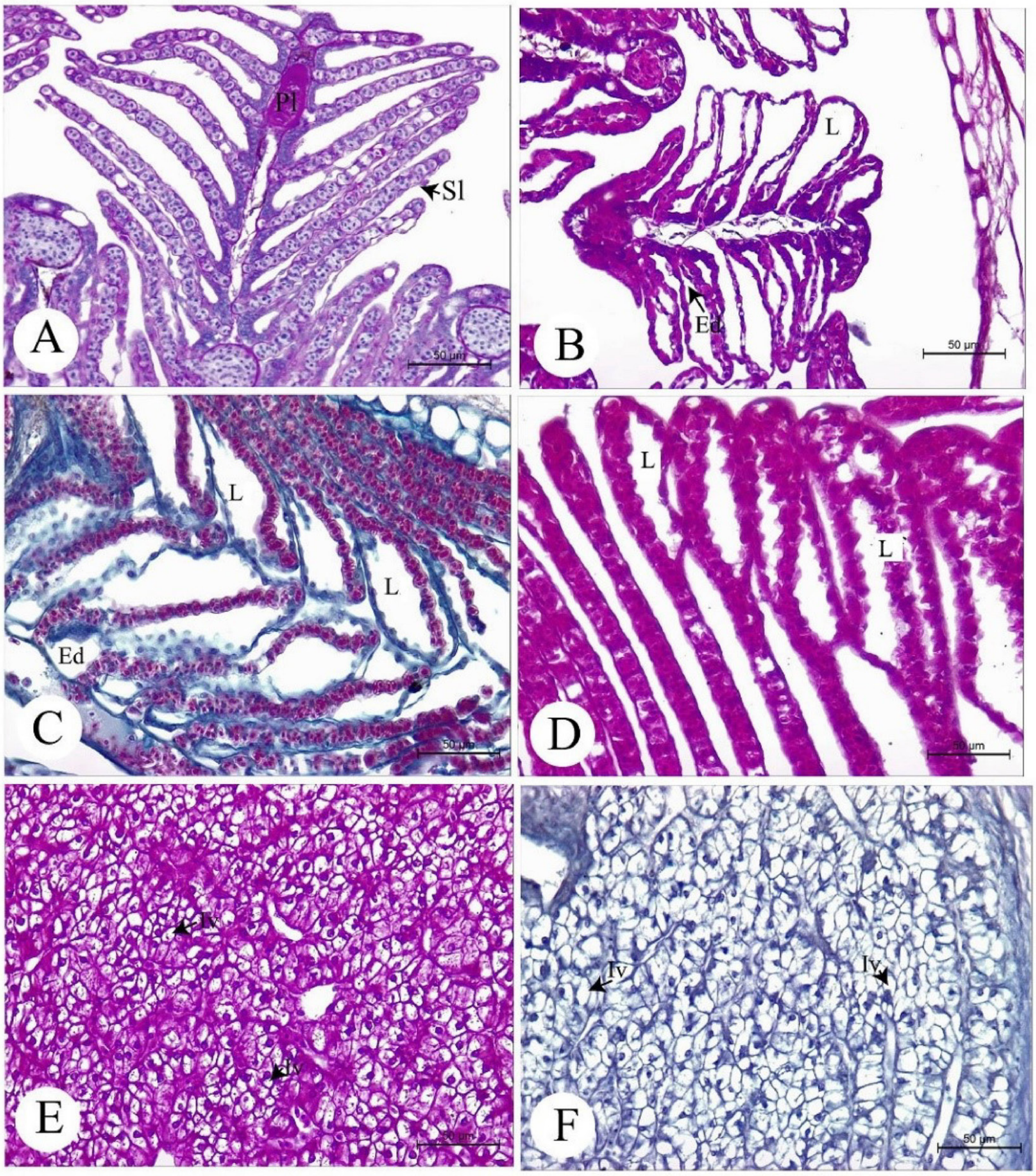


**Figure 1** Light photomicrograph of gill histology (A) consisting of primary lamella (PI) and secondary lamella (SI) of *Hippocampus barbouri* in captivity. Lamellar epithelium lifting (L) and edema (Ed) of the secondary lamellar gills at 14 days (B-D), and intracytoplasmic vacuoles (Iv) were observed in the liver. Note: periodic acid-Schiff (A); Harris's hematoxylin and eosin; (B, D, E); Masson's Trichrome (C).

