Songklanakarin J. Sci. Technol.
44 (1), 281-288, Jan. - Feb. 2022

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

Original Article

Morphological characterization of the digestive system and health status of the invasive shortfin molly *Poecilia mexicana* Steindachner, 1863, in Thailand

Rossanan Aiyala[1], Sinlapachai Senarat[2*], Jes Kettratad[1], Kitipong Angsujinda[3], Wannee Jiraungkoorskul[4], Supparat Kong-oh[2], Gen Kaneko[5], Ezra Mongkolchaichana[6], and Natthakitt To-orn[7]

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

[2] *Department of Marine Science and Environment, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Campus, Sikao, Trang, 92150 Thailand*

[3] *Aquatic Resources Research Institute, Chulalongkorn University, Pathum Wan, Bangkok, 10330 Thailand*

[4] *Pathology Division, Department of Preclinical Sciences, Faculty of Medicine, Bangkokthonburi University, Thawi Watthana, Bangkok, 10170 Thailand*

[5] *College of Natural and Applied Science, University of Houston-Victoria, Victoria, Texas, 77901 United State of America*

[6] *Department of General Education, Faculty of Science and Health Technology, Navamindradhiraj University, Dusit, Bangkok, 10300 Thailand*

[7] *Faculty of Agriculture Technology and Agro-Industry, Rajamangala University of Technology Suvarnabhumi, Huntra, Pranakhon Sri Ayutthaya, 13000 Thailand*

Received: 7 April 2021; Revised: 14 July 2021; Accepted: 15 August 2021

Abstract

The shortfin molly, *Poecilia mexicana* Steindachner, 1863, is an ovoviviparous fish. Unfortunately, the structural characteristics related to the digestive biology of this fish are still poorly known. In the current study, detailed characterization of the digestive system was conducted for *P. mexicana* from natural brackish water in Thailand, based on morpho-histological observation and gut content analysis. Forty fish were collected in February 2020 from a small canal at Samut Prakan province. They were then classified into two size groups for 2.00-4.00 and 4.01-6.00 cm in total length. Results showed that the morphology of the digestive tract is similar in the two groups; it was composed of the terminal mouth, the villiform teeth in both upper and lower jaws, pharyngeal teeth, short esophagus and very long intestine. The intestine coefficients of the two groups were 1.64 ± 0.37 and 2.23 ± 0.38, respectively, suggesting that *P. mexicana* is an omnivorous fish. The gut contained a few prey items in both groups, in which detritus and pieces of plants dominated followed by phytoplanktons, indicating that this fish is a detritus feeder in the small canal. Importantly, many histopathological alterations were recorded, especially necrotic acinar cells and melanomacrophage centers. Although this species is generally considered to be tolerant to environmental changes, these results suggest that environmental problems had caused several lesions (gill, liver and kidney) in *P. mexicana*. Overall, this study provides basic knowledge about feeding ecology of this poorly studied species, warranting further research.

Keywords: digestive histology, gut content analysis, histopathology, ovoviviparous fish

*Corresponding author
Email address: sinlapachai.s@rmutsv.ac.th

1. Introduction

Knowledge of feeding ecology is of great value for understanding ecological roles of a species in aquatic ecosystems. Such information could help the development of sustainable management of fish (Melo Germano *et al*., 2014) and provide a better understanding of trophic levels and food web in the environment (Garvey, Dingledine, Donovan & Stein, 1998). Morphological characteristics of the digestive system, such as intestine coefficient, are useful for understanding the feeding ecology of a species. In addition, gut content analysis is typically considered as the best method (Hyslop, 1980) to understand the feeding ecology because it is a relatively simple approach but a good source of information about the feeding habits (Ducommun, Beltzer, Virgolini & Quiroga, 2010; Kaifu, Miyazaki, Aoyama, Kimura & Tsukamoto, 2013). These methods have been applied successfully in many fishes such as *Mugil cephalus*, *Selene peruviana* and *Eucinostomus currani* (Ramírez-Luna, Navia & Rubio, 2008), *Rastrelliger kanagurta* (Sivadas & Bhaskaran, 2009), *Terapon jarbua* (Manoharan, Gopalakrishnan, Varadharajan, Thilagavathi & Priyadharsini, 2012) and *Cyprinion mhalensis* (Ahmad, Alharthi, Albalawi & Alakel, 2013).

Fishes are among the most feasible organisms for ecological assessment with multiple methods and biomarkers (Frame & Dickerson 2006; NRC 1991; Senarat, Kettratad, Poolprasert, Jiraungkoorskul & Yenchum, 2015; Senarat *et al.*, 2018, 2020). In particular, quantitative histopathology is a suitable and sensitive method to assess the fish health under stressful conditions and for environmental illnesses (Dalzochio, Zimmermann, Petry, Gehlen & da Silva, 2016; Senarat *et al.*, 2020; Wedderburn *et al.*, 2000). There have been many reports of histopathological alterations in fish from polluted areas, such as vacuolar degeneration of hepatocytes and severe lipidosis of *Micropterus salmoides* liver from the polluted Pigeon River, USA (Teh, Adams & Hinton, 1997). Senarat *et al.* (2015) reported the degeneration of kidney and liver of *Hemibagrus filamentus* living in the Tapee River, Thailand, which is located near the agriculture area. Ovarian degeneration and atresia have also been found in fish living in polluted areas (Senarat *et al.*, 2020). Severe alterations including aneurysms, edema, hyperplasia, and lamellar fusion of gill were observed in *Luciobarbus bocagei*, *Pseudochondrostoma* sp., and *Oncorhynchus mykiss* inhabiting a highly toxic area of Northern Portuguese rivers, Portugal, polluted with heavy metals (Fonseca *et al.*, 2017).

