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Full Paper

Ovarian development and alteration in demersal and pelagic fishes of Pranburi River estuary, Thailand

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Abstract: The stressful environment in Pranburi River estuary of Thailand severely impacts the aquatic ecosystem and may be causing reproductive alteration in the estuarine fishes living in this area, although there is little evidence of such deteriorated reproductive system. Thus, we aim to investigate the reproductive system, in particular the ovarian alteration using histological techniques in two groups of fishes: (1) demersal fishes, viz. long-whisker catfish (*Mystus gulio*), dark sleeper (*Butis humeralis*) and maned goby (*Oxyurichthys microlepis*), and (2) pelagic fishes, viz. greenback mullet (*Planiliza subviridis*), green puffer (*Tetraodon fluviatilis*) and Singapore glassy perchlet (*Ambassis kopsii*). Ten samples of each fish were collected during January - March of 2016-2018. Histological observation demonstrates that both demersal and pelagic fishes have an asynchronous oocyte development and maturation, which proceeds through two successive phases: primary growth phase and secondary growth phase. The fishes exhibit significant histopathological alterations in ovaries, including the atretic oocytes in both phases, with irregular shapes and degeneration of yolk granules. Interestingly, all collected demersal fishes possess a significantly higher number of atretic oocytes compared to the pelagic fishes. The observed abnormalities seem to be associated with the environmental stress, which may be more severe in the benthic region of the estuary.

Keywords: estuarine fishes, microanatomy, oogenesis, pelagic fishes, demersal fishes, Pranburi River estuary, Thailand

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INTRODUCTION

Pranburi River estuary of Thailand is recently in a critical condition as a result of untreated water released from communities, fisheries, aquaculture and other industries. The high aquatic pollution in this estuarine region is characterised by numerous pollutants, especially heavy metals and petroleum hydrocarbons accumulated in sediment and water [1, 2]. In particular, total lead found in water of this estuary was about 0.5 mg/L [1], which is considerably higher than that in the World Health Organisation guideline for drinking water (0.01 mg/L) [3]. It is possible that this high pollutant level could have negative effects not only on the ecosystem status but also on the health and wellness of aquatic organisms.

An animal sentinel system provides information on adverse effects of contaminants on the specific environment. The animals used in this purpose are called 'sentinel species' and recognised as a key to the environmental health assessment [4]. Evaluating or monitoring appropriate biological markers using the sentinel species could help keep track of the degree of environmental hazards and the exposure of species to environmental contaminants. Histopathological methods have been commonly used is this field as the most accurate and powerful tool for assessing the fish health under specific environmental exposure in both the laboratory and field studies [5-8]. Pathologists typically use organs sensitive to effects of environmental stressors, especially the ovarian tissue, the primary organ of reproduction [6, 9-13]. Understanding the pathology of ovarian tissue has helped in assessing the female reproductive health as well as the monitoring of unsuccessful reproduction as reported by many investigators [11-14].

For this current study, six important fishes were selected as sentinel species for health assessment based on their different habitats: demersal fishes (*Mystus gulio, Butis humeralis* and *Oxyurichthys microlepi*s) and pelagic fishes (*Chelon subvitidu, Tetraodon fluviatilis* and *Ambassis kopsii*). These fishes potentially play roles in the estuarine ecosystem and are commonly found in overlapping areas in the estuary affected by environmental contaminants. The objective of the study is to address the ovarian structure in six fish species together with their histopathological information to assess the ovarian health status. This can unveil what is happening in fish biology under a contamination circumstance and help understand the impact of environmental stress caused by human community's practices on the environment and aquatic organisms.