## Histopathology of livers

Necrosis and intracytoplasmic vacuoles, also called "hepatocellular lipidosis", in juvenile livers occurred at 14-days juvenile stage and at 30-days adult stage (100 % prevalence) (Figures 1E-1F).

## Histopathology of kidneys

Both 14 and 30-days stages exhibited histological changes in the renal degeneration (100% prevalence) throughout the melanomacrophage aggregates (100% prevalence, Figures 2A-2B).

## Melanomacrophage centers (MMCs) and infection of trematode parasites

We observed MMCs in the mesentery in 30-days adult stage (33.3 % prevalence) (Figures 2A-2B). Interestingly, trematode parasites were found only in those at stage 30-days. Also, the location of the parasites was observed in kidney tissue at 30-days stage (33.3 % prevalence, Figures 2C-2D), and that the degeneration of renal tubules in the kidney was also found near the site of trematode infection (Figure 3).

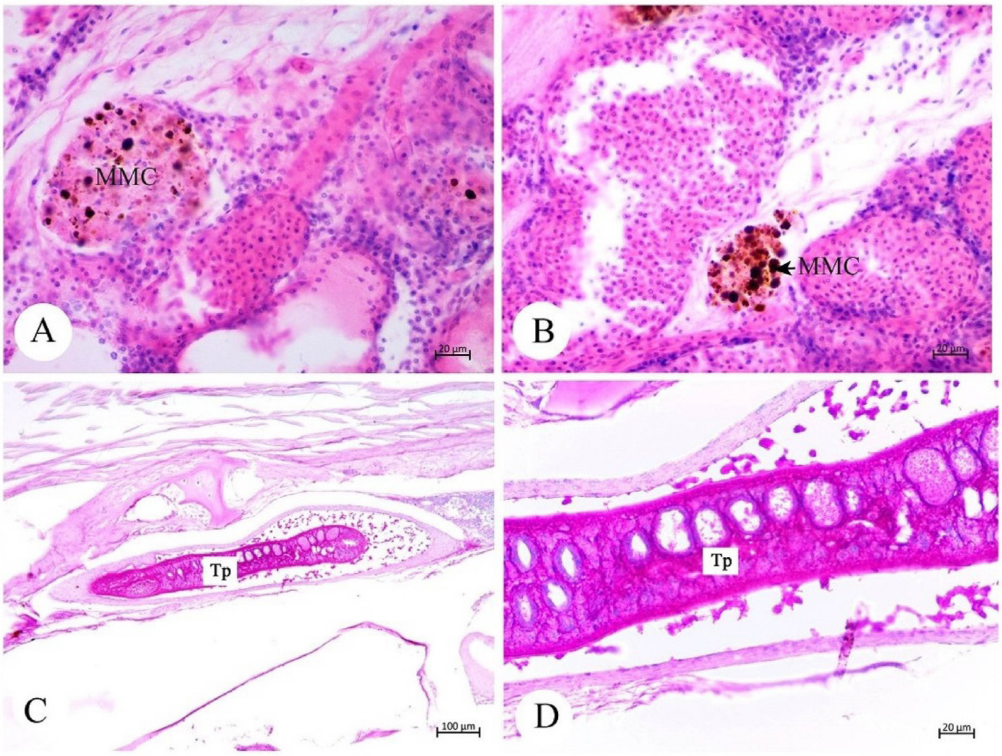


**Figure 2** Light photomicrograph of melanomacrophage centers (MMCs) and trematode parasites of *Hippocampus barbouri* in captivity. A-B: MMCs at 30-days. C-D: Trematode parasites (Tp) at 30-days in kidney tissue. Note: A-F: Harris's hematoxylin and eosin.