The shortfin molly *Poecilia mexicana* is an ovoviparous live-bearing poecilid that inhabits a wide range of habitats with freshwater, blackish water and seawater. This fish is a popular ornamental fish because of its fascinating abilities, including high survival in freshwater, tolerance to brackish water, rapid reproduction, and easy breeding to produce a lot of fries (Bragance, Ramos-Junior, Guimaraes, & Ottoni, 2019). However, these abilities can also degrade the local ecological structure (Bragance *et al.*, 2019) if they have an overlap in the food web with existing species. A previous study has suggested that the principal diet of *P. mexicana* from central Mexican Gulf consists of algae (Rafael, Arturo, & Horacio, 2015); however, basic information about the digestive biology and health status of this fish in Thailand is very limited. It should be emphasized that fish in the same genus, and even the same species from different habitats, can have different feeding ecology in different environments (Kaifu *et al.*, 2013). We therefore aimed to investigate the digestive biology of *P. Mexicana* invading the small brackish water in Samut Prakan province, Thailand. Because this area is close to industrial and urban areas, we also conducted a health assessment using histopathology. The results help us understand the feeding ecology of this species in conjunction with its health status in the local aquatic ecosystem.

2. Materials and Methods

Forty individuals of *P. mexicana* were obtained from Suksawadi Canal (No. 84), Samut Prakan province, Thailand, using a fine mesh in a single sampling trip in February 2020. All fish were euthanized by rapid cooling (Wilson, Bunte, & Carty, 2009), by immersion in cold water (2 to 4 °C) from water/ice 1:1 mix for about 20 min. Total length (TL), total weight (TW) and standard length (SL) were measured from all fish. Environmental parameters in this study include average salinity 10.5 ± 0.03 ppt, average temperature 30.2 ± 0.12 ºC and average pH 7.50 ± 0.01. 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 animals, prepared by the Chulalongkorn University (Protocol Review No. 1923026).

2.1 Morphology and histology of digestive system

The whole fish (n = 5 each; 10 representative individuals were separated into two size groups, 2.00-4.00 cm and 4.01-6.00 cm, hypothesizing different digestive morphology and gut contents) was kept in Davidson's fixative solution for 48 hr at room temperature and then transferred to 70% EtOH. To study the morphology and histology of the digestive system, all samples were investigated for their gross anatomies and processed by the histological procedures described by Presnell & Schreibman (1997) and Suvarna, Layton & Bancroft (2013). Paraffin blocks were longitudinally sectioned to 4 µm thickness using a microtome (Leica Biosystems) and then stained with Harris's hematoxylin and eosin (H&E). Analysis of the H&E-stained sections was conducted for the digestive system and other important organs including gill, kidney, brain, and spleen under a light microscope. Photographs were taken using an Olympus CX31 digital camera, Germany.

2.2 The gut content analysis

Thirty fish were fixed as a whole in Davidson's fixative solution for 48 hr at room temperature and then were kept in 70 % EtOH. After the fixation, the lengths of their digestive tracts were measured to determine the intestine coefficient (IC). The IC was calculated as intestine length (IL)/SL (Angelescu & Gneri 1949). The gut contents were then longitudinally dissected out from all the fish and observed under Dino Eye Piece AM-423C equipped with a digital camera. The composition of gut contents was identified according to the guidelines of Tomas (1997) and Casanova & Boltovskoy (1999). The percentages by volume (%V) were computed following the standard guidelines of Pinkas, Oliphant & Iverson (1971) and Hyslop (1980).

3. Results and Discussion

3.1 Morphometric observations

All morphometric data of *P. mexicana* are shown in Table 1. Based on the total length of fish, we classified them into two size groups including 2.00-4.00 and 4.01-6.00 cm. The average total lengths of these groups were 3.37 ± 0.30 cm and 4.71 ± 0.44 cm, respectively.

3.2 Morphology and gravimetric analysis of digestive system

A small terminal mouth was observed with close-set villiform teeth in both upper and lower jaws (Figures 1A-1B). The gill raker was projected from the branchial arch (Figure 1C), and the number of gill rakers varied from 21 to 23 slits. The shape of gill rakers was similar to short conical knobs, which is known to be associated with the feeding habit preferring small prey (Bentz, 1976; Gibson, 1988; King & Macleod, 1976; Mok & Munro, 1991; Rodriguess & Menin, 2005).

