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Research article

Monitoring of reproductive health in the striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) from the Saen Saep Canal, Thailand

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Abstract

Khlong Saen Saep is one of the most important and large canal systems in Bangkok, Thailand. However, the emergence of heavily polluted water has occasionally been reported due to the domestic sewage and industrial effluents discharged into the system. This situation may affect the reproductive status of aquatic lives residing in this canal. To evaluate the reproductive status of the canal inhabitant, the striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878)—a common dweller of the Saen Saep Canal—was collected from two selected stations, the Bumpen Nua Temple and the Kamalun Islam Mosque. The fish gonads were then anatomically and histologically examined. The results indicated that neither parasites nor ovary with external abnormality were found. Nevertheless, several histological alterations were detected, including vacuolar degeneration of previtellogenic oocytes, accumulation of melanomacrophage centers (MMCs), and atretic oocytes. Moreover, severe histopathological changes, such as the eosinophilic cytoplasm of spermatogonia, MMCs, blood congestion, and syncytium of spermatozoa, were observed in the fish testis. Our study suggested that the environmental stress and pollutions previously reported from the Saen Saep Canal possibly underpin the gonadal impairments of *P. hypophthalmus* examined and may have adverse impact on the reproductive health of others living in this canal system.

Keywords: Histopathology, Pollutant, Reproductive system, Sentinel species, Water quality

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INTRODUCTION

An economically important waterway of Bangkok, Thailand, Khlong Saen Saep is one of the most prominent canals that provides a great support to people living in both urban and rural areas in the form of public transportation and a source of water for domestic, agricultural, and industrial utilization (Pollution Control Department, 2018, 2019). However, overexploitation of this canal by many anthropogenic activities results in its gradual deterioration as shown by the emergence of severe pollution and inhospitable condition (Pollution Control Department, 2018, 2019; Sawat-Uea, 2010). Previous investigations showed that various chemical contaminants, particularly heavy metals, were found and only small amount of dissolved oxygen (DO) of approximately 2.4 mg/L in average could be detected from this canal (Department of Drainage and Sewerage, 2020). In addition, different levels of DO were recently reported from various areas of this canal, such as 1.57 mg/L in Bumpen Nua Temple and 3.72 mg/L in Kamalun Islam Mosque (Puttipong et al., 2020). Given the standard DO concentration of 3.0 mg/L for inhabitable system of aquatic lives, it is highly possible that the Saen Saep Canal has become an environmentally sensitive area. Therefore, living in such ecosystem can cause harmful effects on the health of aquatic animals.

Due to their susceptibility and ease of sample collection, fishes are among one of the most popular organisms, which have long been widely used as sentinel species for monitoring ecological changes (Beeby, 2001; Christophe et al., 2015; Frame and Dickerson, 2006; National Research Council, 1991; Senarat et al., 2015, 2018a, 2018b, 2019, 2020; Singkhanan et al., 2019). Their responses in different aspects (e.g., anatomical, histological, physiological, and behavioral ones) not only reflect various environmental crises, such as water pollution, chemical contamination, and oxygen depletion, but also provide us a good early warning system for a forthcoming ecological catastrophe (Beeby, 2001). Reproductive histopathology has often been chosen as an indispensable approach in determining the reproductive status of a fish population and also a valuable biomarker in evaluating the health of a given ecosystem (Auró de and Ocampo, 1999; Dalzochio et al., 2016). This methodology involves careful examination on cellular and histological changes observed in gonadal tissues of the selected sentinel species (Lawrence and Hemingway, 2003; Senarat et al., 2015, 2017, 2018a, 2018b, 2020; Velmurugan et al., 2007). Louiz et al. (2009) investigated effects of chemicals on the reproductive organs of *Gobius niger* living in Lake Bisertee and found the existence of multiple lesions in gonadal tissues of the examined fish, suggesting the reduced reproductive health and implying the presence of other serious environmental problems.