MATERIALS AND METHODS

Fish Collection and Study Sites

Fixed fish samples at the Fish Biology and Aquatic Health Assessment Laboratory, Department of Marine Science, Chulalongkorn University were used. These samples were originally collected for our previous studies [15, 16] by beach seine net (mesh size 1 cm) in the Pranburi River estuary (N12º 24.314' E099º 58.597'), Thailand, during January-March 2016-2018. We randomly obtained two groups of fishes from the fixed samples (N = 10 for each species): (1) demersal fishes i.e. long-whisker catfish (*Mystus gulio*, 15.2 ± 0.98 (SD) cm in total length), dark sleeper (*Butis humeralis*, 9.1 ± 1.20 cm), and maned goby (*Oxyurichthys microlepis*, 12.9 ± 1.2 cm); (2) pelagic fishes, i.e. greenback mullet (*Planiliza subviridis*, 15.2 ± 1.22 cm), green puffer (*Tetraodon fluviatilis*, 10.6 ± 1.2 cm), and Singapore glassy perchlet (*Ambassis kopsii*, 3.3 ± 1.5 cm). To collect the ovary, an abdominal incision was made and then the fish was cut from the cloacal opening towards the anterior region. Ovarian tissues were dissected out and placed in 70%

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EtOH for morphological and histological observations to assess signs of ovarian/oocyte abnormalities.

Histological and Histochemical Observations

To confirm the histological structure of the ovaries, the fixed ovarian tissues were processed according to standard histological techniques [17, 18]. Paraffin sections of 4-µm thickness were stained with haematoxylin–eosin and Masson's trichrome staining method [17, 18]. Histological sections of the ovarian structure and oogenesis of six fish were viewed under the light microscope and diagnosed based on the modified criteria of Uribe et al. [19]. The sections were photographed with a Leica TE750 camera (Heidelberg, Germany).

Histopathological Assessment and Counting of Atretic Oocytes

The histopathological diagnosis/alterations of the ovarian tissue were determined under a Leica light microscope equipped with a digital camera according to the guidelines of Dietrich and Krieger [6] and Blazer [14]. The atretic follicles were diagnosed based on Senarat et al. [20]. Numbers of atretic follicles were quantified from three sections (50 oocytes/fish per section) in the middle area of three ovarian sections under a light microscope (10x and 40x) and averaged as mean ± SD.

Statistical Analysis

A two-sample t-test was used to compare the average number of atretic oocytes in demersal and pelagic fishes between sites A and B in Pranburi River estuary. The statistics were calculated using Statistical Package for the Social Sciences (SPSS) software (version 16.0).

RESULTS AND DISCUSSION

Parameters of Environmental Factors

Environmental factors were recorded to assess the water quality in the study areas (Table 1). These data were originally received from Mitparian [21]. The depth, water temperature and pH were quite similar between sites. It is noted that the dissolved oxygen (OD) was higher at site A than site B, fitting well with the fact that site A was located in the mangrove forest while site B being in the urban area received wastewater from the communities. In addition, site B was lower in salinity (27.9 ppt) than site A (30.7 ppt) as a result of the inflow of freshwater from Pranburi River.

Table 1. Environmental factors at Pranburi River estuary [21]

Parameter	Site	
	A	B
Depth (m)	0.5	0.5
Water temperature (˚C)	25.3	25.7
Dissolved oxygen (mg/L)	5.65	5.00
pH	7.65	7.69
Salinity (ppt)	30.7	27.9

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Histological Structure of Ovarian Tissue and Oogenesis

The reproductive feature of nine estuarine fishes has been recorded [10]; however, it has never been investigated for the three demersal and three pelagic fishes which we selected as sentinel species in Pranburi River estuary. Representative images of mature ovarian tissues of the six teleosts are shown in Figure 1. The ovarian surface is basically covered with a thin layer of tunica albuginea while the parenchymal ovaries carry oocytes at different developmental stages (Figure 1). We observe the asynchronous development of oocytes in all examined fishes, similar to what is seen in other teleost fishes [22, 23]. All of the selected six species are also known to exhibit protracted spawning period with multiple spawning, which is typically associated with the asynchronous development of oocytes. Histologically, we can classify the oocytes of the six fish species into two distinct developmental phases: the primary growth phase and secondary growth phase (representative Figures 1A, 1D, 1G).