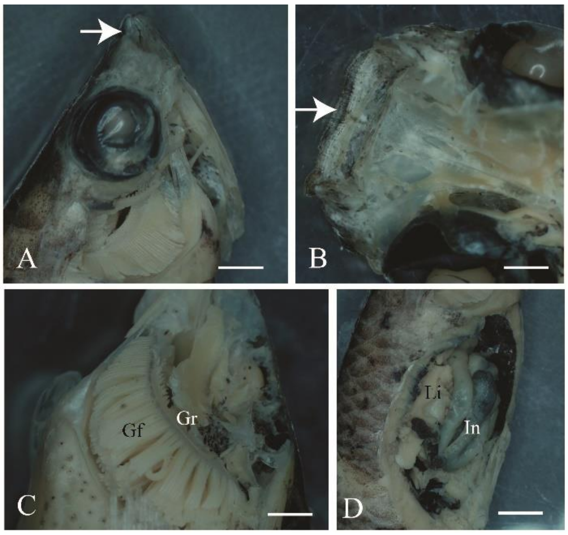

Figure 1. Morphological observation of the digestive system in *Poecilia mexicana*. Representative images showing the terminal mouth (A, arrow) and teeth arrangement of upper jaw (B, arrow). A short conical knob-like gill raker (Gr) and gill filament (Gf) were observed (C). Within the peritoneal cavity (D), the liver (Li) and long intestine (In) were found.

Well-developed pharyngeal teeth were found in the pharynx, and a straight and thick esophagus was connected with the pharynx. No stomach was anatomically identified. A long and thin intestine was clearly found (Figure 1D). The average length of digestive tract (from esophagus to intestine) is shown in Table 1. The intestine coefficient (IC) was 1.64 ± 0.37 and 2.23 ± 0.38 for the 2.00-4.00 cm and 4.01-6.00 cm groups, respectively. It is well known that IC reflects the trophic level in fish; the IC is reported to be 1.25 in carnivorous *Leporinus friderici* and 1.14 in *L. taeniofasciatus* (Albrecht, Ferreir & Caramaschi, 2001), whereas the IC is above 2.0 in omnivores *Ctenopharyngodon idellus* (Nie & Hong, 1963). The IC values of 1.64 - 2.23 suggest that *P. mexicana* is an omnivorous fish.

3.3 Histology of the digestive tract and accessory organs

We also histologically examined the digestive system of *P. mexicana*. It consisted of two distinct parts, the digestive tract (oral cavity to the intestine) and the accessory organs (liver, pancreas and gall bladder).

The prominent teeth in the stratified epithelium were covered the maxillary (upper jaw) and the mandible (lower jaw) (Figure 2A). This teeth arrangement is similar to those of some other fishes such as *Sungei Buloh* (Mok & Munro, 1991) and *Neostethus lankesteri* (Palasai, 2016) that feed on small aquatic animals (Mok & Munro, 1991), suggesting that small aquatic animals are the major prey of *P. mexicana*. The barely developed tongue supported by the hyaline cartilage (Figure 2B) closely resembled the structure of herbivore (Senarat *et al.*, 2015). This is in contrast to carnivorous *Esox Lucius*, whose tongue is well developed containing apex, body and root (Sedeghinzad *et al.*, 2014). The pharynx contained pharyngeal teeth plates (Figure 2C) and prominent pharyngeal villiform teeth (Figures 2D). The pharyngeal teeth are known to act as a grinding mill (Tibbetts & Carseldine, 2005). Hence, we speculate that *P. mexicana* might masticate the prey rather than directly swallowing them into the intestinal tract. Between the pharynx and esophagus, there was a clearly observable taste bud (Figure 2E). At the junction of pharynx and esophagus, the stratified squamous epithelium of the pharynx abruptly changed to the simple squamous epithelium (Figure 2D). Several mucous-secreting cells were observed in esophagus (Figure 2E). Histologically, esophagus was a narrow tube consisting of four main layers: the mucosa, submucosa, muscularis, and serosa (Figure 2F). The clear longitudinal fold was abundantly observed, forming finger-like projections consisting of mucosa and submucosa (Figure 2F). The mucosal layer contained squamous

Table 1. Summary of average morphometric data (mean±SD) on *Poecilia Mexicana* from Thailand

	Total length (TW)	Standard length (SL)	Total weight (TW)	Intestine length (IL)	Intestine coefficient (IC)
Group 1 (2.00-4.00 cm. in total length, n = 12)	3.37±0.30	2.64±0.22	0.59±0.13	5.52±1.32	1.64±0.37
Group 2 (4.01-6.00 cm. in total length, n = 18)	4.71±0.44	3.65±0.35	1.63±0.48	10.59±2.34	2.23±0.38