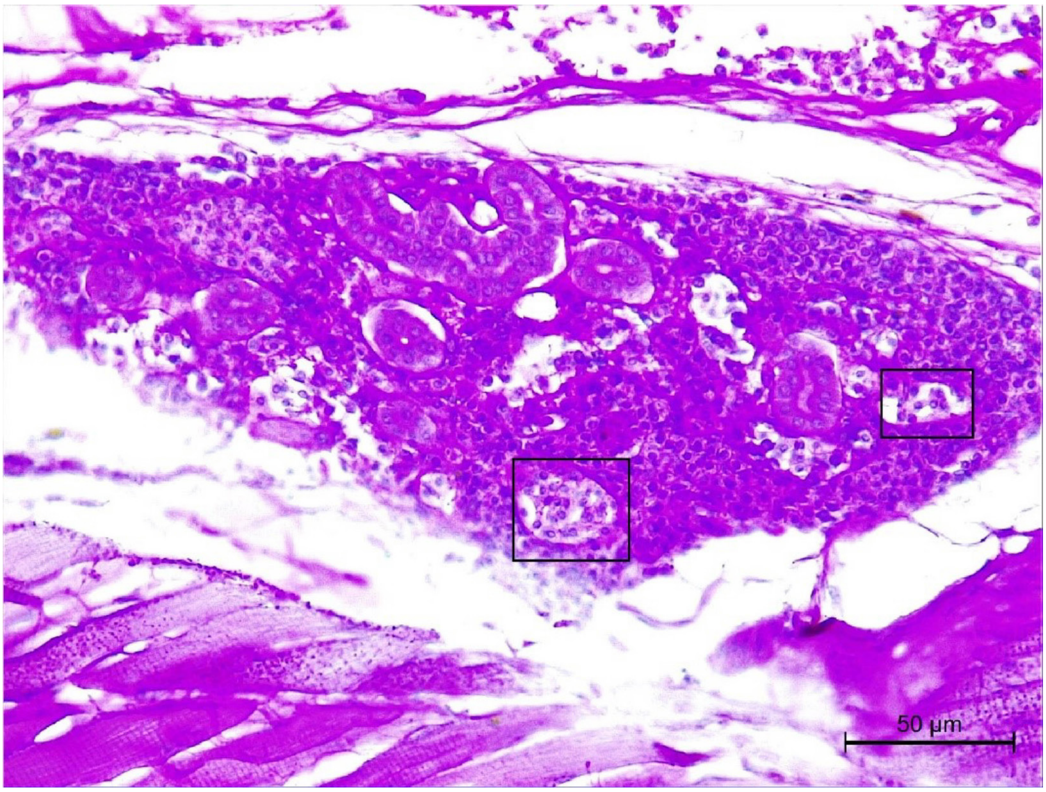


**Figure 3** Light photomicrograph of the degeneration of the renal tubule (square) of *Hippocampus barbouri* in captivity; representative figure at 30-days. Note: periodic acid-Schiff.

## DISCUSSION

Due to a lack of information on *H. barbouri* health under captive conditions, one requirement was to identify histopathological alterations of captive broodstock *H. barbouri* for documentation and help in applying to aquaculture development. This method has been routinely recommended and used to assess the general health status of fishes under both laboratory and field conditions (Blazer, 2002; OECD, 2009; Dietrich and Krieger, 2009; Senarat et al., 2015; Senarat et al., 2018a; Senarat et al., 2018b; Senarat et al., 2019; Senarat et al., 2020c).

Intense lamellar epithelium lifting and intraepithelial edema of the secondary gills in *H. barbouri* were observed. This indicates that the gill may be a more specific and sensitive organ in *H. barbouri*. It was reported that these lesions serve as a mechanism of defense by increasing the structural distance between the epithelium and pollution or low-quality environmental condition. This notion was supported by several studies on the gills of teleost fish (Bury et al., 1998; Sola et al., 1995), for example, *Cyprinus carpino* after exposed to 10 mg/L Thiamethoxam (Georgieva et al., 2014), *Acanthopagrus latus* after treated to $HgCl_2$ concentrations (Hassaninezhad et al., 2014) and *Poecilia reticulate* exposed to chlorpyrifos (de Silva and Samayawardhena, 2002). Additionally, some reports have shown that histopathological lesions on the gills could develop due to sensitization of the mucosal membrane lining the lamellae, with the activity of mucous and chloride cells towards the water supplied to the fishes (Boyd et al., 1980), which needs to be observed of *H. barbouri* in further study. Although here we found a few lesions in the gill, the overall histopathological conditions indicate impaired gills in captive *H. barbouri* which reflect a reduced physiological function (the respiration, excretory and osmoregulatory functions).