The striped catfish *Pangasianodon hypophthalmus* is an economically important freshwater and fast-growing fish in Southeast Asia, particularly in Thailand (Neilson et al., 2018; van Zalinge et al., 2002; Wongroj and Siriwattanarat, 2021). Its abundance and long lifespan makes this fish a suitable sentinel species, especially for long-term monitoring (Wongroj and Siriwattanarat, 2021). Furthermore, *P. hypophthalmus* is widely found along the Saen Saep Canal where the environmental problems have occasionally been reported (Riyakan, 2013; Wongroj and Siriwattanarat, 2021). Therefore, the striped catfish dwelling in this canal can potentially reflect any environmental fluctuations occurring over a certain period of its exposure. In this study, we assessed the reproductive health of adult *P. hypophthalmus* from the Saen Saep Canal based on histopathological examination. Results obtained from our study reveal the current reproductive status of this fish, which can be considered a warning sign for us to the beginning of proper water management and to solve the environmental problems existing in this canal.

MATERIALS AND METHODS

Study areas and sample collection

Fresh gonads of adult *P. hypophthalmus* were obtained from previous study of Puttipong et al. (2020). All fish were randomly captured using fishing net and hook from two sampling stations, the Bumpen Nua Temple (13.800448 °N, 100.710106 °E) and the Kamalun Islam Mosque (13.8348046 °N, 100.7725242 °E), of the Saen Saep Canal. Both sampling sites are 7.75 km apart and located in different environmental conditions (Figure 1A-1C). The former is in the middle of community areas as it is densely surrounded by households, markets, schools, temples, and factories (Figure 1D). In contrast, location of the latter is in a sparsely populated region with a majority of landuse for accommodation and agricultural fields as shown in Figure 1E.

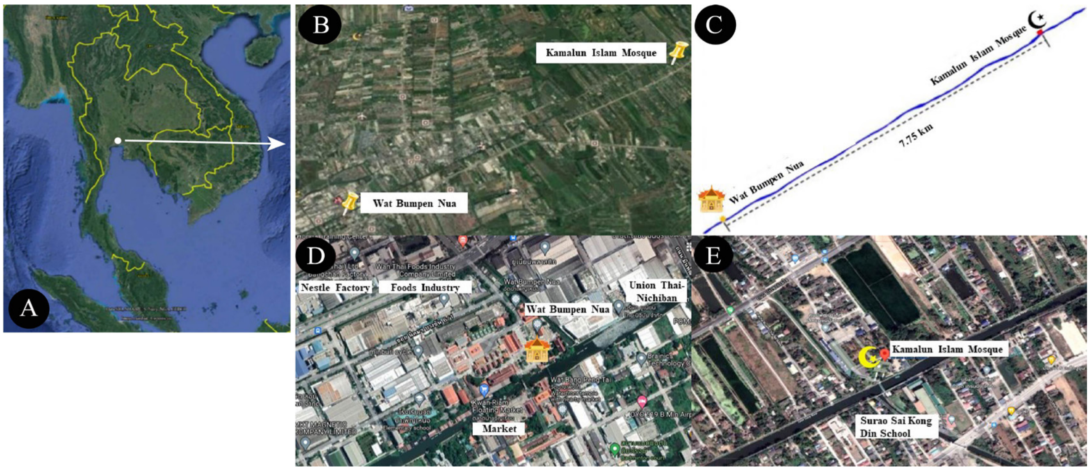

Figure 1 Images showing studied sites located along the Saen Saep Canal, Bangkok, Thailand from which the striped catfish *Pangasianodon hypophthalmus* were collected. A: Map of Thailand generated by Google Earth Pro (Google LLC., 2018) demonstrating location of the Saen Saep Canal. B: A satellite image showing the locations of the Bumpen Nua Temple and the Kamalun Islam Mosque. C: A schematic drawing illustrating the relative location and distance between the two sampling sites. D-E: Satellite images demonstrating the overview environmental conditions in the vicinity of the Bumpen Nua Temple (D) and the Kamalun Islam Mosque (E).

Seven individuals of adult *P. hypophthalmus* were collected from each studied station. 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. 1923008).