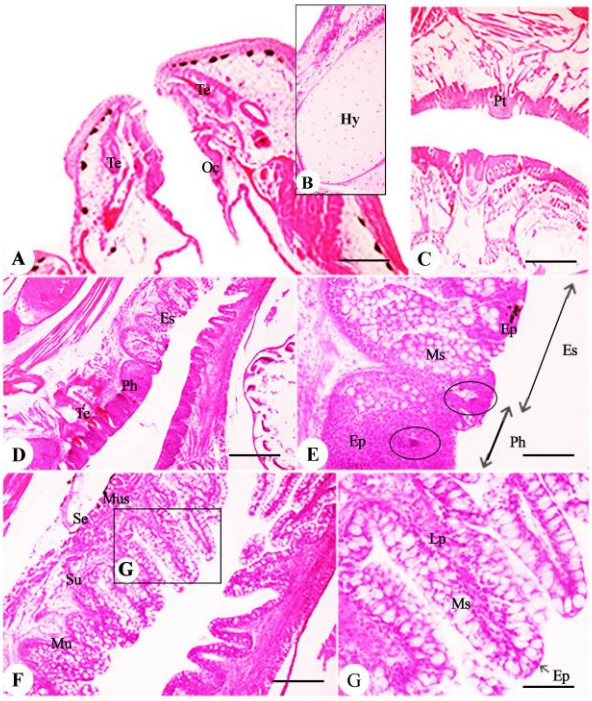

Figure 2. Light microscope observations of the digestive tract of *Poecilia mexicana*. Representative images of mouth lining with teeth (Te) within the oral cavity [Oc] (A), hyaline cartilage (Hy) of tongue (B), pharyngeal teeth [Pt] (C), conjunction between pharynx (Ph) and esophagus (Es) showing several teeth (Te) (D), and high magnification showing the transition of stratified epithelium (Ep) in pharynx to simple squamous epithelium (Ep) in esophagus (Es) (E). Esophageal wall was composed of four layers including mucosa (Mu), submucosa (Su), muscularis (Mus) and serosa (Se) (F). The simple squamous epithelium (Ep), mucous secreting cell (Ms) and lamina propria (Lp) of mucosal esophagus were also observed (G). Circles = taste buds

epithelium, mucous-secreting (goblet) cells and lamina propria, a thin layer of connective tissue (Figure 2G). The goblet cells are considered to play a role in lubrication, immunological responses against bacterial infections, and osmoregulation (Harder, 1975; Cataldi, Cataudella, Monaco, Rossi & Tancioni, 1987). A rich anastomosing network of muscularis (Figure 2F) had two layers of smooth muscle: an inner circular layer and an outer longitudinal layer, which play a major role in the movement of food items.

The intestine was classified into anterior and posterior intestines (Figure 3). The composition of intestinal wall was similar to that of esophagus, but the mucosal layer of the anterior intestine was covered with a simple ciliated columnar epithelium and contained a higher number of mucus-secreting cells than esophagus (Figures 3A-3B). Compared to the anterior intestine, the posterior intestine had less folding and rarely formed a finger-like structure, but had a high number of mucus-secreting cells in the mucosal surface observed (Figure 3C-3D). Since the intestinal mucus-secreting cells are known to play a key role in lubrication, transport of food particles, defecation, and protection of the epithelial layer rather than absorptive functions (Murray, Wright, & Goff, 1994; Purushothaman *et al.*, 2016), the involvement of increased mucous-secreting goblet cells may be a protective barrier for mucosal layer and lubrication leading to fecal expulsion (Murray *et al.*, 1994).

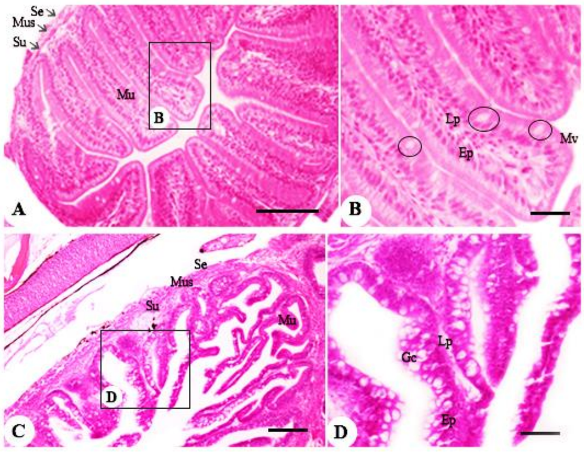

Figure 3. Light microscope observations of the anterior intestine (A-B) and posterior intestine (C-D) of *Poecilia mexicana*. Abbreviations: Ep = epithelium, Lp = lamina propria, Mv = microvilli, Mu = mucosa, Mus = muscularis, Se = serosa, Su = submucosa, circles = taste buds

3.4 Accessory organs

The liver and pancreas of *P. mexicana* were considered to be accessory digestive organs. The liver plays multiple roles including glycogen storage, detoxification and metabolism (Genten *et al.*, 2009). The hepatic cords or lobules were extremely difficult to identify in the liver tissue, but a complex network of hepatocytes and the central vein was observed (Figure 4A). It is well-known that glycogen is an energy reserve found in hepatocytes, as reported in *Ictalurus punctatus* (Hunton & Pool, 1976), *Oreochromis niloticus* (Vicentini *et al.*, 2005) and *Hippocampus barbouri* (Senarat *et al.*, 2021). An anastomotic network of sinusoids was formed between hepatocytic cords (Figure 4A). The pancreas was histologically inserted into the hepatic tissue, forming the "hepatopancreas" (Figure 4B). The pancreatic exocrine glands were observed as a small island of acinar cells or secretory acini (Figure 4B). A key role of this cell is to produce digestive enzymes for the breakdown of food (Buddington & Kuzmina, 2000). Portal afferent veins were also found.