Two stages are identified in the primary growth phase: perinucleolar stage and oil droplet and cortical alveolar stage. Oocytes in the first stage have quite similar characteristics among all species tested, being composed of a central nucleus called germinal vesicle with several peripheral nucleoli inside. Images from *M. gulio* (Figures 1A-C), *B. humeralis* (Figures 1D-F) and *O. microlepis* (Figures 1G-I) are shown as examples. In addition, the ooplasm stains well with hematoxylin and exhibits a strong basophilic feature, indicating the presence of a high amount of RNA and ribosome [24]. Oil droplets and cortical alveoli in the ooplasm were dispersed along the nuclear membrane and periphery of the oocytes, as shown in the representative images from *M. gulio* (Figure 1B), *B. humeralis* (Figure 1E) and *O. microlepis* (Figure 1H). It has been shown that the secretion of enzymes and proteins from the cortical alveoli functions to prevent polyspermy [25]. The presence of this structure in the tested fish indicates the conserved mechanism of 'slow block to polyspermy' as demonstrated in other teleosts and amphibians. Overall, all of these characteristics of the primary oocyte are clearly similar to those of other well-studied teleosts [25, 26].

The secondary growth phase is classified into two successive stages: secondary growth stage and full-grown oocyte stage. Examples are those from *M. gulio* (Figures 1A-C), *B. humeralis* (Figure 1D) and *O. microlepis* (Figures 1G, 1I). Secondary growth oocytes exhibit acidophilic yolk globules at the periphery of the ooplasm, being found in both demersal and pelagic species. The nucleus has an eccentric shape and is obviously folded. The zona pellucida is formed as an acellular acidophilic layer and distinctly striated in all fishes. The nutrients, supposed to be mainly vitellogenin, are accumulated in the yolk granules which progressively increase in number and size; the fusion of the yolk granules is observed only in *O. microlepis* (Figures 1G, 1I).

Macroscopic Abnormality and Histopathological Alteration of Ovarian Tissues

No macroscopic abnormality is observed in the ovarian tissues of both demersal and pelagic fishes. Based on histological observation, most distinct degenerative changes in the oocytes in all examined fishes are atretic oocytes which are broken down and exhibit an irregular shape along with the degeneration and disorganisation of the yolk granules (Figures 2A-G). All of *C. subvitidu* (Figure 2A), *A. kopsii* (Figure 2B), *T. fluviatilis* (Figure 1H), *B. humeralis* (Figure 2C), *O. microlepis* (Figure 2D) and *M. gulio* (Figures 2F-G) exhibit similar abnormality of oocytes and follicle complex, as also described in some other fishes [14, 28]. The abnormality observed in this study might be related to an unfavourable environment during the study period [14, 29-31]. At