Anatomical and histological observations of gonads

Gonads were anatomically observed and photographed with a Canon PowerShot G11 (Canon Hongkong Company, Hongkong). Three pieces of gonadal tissues, each with 2 cm^3 in dimension were prepared and fixed in Davidson's fixative for 48 hours at room temperature. The fixed tissues were then processed with standard protocol for a histology preparation (Presnell and Schreibman, 1997; Suvarna et al., 2013). The paraffin embedded tissues were sectioned at 4-µm thickness and stained with Harris's hematoxylin and eosin (H&E) (Presnell and Schreibman, 1997; Suvarna et al., 2013). All histological slides of gonads were examined for sex determination, gonadal structures, and histopathological alterations.

Evaluation of gonadal histopathology was derived from microscopic observation at 40x magnification. Three slides of histological section were thoroughly examined for each pathological condition. The observed histopathology was then photographed using a ZEISS Axio Lab.A1 light microscope and an iSolution digital camera (Carl Zeiss Microscopy GmbH, Germany). The percent prevalence (P) of each histopathological alteration was calculated by the formula: $P = (N/S) \times 100$, where N is number of fish with histopathological symptom and S is the total number of fish in each sex. The prevalence values reported were calculated separately for each sampling station.

Data analyses

The fit of the distributions to datasets was assessed by the Shapiro-Wilk normality test due to our sample size of less than 50. Statistical significance of the percent prevalence of all lesions observed was determined using Two Independent Samples test with Mann-Whitney U implemented. The analysis was conducted using IBM SPSS Statistics Data Editor (The IBM SPSS Software, Armonk, New York, USA). A p value of 0.05 or less was considered statistically significant.

RESULTS

Histology for sex determination

The sex of *P. hypophthalmus* was determined based on anatomical and histological observations. The details of male and female fish were shown in Figure 2 and Table 1.

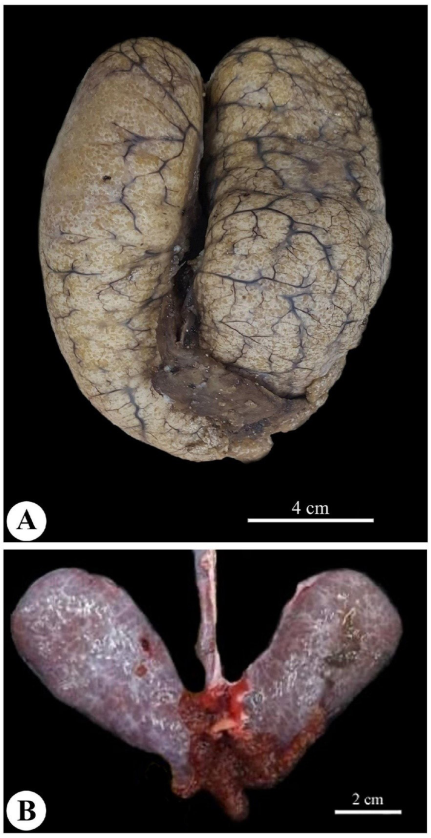

Figure 2 The anatomical observation of representative ovary (A) and testis (B) of the striped catfish *Pangasianodon hypophthalmus* collected from the Saen Saep Canal.

Table 1 Percent prevalence of histopathological alterations found in gonadal organs of adult *Pangasianodon hypophthalmus* collected from the Saen Saep Canal, Bangkok, Thailand during February to March 2019.

Histopathological alterations	Percent prevalence	
	Bumpen Nua Temple (n = 7)	Kamalun Islam Mosque (n = 7)
Ovary	**n = 2**	**n = 4**
Vacuolar degeneration	100	-
Atresia in previtellogenic stage	-	50
Melanomacrophage centers	100	100
Testis	**n = 5**	**n = 3**
Eosinophilic cytoplasm of spermatogonia	40	66.67
Melanomacrophage centers	100	100
Blood congestion	100	100
Syncytium of spermatozoa	-	100

Histopathology

Our anatomical examination revealed that the existence of parasites and gonads with external abnormality were not observed in the fish sampled from the Bumpen Nua Temple and the Kamalun Islam Mosque (Figure 2A-2B). However, at histological level, a sign of severe vacuolar degeneration of previtellogenic oocyte was found with 100% prevalence and also only in the fish collected from the temple (Figure 3A-3B, Table 1). In contrast, atresia of previtellogenic stage was observed in those of the Kamalun Islam Mosque (Figure 3C, Table 1) although the late vitellogenic ones were normally structured (Figure 3D-3E). Moreover, accumulation of melanomacrophage centers (MMCs) was identified in the fish from both stations with 100% prevalence (Figure 3C, Table 1).