3.5 Gut content analysis

Our observations showed that *P. mexicana* with standard length of 2.0-4.0 cm feeds on various types of food (Figure 5A). The detritus or pieces of plants accounted for the largest proportion (99.98%). Cyanobacteria, diatoms and dinoflagellates were minor contributions that occupied only 0.02%. Within the minor composition, diatoms showed the highest proportion (50.73%), followed by cyanobacteria (39.67 %) and dinoflagellates (9.59%). These data are similar to those on *P. mexicana* living in the central Mexican Gulf (Rafael *et al.*, 2015). No eggs or juvenile fish were observed in *P. mexicana* gut. This situation was similar in the fish with standard length of 4.1-6.0 cm (Figure 5B). Diatoms were the

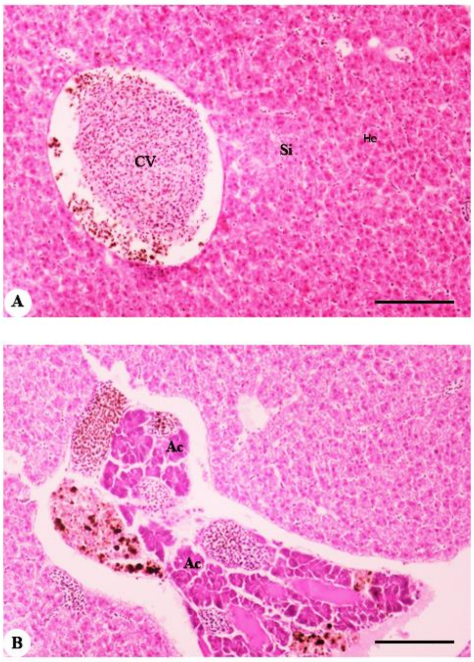

Figure 4. Light microscope observations of the liver (A) and pancreas (B) of *Poecilia mexicana*. Abbreviations: Ac = acinar cell, Cv = central vein, He = hepatocyte, Si = sinusoid

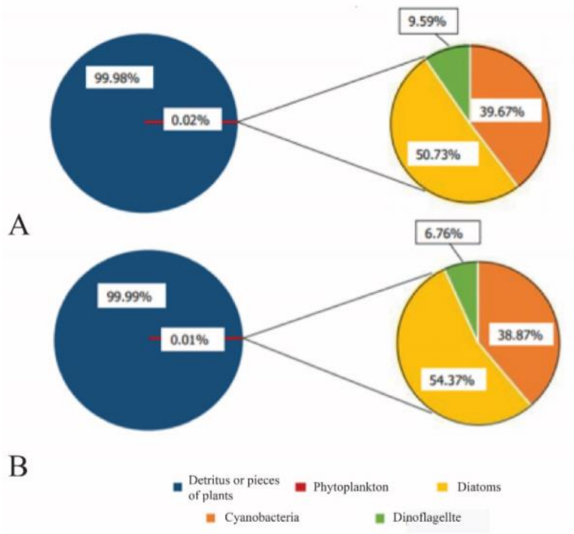

Figure 5. Gut content analysis of *Poecilia mexicana* grouped by lengths in 2.0-4.0 cm (A) and in 4.1-6.0 cm (B)

most abundant (54.37 %), followed by cyanobacteria (38.87 %) and dinoflagellates (6.76 %). Livinsgton (1982) reported that the juvenile molly fish do not have a specific dietary requirement, but adult fish select specific food items. Results from this study suggest that adult *P. mexicana* can also survive on non-specific diets. Diatoms occupied the higest percentage of phytoplankton, possibly reflecting their dominance in the river, where the nutrients are supplied from river discharges, sewage, and water circulation process (Thongdonphum, Meksumpun, Meksumpun, Sawasdee, & Kasemsiri, 2010). Pollution Control Department (2019) also reported that the biochemical oxygen demand (BOD) of this river and NH_3-N content 1.8 - 8.2 mg/L and 0.24 - 3.19 mg/L, respectively, reflecting high contents of dissolved organic matter. The high abundance of diatoms in the *P. mexicana* gut hence may be related to high nutrient loads by anthropogenic activities. Overall, results of the gut content analysis suggest that *P. mexicana* could be a detritus feeder in the small canal in Thailand, which is different from previous observations. Namely, Rafael *et al.* (2015) reported that *P. mexicana* inhabiting in the central Mexican Gulf is primarily herbivore consumer, whereas this species is planktophagous and insectivorous in a temporary pool near Tlacotalpan, in the Mexican southeast (Tessy, Sharon, & Omar, 2021). Since the diet composition of this fish varies depending on the environment, this species should have the ability to adapt to different trophic levels (Trujillo-Jiménez & Toledo, 2007).