Hepatocellular lipidosis in juvenile livers was found at 14 and 30-days (100 % prevalence) (Figures 1E-1F). The occurrence of the hepatocellular lipidosis and cellular degeneration of hepatocytes was known to be related to liver damage, as well as a consequent loss of liver function (Greenfield et al., 2008). A similar mechanism was previously suggested by Hinton and Laure´n (1990), who postulated that a derangement in lipid and protein metabolism (lipidosis) might play a negative role in the abnormal accumulation of triglycerides in the hepatocytes. It was reported that the appearance of the hepatocellular lipidosis is caused by several factors, for example, chlorinated hydrocarbons and other contaminants (Hendricks et al., 1984; Hinton et al., 1992; Robertson and Bradley 1992; Schrank et al., 1997), including PCBs (Anderson et al., 2003; Teh et al., 1997). However, liver histopathology is also related to old age, poor nutritional value of food sources, and/or other environmental conditions (Hinton et al., 1992; Robertson and Bradley, 1992). These ideas were in accordance with liver histopathological observations from *Sparus aurata* fed on the different dietary lipid contents (Caballero et al., 1999; Zilberg and Munday, 2002). Therefore, based on the hepatic histopathological condition of *H. barbouri* under captivity, contamination by pollutant(s) and other factors as above (Hinton and Laure´n 1990) may play some role in deteriorating health of the seahorse in aquaculture.

The kidney is one of the major organs affected from contaminated aquatic environments, which can be visualized through histological observation. The renal degeneration of *H. barbouri* exhibited both 14 and 30 days, and it was similar to histological damage of teleostean renal tissues exposed to carbofuran (Yenchum, 2010), ammonia (Aysel et al., 2008), and methyl mercury exposure (Melaa et al., 2007). This underscores the importance of the kidney in the excretion of metal and hydrophilic pollutants as shown by Pritchard and Bend (1984); Meinelt et al. (1997); Takashima and Hibya (1995). In addition, renal tissues may be used as a histopathological biomarker to observe pollutants in captivity. Further investigations are needed to accurately measure contaminants such as metals and other chemical pollutants in captive water conditions, and the relation to histological damage to renal tissue and their functions.

Melanomacrophage centers (MMCs), accumulation of pigmented phagocytes functioning in phagocytosis of erythrocytes and infectious materials, play central roles in several vertebrates' immune system (Takashima and Hibya, 1995; Steinel and Bolnick, 2017). In teleost fishes, the immune responses to parasites, pollutants, as well as stress, can trigger the emergence of MMCs, which can be used as a histological indicator for pathogen infection (Alvarez-Pellitero et al., 2007; Robert, 2012; Sitja-Bobadilla, 2008). The appearance of MMCs together with trematode parasites was found, supporting the notion that MMCs occurred when fish hosts were infected with parasites. In addition, we observed that the location of MMC accumulation was not in close proximity to the parasites, which were protected by thick cyst wall.

It is possible that the presence of MMCs was a good indication of general stress in this seahorse. This was in agreement with previous observations, which can help in better understanding the dynamics of fish health either in the wild or captivity (Steinel & Bolnick, 2017; Tsujii & Seno, 1990).

# CONCLUSION

We provided evidence of various histopathological observations in several vital organs from the zebra-snout seahorse, *H. barbourin* under captivity, including edema of the gills, hepatocellular lipidosis and kidney degeneration, and infection of parasites. In addition, different life history stages of seahorse exhibited a distinct prevalence of disease. From our findings, we propose that the presence of histopathological lesions here reflect an unhealthy status of juvenile to adult stages of the seahorse under captive culture. As a conservation and fisheries management tool, successful rearing and aquaculture of healthy stocks are needed to offset overharvest and habitat degradation. In captive and other aquaculture settings, the regular monitoring of water quality and providing optimal nutrition for *H. barbouri* is required and should be continuously assessed by the Phuket Biological Center (PMBC), Thailand in order to reach successful captive breeding of this species.

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# CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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