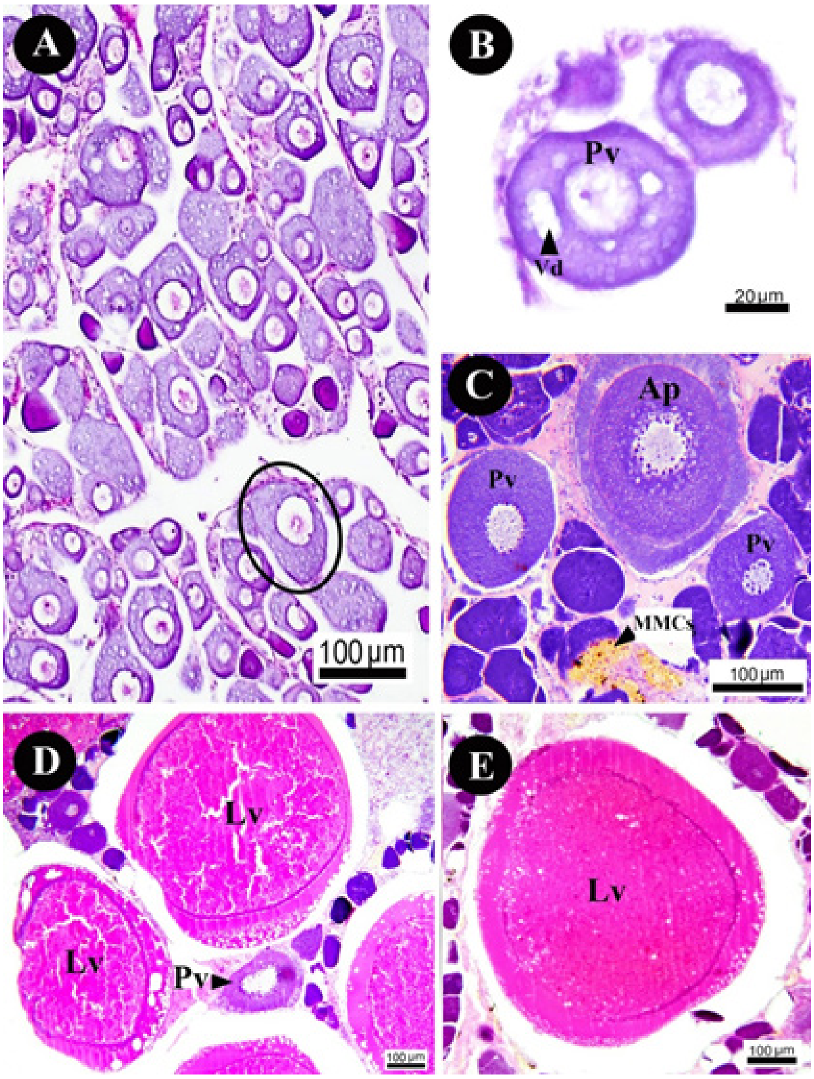

Figure 3 Light photomicrographs showing the oocyte histology and its histopathology found in the striped catfish *Pangasianodon hypophthalmus* collected from the Saen Saep Canal. A: Overview of ovarian parenchyma containing the previtellogenic stage (circle). B: Vacuolar degeneration (Vd) at higher magnification. C: Atresia of previtellogenic stage (Ap) and the presence of melanomacrophage centers (MMCs). D-E: Normal feature of late vitellogenic stage (Lv). Abbreviation: Pv = previtellogenic stage.