3.6 Histopathological examination

Our histolopathological examination showed that coagulative necrosis in the liver was rare in both groups with 20% prevalence (Figures 6A-6B), but acinar necrosis was prominent (100%, Figure 6C). The renal degeneration in the kidney (Figure 6D), as well as edema (Figure 6E) and aneurysms in gill (Figure 6F) were found in about 20% of the specimens. It is reported that exposure to high levels of heavy metals could damage the gills causing edema and aneurysms (Fonseca *et al.*, 2016). These are suggested to be defensive mechanisms (Arellano, Storch, & Sarasquete, 1999; Nimet, Neves, Viana, Amorim, & Delariva, 2020).

The melanomacrophage center (MMC) is a group of brown cells commonly embedded in various organs including pancreas (Figure 7A), mesentery (Figure 6B), granuloma (Figures 7C-7D), brain (Figure 7E), kidney (Figure 7F-7G) and spleen (Figure 7I). MMCs were found in both examined groups in this study. It is possible that MMCs are indicative of environmental contaminant exposure and infection. Roganovic *et al.* (1998) reported that increased MMCs are associated with contaminant exposure and parasitic infestation; the MMCs are also linked to other factors such as sex and stock density (Steinel & Bolnick, 2017).

Integrating the histopathological changes observed in the liver, pancreas, kidneys and gills of *P. mexicana*, the fish used in this study may be exposed to chemical contaminants or environmental stress. However, very little is known of the current condition of this river because of the lack to environmental studies. Only aforementioned BOD and NH_3-N contents are reported, which should be associated with high nutrient loads and anthropogenic activities. Further studies on the water quality along with the histopathological analysis on *P. mexicana* living in the small canal are warranted.

4. Conclusions

In this study, we showed morphological and histological characteristics of the digestive system in *P. mexicana*. This fish is considered to be a detritus feeder and generalist feeder in the small canal of Thailand because the detritus or pieces of plants accounted for the highest proportion of the gut content (99.98%). The feeding ecology of *P. Mexicana* thus does not clearly indicate the direct impact of this invasive species in the trophic system of the canal, but further study will be required in the fields of population ecology and reproductive biology to fully understand its ecological role. Histopathological alterations, especially necrotic acinar cells of pancreas and MMCs, suggest potential health problems of this species in the habitat studied.

Acknowledgements

This research was supported by the Department of Marine Science and the Faculty of Science, Chulalongkorn University. The authors are thankful to Mr. Somnuk Guta from Department of Pathobiology, Faculty of Science, Mahidol University, for his technical assistance.

References

Ahmad, Z., Alharthi, I., Albalawi, H., & Alakel, A. (2013). Studies on the feeding ecology of *Cyprinion mhalensis* dwelling in Wadi Bua, Taif, Saudi Arabia. *Pakistan Journal of Zoology*, *45*(2), 351-358.

Albrecht, M. P., Ferreir, N. F. N. & Caramaschi, E. P. 2001. Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; Anostomidae). *Journal of Fish Biology*, *58*, 419-430.

Angelescu, V., & Gneri, F. S., (1949). Adaptaciones del aparato digestivo al regime alimentício en algunos peces del rio Uruguay y del rio de La Plata. *Revista del Instituto Nacional de Investigación de las Ciencias Naturales y Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Ciencias zoológica*, *1*, 161-281.

Arellano, J. M., Storch, V., & Sarasquete, C., (1999). Histological changes and copper accumulation in liver and gills of the Senegales sole, *Solea senegalensis*. *Ecotoxicology and Environmental Safety*, *44*(1), 62–72.

Bentz, K. L. M., (1976). Gill arch morphology of the Cape Hakes *Merluccius capensis* Cast and M. *Paradoxus Franca*. *Fish Bulletin South Africa*, *8*. 17-22.

Bragance, P. H. N., Ramos-Junior, C. C., Guimaraes, E. C., & Ottoni, F. P., (2019). Identification of the Mexican Molly, *Poecilia mexicana* (Cyprinodontiformes: Poeciliidae), introduced in Brazil through α-taxonomy and DNA barcoding. *Cybium*, *43*(4), 331-340.

Buddington, R., & Kuz'mina, V. (2000). Digestive system. In G. K. Ostrander (Ed.), *The laboratory fish* (pp. 379–384). San Diego, CA: Academic Press.

Casanova, J., & Boltovskoy, D., (1999). *South Atlantic Zooplankton*. Leiden, The Netherlands: Backhuys.

Cataldi, E., Cataudella, S., Monaco, G., Rossi, A., & Tancioni, L., (1987). A study of the histology and morphology of the digestive tract of the sea-bream, *Sparus aurata*. *Journal of Fish Biology*, *30*(2), 135-145.

Ducommun, M. D., Beltzer, A. H., Virgolini, A. L. R., & Quiroga, M. A., 2010. Feeding ecology of *Cocoi Heron* (Ardea cocoi) in the flood valley of the Parana River. *Avian Biology Research*, *3*(3), 115-121.

Dalzochio, T., Zimmermann, G., Petry, I. E., Gehlen, G., & da Silva, L. B. (2016). The use of biomarkers to assess the health of aquatic ecosystems in Brazil: A review. *Journal Aquatic Research*, *8*, 289-298.