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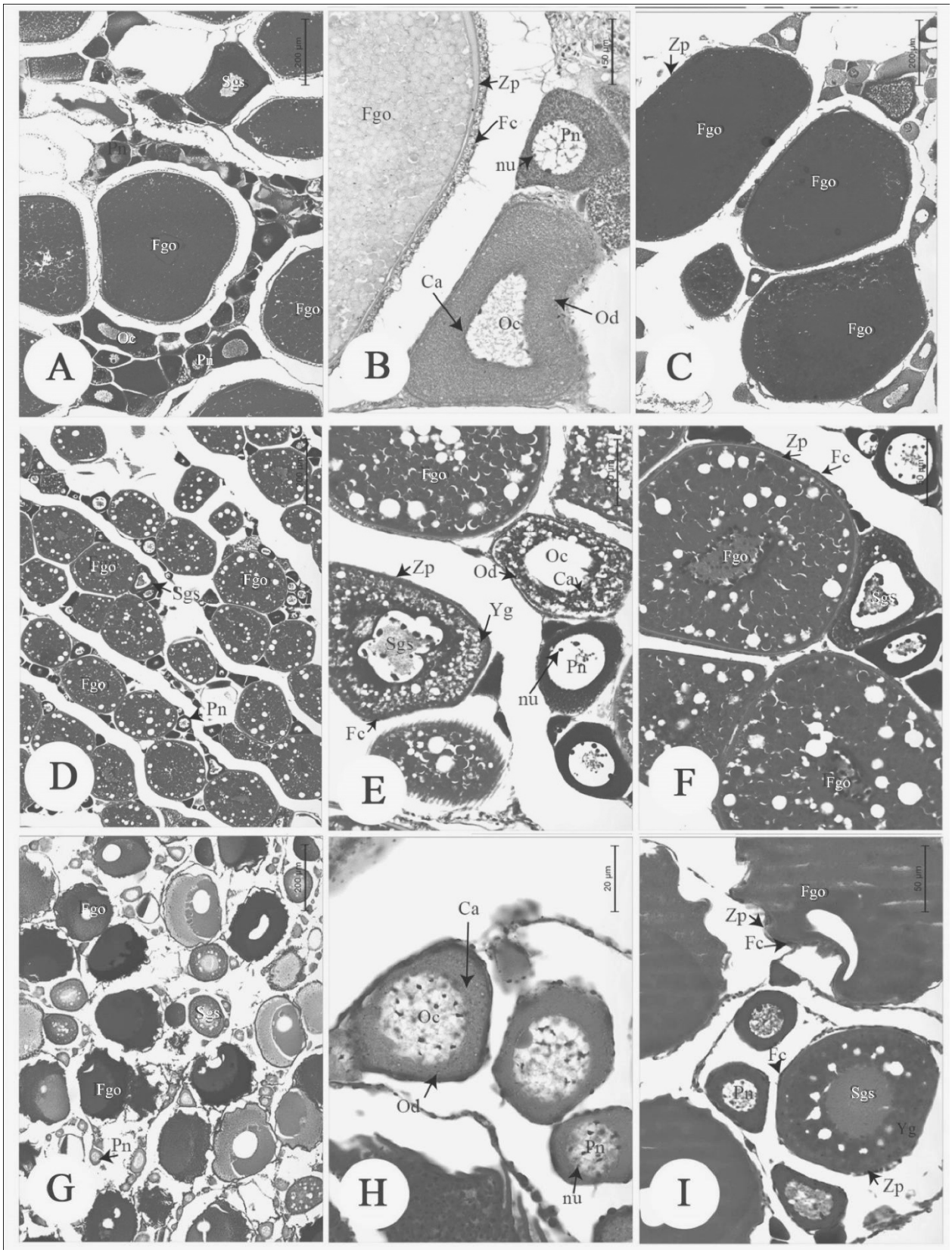

Figure 1. Representative light photomicrographs of oogenesis during primary growth phase (perinucleolar (Pn) and oil droplets and cortical alveolar step (Oc)) and secondary growth phase (secondary growth step (Sgs) and full-grown oocyte step (Fgo)) in *Mystus gulio* (Figures A-C), *Butis humeralis* (Figures D-F) and *Oxyurichthys microlepis* (Figures G-I). Ca = cortical alveoli, Fe = follicular cell, Od =oil droplet, nu = nucleolus, Yg = yolk granule, Zp = zona pellucida.