Testicular histopathology of the fish from the two stations was shown in Figure 4 and Table 1. This included an eosinophilic cytoplasm of spermatogonium, MMCs, and blood congestion observed in fishes from all stations (Figure 4A-4B). However, the syncytium of spermatozoa was found only in the mosque fish with 100% prevalence (Figure 4C-4D, Table 1).

Statistical analysis showed no significant difference in the percent prevalence of all lesions found in the fish sampled from the mosque and the temple.

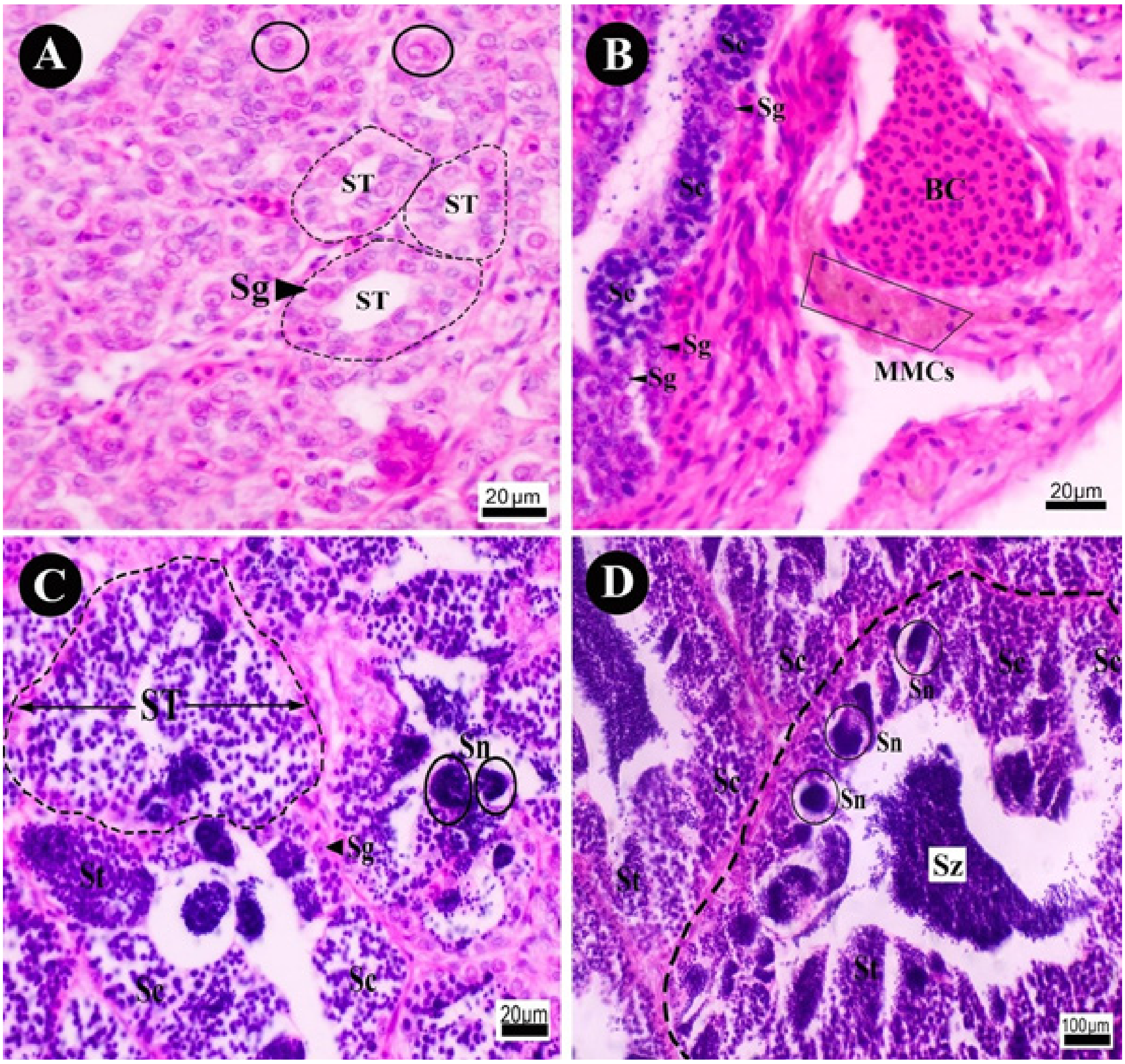

Figure 4 Light photomicrographs showing the spermatogenic histology and its histopathology found in the striped catfish *Pangasianodon hypophthalmus* collected from the Saen Saep Canal. A: The eosinophilic cytoplasm of spermatogonia (circles). B: Blood congestion (BC) and the formation of melanomacrophage centers (MMCs). C-D: The syncytium of spermatozoa shown in circles (Sn). Abbreviations: Sc = spermatocyte, Sg = spermatogonium, ST = seminiferous tubules, St = spermatid, and Sz = spermatozoa.

DISCUSSION

In this study, several histopathological changes were revealed from gonadal tissues of *P. hypophthalmus* collected from both the Bumpen Nua Temple and the Kamalun Islam Mosque of the Saen Saep Canal. This suggested unknown factors, affecting reproductive health of the fish. In addition, a number of histological incidence, including blood congestion, eosinophilic cytoplasm of spermatogonia, and melanomacrophage centers, were concurrently found in *P. hypophthalmus* from both sampling sites and no significant difference in the percent prevalence of all observed lesions from both stations was statistically documented. Therefore, it could be speculated that *P. hypophthalmus* populations at least in these two areas have currently encountered adverse environmental conditions derived from the water quality of the Saen Saep Canal.

Our study showed the vacuolar degeneration of previtellogenic oocytes observed in all fishes collected from the Bumpen Nua Temple. Such degeneration might have been occurred at the early stage of germ cell development as a result of long-term exposure of the fish to unknown pollutants. As located in a densely populated region, it is highly possible that water quality around the Bumpen Nua Temple has consistently been polluted by sewage discharged from surrounding households, shops, markets, schools, and factories. Sukharomana (2009) reported that 86.6% of wastewater released into the Saen Saep Canal was from houses and local shops, followed by automobile