Fonseca A. R., Fernandes, L. F. S., Fontainhas-fernandes A., Monteiro, S. M., & Pachecobeet, F. A. L., (2016). From catchment to fish: Impact of anthropogenic pressures on gill histopathology. *Science of the Total Environment*, *550*, 972-986.

Frame, L., & Dickerson, R. L. (2006). Fish and wildlife as sentinels of environment contamination. In D. O. Norris & J. A. Carr (Eds.), *Endocrine disruption: Biological bases for health effects in wildlife and humans* (pp. 202–222). New York, NY: Oxford University Press.

Garvey, J. E., Dingledine, N. A., Donovan, N. S., & Stein, R. A., (1998). Exploring spatial and temporal variation within reservoir food webs: Predictions for fish assemblages. *Ecological Applications*, *8*, 104-120.

Genten, E., Dangay, A., 2009. *Atlas of fish histology*. Enfield, NH: Science.

Gibson, R. N., (1988). Development, morphometry and particle retention capability of the gill rakers in the herring *Clupea harengus* L. *Journal of Fish Biology*, *32*, 949-962.

Harder, W., (1975). The digestive tract. In anatomy of fishes, part 1. Stuttgart: Schweizerbart'sche Verlags buchhandlung.

Hinton, D. E., & Pool, C. R. (1976). Ultrastructure of the liver in channel catfish, Ictalurus punctatus (Rafinesque). *Journal of Fish Biology, 8*(2), 209-219.

Hyslop, E., (1980). Stomach content analysis – a review of methods and their application. *Journal of Fish Biology*, *7*, 411-429.

Kaifu, K., Miyazaki, S., Aoyama, J., Kimura, S., & Tsukamoto, K., (2013). Diet of Japanese eels Anguilla japonica in the Kojima Bay-Asahi River system, Japan. *Environmental Biology of Fishes*, *96*(4), 439-446.

King, D. P. F., & Macleod, P. R. (1976). Comparison of the food and filtering mechanism of Pilchard (*Sadinops ocellata*) and Anchovy (*Engraulis capersis*) of South-West Africa. *Investigational report - Sea Fisheries Branch*, *111*, 29.

Livinsgton, R. J. (1982). Trophic organization of fishes in a coastal seagrass system. *Marine Ecology Progress Series*, *7*(1), 1–12.

Manoharan, J., Gopalakrishnan, A., Varadharajan, D., Thilagavathi, B., & Priyadharsini, S., (2012). Stomach content analysis of *Terapon jarbua* (Forsskal) from Parangipettai coast, South East Coast of India. *Advances in Applied Science Research*, *3*(5), 2605-2621.

Melo Germano, R., Stabille, S. R., Britto Mari, R., Pereira, J. N. B., Faglioni, J. R. S., Neto, M., & Hubner, M., (2014). Morphological characteristics of the *Pterodoras granulosus* digestive tube (Valen ciennes, 1821) (Osteichthyes, Doradidae). *Acta Zoology*, *95*(2), 166-175.

Mok, E. Y. M., & Munro, A. D., (1991). Observations on the food and feeding adaptations of four species of small pelagic teleosts in streams of the *Sungei Buloh* mangal, Singapore. *Raffles Bulletin of Zoology, 39*(1), 235-257.

Murray, H. M., Wright, G. M., & Goff, G. P., (1994). A comparative histological and histochemical study of the stomach from three species of pleuronectid, the Atlantic halibut, *Hippoglossus hippoglossus*, the yellowtail flounder, *Pleuronectes ferruginea*, and the winter flounder, *Pleuronectes americanus*. *Canadian Journal of Zoology*, *72*(7), 1199-1210.

Nie, D. S., & Hong, S. F., (1963). The histology of the digestive tract of the grass carp (*Ctenopharyn godonidellus*). *Acta Hydrobiologia Sinica*, *3,* 1-25.

Nimet, J., Neves, M. P., Viana, N. P., Amorim, J. P. A., & Delariva, R. L. (2020). Histopathological alterations in gills of a fish (*Astyanax bifasciatus*) in neotropical streams: negative effects of riparian forest reduction and presence of pesticides. *Environmental Monitoring and Assessment*, *192*, 58

National Research Council. (1991). *Animals as sentinel of environmental health hazards*. Washington, DC: National Academy Press.

Palasai, A. 2016. Feeding ecology of priapiumfish *Neostethus lankesteri* regan, 1916 in Sirinart Rajini mangrove ecosystem learning center and Pranburi river, Prachuap Khiri Khan province. Retrieved from http://cuir.car.chula.ac.th/bitstream/123456789/64942/1/5672147423.pdf

Pinkas, L., Oliphant, M., & Iverson, L., (1971). Food habits of Albacore Bluefin Tuna and Bonito in California waters. California Depart. *Fish Game: Fisheries Bulletin*, *152*, 1-105.

Pollution Control Department. (2019). Thailand state of Pollution Report 2014. Bangkok, Thailand: Author.

Presnell, J. K., & Schreibman, M. P., (1997). *Humason's animal tissue techniques* (5th ed.). Baltimore, MD: The Johns Hopkins University Press.