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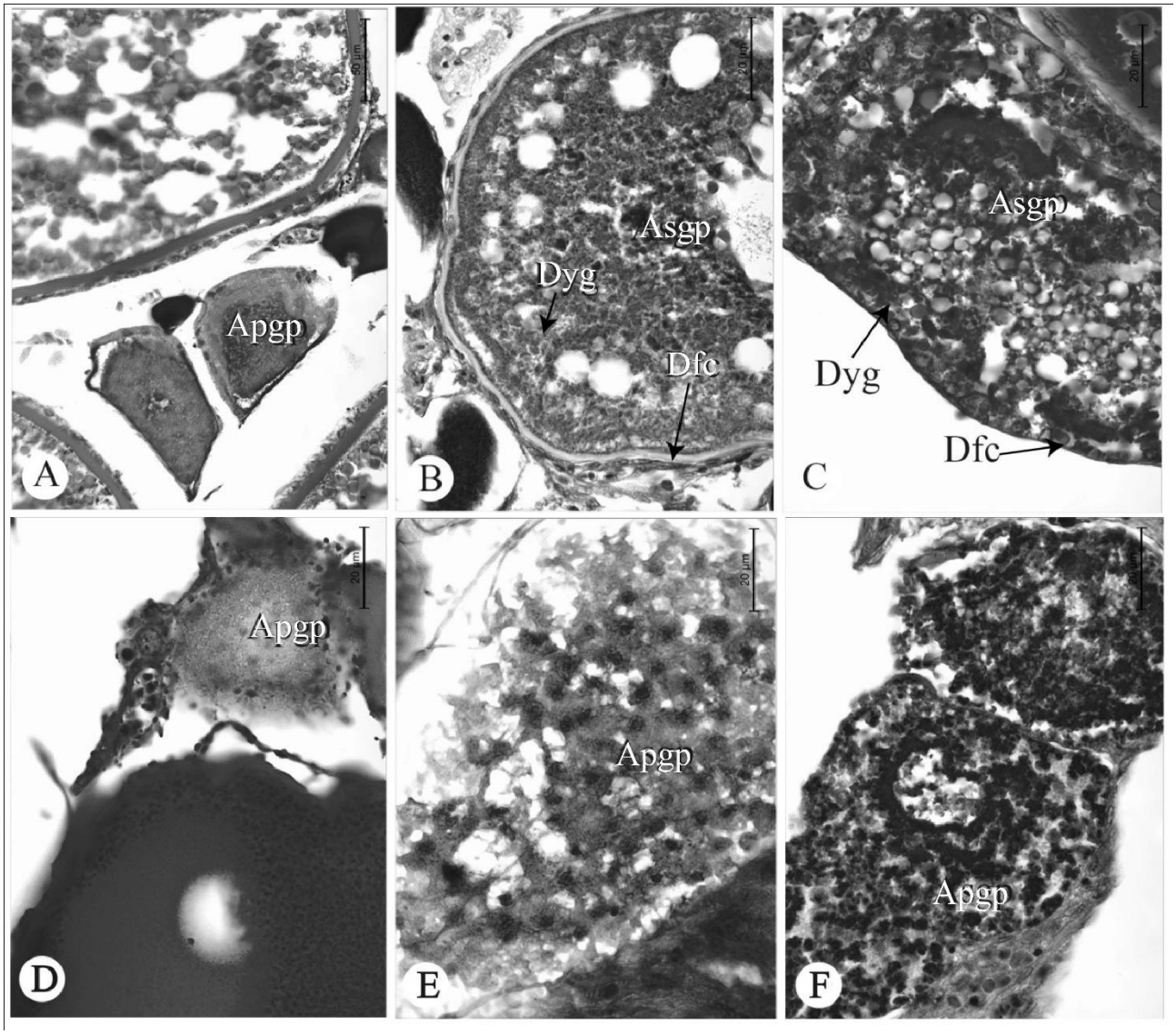

Figure 2. Representative light photomicrographs of atretic oocytes at primary growth phase (Apgp) and secondary growth phase (Asgp): *Chelon subvitidu* (A), *Ambassis kopsii* (B), *Butis humeralis* (C), *Oxyurichthys microlepis* (D), *Mystus gulio* (E-F). Dfc = degeneration of follicle cell, Dyg = degeneration of yolk granule

Pranburi River estuary, the water released from the agricultural area might also flow into the river stream. It has been shown by Spanò et al. [11] that the ovarian degeneration and increasing atresia in the ovary of *Carassius auratus* occurred after exposure to 100 and 1000 g/L of atrazine, a commonly used herbicide. Also, Tillitt et al. [13] reported that the adult fathead minnow, *Pimephales promelas*, exposed to different concentrations of atrazine (5 µg/L and 50 µg/L) showed different degrees of follicle atresia in a dose-dependent manner. The present data underscore the need of detailed water quality assessment targeting not only heavy metals and petroleum hydrocarbons, but also herbicides from agricultural run-off.