repair shops (6%), industrial factories (3.7%), and gas stations (2.7%). Moreover, our previous study demonstrated higher level of biochemical oxygen demand (BOD), electrical conductivity (EC), and total dissolved solid (TDS), but lower concentration of dissolved oxygen (DO) documented from the Bumpen Nua Temple water area than those of the Kamalun Islam Mosque (Puttipong et al., 2020). These parameters suggested poorer water quality of the former sampling site, which might have induced the lesion found. Various chemicals are known to have pathological effects on aquatic lives as reported by Ateeq et al. (2006) when similar vacuolation could be induced in the walking catfish, *Clarias batrachus* after its exposure to herbicides—2, 4-dichlorophenoxyacetic acid and butachlor.

Although atresia is a normal physiological process among fishes, it is also known as a pathological condition after exposure to environmental contaminants (Blazer, 2002; Cross and Hose, 1988; Johnson et al., 1988; Kirubagaran and Joy, 1988). In our study, we found this condition in previtellogenic oocytes in 50% of *P. hypophthalmus* sampled from the Kamalun Islam Mosque. As shown in Figure 1E, this area is surrounded by households and large patches of agricultural fields. It is possible that agricultural runoffs contaminated with pesticides might have partly been involved in atresia observed in this study. Suppadit and Phoochinda (2005) reported that agricultural wastewater, often contaminated with chemicals, insecticides, and organic compounds, comprised one of the three main sources of the Saen Saep Canal water pollution, in addition to domestic sewage and industrial effluents. Furthermore, Spanò et al. (2004) demonstrated the incidence of an ovarian degeneration and atresia in the ovary of the goldfish, *Carassius auratus* after its exposure to 100 and 1000 g/L of an herbicide atrazine. Similarly, the emergence of atresia in perinucleolar stage was observed after exposing the adult fathead minnow, *Pimephales promelas* to 5 µg/L and 50 µg/L of atrazine (Tillitt et al., 2010). Senarat et al. (2020) also reported that the atresia of oocytes could be found in various fishes living in polluted areas of Thailand. Moreover, it has been suggested that assembly of atretic oocytes, especially in previtellogenic follicles, is found to be associated with exposure of fishes to environmental illness (Blazer, 2002). The atretic condition found in this study might reveal the on-going environmental pollution in the Saen Saep Canal, especially in the mosque area.