Purushothaman, K., Lau, D., Saju, J. M., Musthaq Sk, S., Lunny, D.P., Vij, S., & Orban, L., (2016). Morpho-histological characterisation of the alimentary canal of an important food fish, Asian seabass (*Lates calcarifer*). *PeerJ*, *4*, e2377.

Rafael, C. L., Arturo, R. R., & Horacio, C. G., (2015). Some ecology features of *Poecilia mexicana* Steindachner, 1863 (Osteichthyes: Poeciliidae) from Alvarado Lagoonal System, Veracruz, Mexico. *American Journal of Life Sciences*, *3*(2), 76-84.

Ramírez-Luna, V., Navia, A. F., & Rubio, E. A., (2008). Food habits and feeding ecology of an estuarine fish assemblage of northern Pacific Coast of Ecuador. *Pan-American Journal of Aquatic Sciences*, *3*(3), 361-372.

Rodriguess, S. S., & Menin, E., (2005). Anatomia da cavidade bucofaringeana de *Conorhynchos conirostris* (Valen ciennes, 1984) (Siluriformes). *Ceres, 52*, 843-862.

Roganovic-Zafirova, D., & Jordanova, M., (1998). Histopathological analysis of liver from Ohrid roach (*Rutilus rubilio ohridanus*) collected in Gransnica, a contaminated lake site of Lake Ohrid. *Maced Ecology*, *5,* 530-544.

Sedeghinzad, J., Rahmati-holasoo, H., Fayyza, S., & Zargar, A. (2014). Morphological study of the northern pike *Esox lucius* tongue. *Anatomical Science International*. doi:10.1007/s12565-014-0254

Senarat, S., Kettratad, J., Poolprasert, P., Jiraungkoorskul, W., & Yenchum, W. (2015). Histopathological findings of liver and kidney tissues of the yellow mystus, *Hemibagrus filamentus* (Fang and Chaux, 1949), from the Tapee River, Thailand. *Songklanakarin Journal of Science Technology*, *37*(1), 1-5.

Senarat, S., Kettretad, J., Poolprasert, P., Tipdomrongpong, S., Plumley, F. G., & Jiraungkoorskul, W. (2018). Health status in wild and captive *Rastrelliger brachysoma* from Thailand: Histopathology. *Songklanakarin Journal of Science Technology*, *40*(5), 1090-1097.

Senarat, S., Kettratad, J., Siriwong, W., Bunsomboonsakul, S., Kenthao, A., Kaneko, G., . . . Jiraungkoorskul, W. (2020). Oogenesis and ovarian health problems in economically important fishes from different habitats potentially affected by pollution in Thailand. *Asian Fisheries Science*, 33 (3), 274-286.

Senarat, S., Sujittosakul, R., Kettratad, J., Pairohakul, S., Kaneko, G., & Jiraungkoorskul, W. 2021. Ultrastructure of hepatocyte and liver ontogeny of the Indo-Pacific seahorse *Hippocampus barbouri* Jordan & Richardson 1908. *Journal of Advanced Veterinary Research, 11*: 136-140.

Sivadas, M., & Bhaskaran, M., (2009). Stomach content analysis of the Indian mackerel *Rastrelliger kanagurta* (Cuvier) from Calicut, Kerala. *Indian Journal of Fisheries, 56*(2), 143-146.

Steinel, N. C., & Bolnick, D. l., (2017). Melanomacrophage centers as a histological indicator of immune function in fish and other poikilotherms. *Frontiers in Immunology*, *8*, 827.

Suvarna, S. K., Layton, C., & Bancroft, J. D., (2013). *Bancroft's theory and practice of histological techniques*. Canada: Elsevier.

Teh, S. J., Adams, S. M., & Hinton, D. E., (1997). Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquatic Toxicology*, *37*(1), 51-70.

Thongdonphum, B., Meksumpun, S., Meksumpun, C., Sawasdee, B., & Kasemsiri, P., 2013. Predictive model for biochemical component of phytoplankton in the river and estuarine systems of the Mae Klong River, Thailand. *International Journal of Engineering Research and Development*, *4*(1), 13-18.

Tibbetts, I. R., & Carseldine, L., 2005. Trophic shifts in three subtropical Australian halfbeaks (Teleostei: Hemiramphidae). *Marine and Freshwater Research, 56*(6), 925-932.

Tomas, C. R., (1997). *Identifying marine phytoplankton*. Florida, FL: Academic Press.

Vicentini, C. A., Franceschini-Vicentini, I. B., Bombonato, M. T. S., Bertolucci, B., Lima, S. G., & Santos, A. S. (2005). Morphological study of the liver in the teleost Oreochromis niloticus. *International Journal of Morphology, 23*(3), 211-216.

Wedderburn, J., Mcfadzen, I., Sanger, R.C., Beesley, A., Heath, C., Hornsby, M., & Lowe, D., (2000). The field application of cellular and physiological biomarkers, in the mussel *Mytilus edulis*, in conjunction with early life stage bioassays and adult histopathology. *Marine Pollution Bulletin, 40*(3), 257-267.

Wilson, J. M., Bunte, R. M., & Carty, A. J. (2009). Evaluation of rapid cooling and tricainemethanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science, 48*(6), 785-789.