The number of atretic oocytes is summarised in Figure 3, comparing sites A and B as well as demersal and pelagic fishes. Fishes from site B show significantly higher abundance of the atretic follicles than those from site A irrespective of the oocyte development phases (*M. gulio*, *B. humeralis* and *O. microlepis*) (Figure 2), although atretic follicles are common in both groups. It is likely that demersal fishes are more heavily exposed to environmental contaminants than pelagic

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fishes since they generally have a higher number of atretic oocytes. In Pranburi River estuary lead and petroleum hydrocarbons are known to be present as contaminants in the sediment [1, 2]. Some heavy metals can attach strongly to soil particles. While heavy metals at this state are sometimes considered to be inactive [32], they might persistently affect demersal fishes more than pelagic fishws in the estuary. In addition, some heavy metals exhibit moderate lipid solubility and can thus penetrate into animal tissues [33] and accumulate in the lipid-rich tissues, especially the gonadal tissue [34, 35]. This could probably explain why the atretic oocytes are observed mostly at the secondary growth stage (high lipid accumulation) of demersal fishes. Altogether these could deteriorate the reproduction of the demersal fishes in a more severe manner compared to pelagic fishes. More observation in further to monitor the heavy metal contamination in various sampling sites and its relation to ovarian bioaccumulation in wild fishes and reproductive histopathology is required. Pesticides and heavy metals such as chromium, arsenic and lead have also been associated with the gonadal impairments [36, 37].

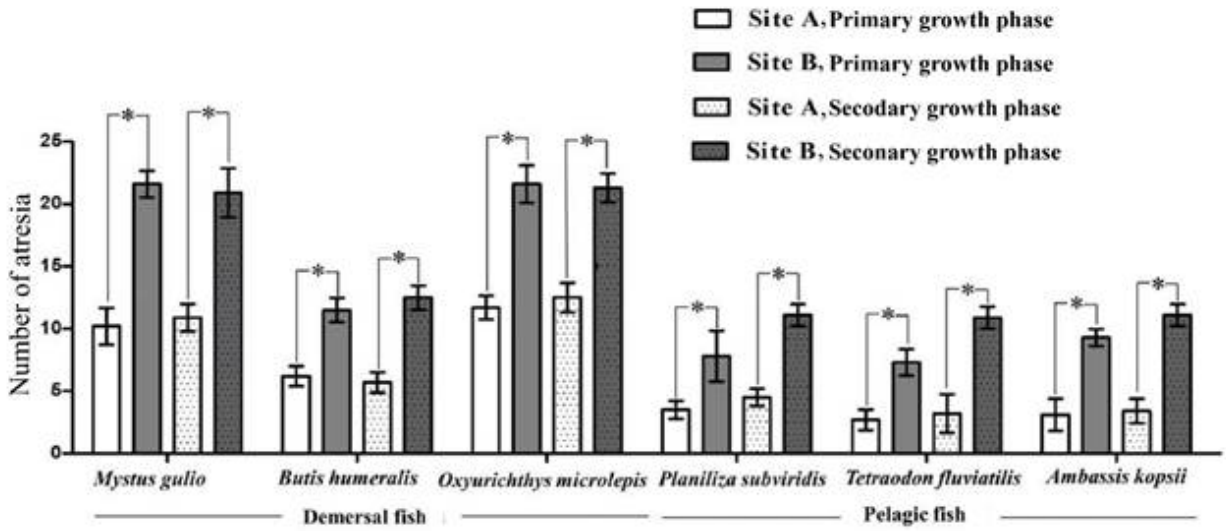

Figure 3. Numbers of atresia at primary growth phase (PGP) and secondary growth phase (SGP) (mean± SD) of demersal fishes and pelagic fishes from site A and site B. Values represent mean ± SD; significantly different at *P<0.05.

On the other hand, there is relatively little information on the effect of inadequate salinity on the frequency of atretic oocytes in wild fishes, although it has been reported that the salinity is related to the fertilisation, survival and egg development of fishes [38, 39]. A high proportion of atretic oocytes was observed in all examined fishes from habitat with high salinity (site A). This agrees well with a previous study which reported a reduction in the percentage of impaired eggs in both euryhaline and stenohaline fishes at low salinity [40]. However, more in-depth analysis needs to be done to verify the relationship of salinity and the aberrant development of atretic oocytes. Also, it has been shown that several kinds of pollutants, especially the endocrine-disrupting chemicals, directly affect the reproductive system and induce the abnormality in ovarian tissues in fishes, apart from atretic oocytes and follicular cell degeneration [41].