In our study, several signs of cytopathological changes were observed in reproductive cells of *P. hypophthalmus*. Cytoplasmic vacuolation as occurred in previtellogenic oocytes is an indication of cellular injury (Mumford et al., 2007). This cytological alteration, especially in hepatocytes, can be induced by cellular exposure to heavy metals and insecticides (Meyers and Hendricks, 1982). Such change is normally reversible when affected cells are adapted and toxicity of the induced chemicals is also minimal (Mumford et al., 2007). However, long-time exposure to contaminants can progress the injury to a point of no return and ultimately lead to cell death (Meyers and Hendricks, 1982; Mumford et al., 2007). When happened to germ line cells, this phenomenon not only inhibits normal cellular differentiation, but also reduces gamete heath and quality. Similar consequence will be ensued to the oocytes of *P. hypophthalmus* if aquatic environment of the Saen Saep Canal remains untreated.

Melanomacrophage centers (MMCs) are groups of brown cells, which play an important role in the innate immune system (Agius and Roberts 2003; Louiz et al., 2018). An increase in MMCs often indicates the parasitic infection or contaminant exposure of the affected organisms (Roganovic-Zafirova and Jordanova, 1998). However, descriptive studies showed that formation of MMCs also involves various factors, including life history and environmental changes (Blazer et al., 1997; Kumar et al., 2016; Natalie and Daniel, 2017). Our results demonstrated the presence of the MMCs in all *P. hypophthalmus* examined although the parasitic infestation was not observed in the gonads studied both at anatomical and histological levels. This 100% prevalence of MMCs documented together with the impaired oocytes observed strongly suggested that *P. hypophthalmus* was living under certain environmental stress conditions, especially those derived from some pollutants.

Evidence of eosinophilic cytoplasm of spermatogonia was markedly found. Such phenomenon was due to major degeneration of structural proteins, leading to homogeneous eosinophilic appearance (Young et al., 2005). This intense cytoplasmic eosinophilia is recognized as a cytological characteristic of cellular necrosis (Mumford et al., 2007). Therefore, it is interesting to note that this pathology may lead to the decrease in sperm production and sperm quality as spermatogonia of the striped catfish have encountered cellular degeneration. Furthermore, the occurrence of MMCs and syncytia of spermatozoa observed in male gonads of *P. hypophthalmus* were similar to previous study by Louiz et al. (2009), which reported the same lesions found in *Gobius niger* inhabiting the polluted environment. Although formation of spermatogonial syncytia is widely known across animal phyla, complete cytoplasmic divisions and differentiations are required for subsequent spermiogenesis and spermiation (O'Donnell et al., 2011; Pepling et al., 1999). Emergence of such syncytia would likely hamper the striped catfish from appropriate sperm maturation and potentially lower its reproductive success in the currently living environment, the Saen Saep Canal.

CONCLUSIONS

The present study showed the reproductive impairment and gamete histopathology found in *P. hypophthalmus* living in the Saen Saep Canal. The emergence of atresia in previtellogenic oocytes and eosinophilic cytoplasm of spermatogonia may potentially result in the decreased reproductive success of the fish. Our findings not only reveal the current status of reproductive health in this fish, which has currently been threatened by many anthropogenic activities, but also highlight the urgent necessity in terms of appropriate water management needed for mitigating the on-going water pollution in the Saen Saep Canal, Thailand.

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AUTHOR CONTRIBUTIONS

Conceptualization, T.P., C.C. and J.K.; Methodology, S.S. and TP.; Software, Validation, S.S. and J.K.; Formal Analysis, T.P., S.S. and C.C.; Investigation, S.S.; Resources, J.K. and C.C.; Writing – Original Draft Preparation, S.S.

CONFLICT OF INTEREST

The authors report that no conflicts of interest exist. The authors alone are responsible for the content and writing of this paper

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