CONCLUSIONS

The ovarian development and alterations of demersal and pelagic fish species (3 of each), inhabiting in Pranburi River estuary have been elucidated. The oocyte abnormality was found in all

fishes, possibly leading to decreased total egg production. In addition, demersal fishes exhibit a higher degree of histopathology of the reproductive system than pelagic fishes, which is probably related to polluted and stressful conditions of the sediment of this estuary. Subject to further in-depth analysis of this relationship between pollutants and histopathology, these fishes can be used as sentinel species and good biomarkers for assessing the environmental status.

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REFERENCES

1. V. Cheevaporn and P. Menasveta, "Water pollution and habitat degradation in the Gulf of Thailand", *Marine Pollut. Bull.*, **2003**, *47*, 43-51.
2. G. Wattayakorn, "Petroleum pollution in the Gulf of Thailand: A historical review", *Coast. Mar. Sci*., **2012**, *35*, 234 -245.
3. WHO, "Guidelines for Drinking-water Quality", 4th Edn. incorporating the 1st addendum, World Health Organization, Geneva, **2017**.
4. National Research Council, "Animals as Sentinel of Environmental Health Hazards", National Academy Press, Washington D.C., **1991**.
5. Z. Ayas, G. Ekmekci, M. Ozmen and S. V. Yerli, "Histopathological changes in the livers and kidneys of fish in Sariyar Reservoir, Turkey", *Environ. Toxicol. Pharmacol*., **2007**, *23*, 242-249.
6. D. R. Dietrich and H. O. Krieger, "Histological Analysis of Endocrine Disruptive Effects in Small Laboratory Fish", John Wiley & Sons, Hoboken (N.J.), **2009**, pp.254-265.
7. S. Senarat, J. Kettratad, P. Poolprasert, W. Yenchum and W. Jiraungkoorskul, "Histopathological finding of liver and kidney tissues of the yellow mystus, *Hemibagrus filamentus* (Fang and Chaux, 1949), from the Tapee River, Thailand", *Songklanakarin J. Sci. Technol.*, **2015**, *37*, 1-5.
8. S. Senarat, J. Kettratad, S. Tipdomrongpong, F. G. Plumley, W. Jiraungkoorskul and P. Poolprasert, "Health status in wild and captive short mackerel, *Rastrelliger brachysoma* from Thailand: Histopathology", *Songklanakarin J. Sci. Technol.*, **2018**, *40*, 1090-1097.
9. S. Senarat, W. Jiraungkoorskul and J. Kettretad, "Ovarian histology and reproductive health of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851), as threatened marine fish in Thailand", *Songklanakarin J. Sci. Technol.*, **2017**, *39*, 225-235.
10. S. Senarat, J. Kettratad, W. Siriwong, S. Bunsomboonsakul, A. Kenthao, G. Kaneko, A. Sopon, C. Sudtongkong and W. Jiraungkoorskul, "Oogenesis and ovarian health problems in economically important fishes from different habitats potentially affected by pollution in Thailand", *Asian Fish. Sci.*, **2020**, *33*, 274-286.
11. L. Spanò, C. R. Tyler, R. van Aerle, P. Devos, S. N. M. Mandiki, F. Silvestre, J. P. Thomé and P. Kestemont, "Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*)", *Aquat. Toxicol.,* **2004**, *66*, 369-379.

12. Organisation for Economic Co-operation and Development, "Guidance document for the testing of chemicals", **2006**, https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd125.pdf (Accessed: December 2016).
13. D. E. Tillitt, D. M. Papoulias, J. J. Whyte and C. A. Richter, "Atrazine reduces reproduction in fathead minnow (*Pimephales promelas*)", *Aquat. Toxicol*., **2010**, *99*, 149-159.
14. V. S. Blazer, "Histopathological assessment of gonadal tissue in wild fishes", *Fish Physiol. Biochem.*, **2002**, *26*, 85-101.
15. S. Senarat, J. Kettratad, S. Pairohakul, S. Ampawong, B. P. Huggins, M. M. Coleman and G. Kaneko, "An update on the evolutionary origin of aglomerular kidney with structural and ultrastructural descriptions of the kidney in three fish species", *J. Fish Biol.*, **2022**, *100*, 1283-1298.
16. N. Sukkhee, T. Mitparian, T. Kanjanarakha, S. Senarat, N. Kangwanrangsan, G. Kaneko and J. Kettratad, "Spermatozoon of the wild scalloped perchlet, *Ambassis nalua* (Hamilton, 1822): Ultrastructure and morphometric analysis", *Vet. Integr. Sci*., *20*, 2022, 199-208.
17. J. K. Presnell and M. P. Schreibman, "Humason's Animal Tissue Techniques", 5th Edn., Johns Hopkins University Press, Baltimore, **1997**, pp.17-44.
18. S. K. Suvarna, C. Layton and J. D. Bancroft, "Bancroft's Theory and Practice of Histological Techniques", 7th Edn., Elsevier, San Diego, **2013**, pp.40-95.
19. M. C. Uribe, H. J. Grier and L. R. Parenti, "Ovarian structure and oogenesis of the oviparous Goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (Teleostei, Cyprinodontiformes)", *J. Morphol*., **2012**, *273*, 371-387.
20. S. Senarat, J. Kettretad and W. Jiraungkoorskul, "Classification stages of novel atretic structure in short mackerel *Ratrelliger brachysoma* (Bleeker, 1851) from the Upper Gulf of Thailand", *Songklanakarin J. Sci. Technol.*, **2015**, *37*, 569-573.
21. T. Mitparian, "Reproductive biology and feeding ecology of the grunting toadfish, *Allenbatrachus grunniens* (Linnaeus, 1758)", *MSc Thesis*, **2018**, Chulalongkorn University, Thailand.
22. S. Chellappa, J. T. A. X. Lima, A. Araújo and N. T. Chellappa, "Ovarian development and spawning of serra spanish mackerel in coastal waters of Northeastern Brazil", *Braz. J. Fish Biol.*, **2010**, *70*, 451-456.
23. R. A. Wallace and K. Selman, "Cellular and dynamic aspects of oocyte growth in teleosts", *Amer. Zool.*, **1981**, *21*, 325-343.
24. R. A. Wallace and K. Selman, "Ultrastructural aspects of oogenesis and oocyte growth in fish and amphibians", *J. Electron. Microsc. Tech.*, **1990**, *16*, 175-201.
25. Y. Nagahama, "The functional morphology of teleost gonads", in "Fish Physiology, Reproduction Part A: Endocrine Tissues and Hormones" (Ed. W. S. Hoar, D. J. Randall and E. M. Donaldson), Academic Press, New York, **1983**, Ch.3.
26. K. Selman, R. A. Wallace and V. Barr, "Oogenesis in *Fundulus heteroclitus*. V. The relationship of yolk vesicles and cortical alveoli", *J. Exp. Zool.*, **1988**, *246*, 42-56.
27. N. D. Koç, Y. Aytekin and R. Yüce, "Ovary maturation stages and histological investigation of ovary of the zebrafish (*Danio rerio*)", *Braz. Arch. Biol. Technol.*, **2008**, *51*, 513-522.
28. B. G. M. Jamieson, "Reproductive Biology and Phylogeny of Fishes (Agnathans and Bony Fishes)", Science Publishers, Enfield, **2009**, pp.100-120.

29. J. N. Cross and J. E. Hose, "Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off southern California", *Mar. Environ. Res.*, **1988**, *24*, 185-188.
30. L. L. Johnson, E. Casillas, T. K. Collier, B. B. McCain and U. Varanasi, "Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington", *Can. J. Fish. Aquat. Sci.*, **1988**, *45*, 2133-2146.
31. R. Kirubagaran and K. P. Joy, "Toxic effects of mercuric chloride, methylmercuric chloride, and emisan 6 (an organic mercurial fungicide) on ovarian recrudescence in the catfish *Clarias batrachus* (L.)", *Bull. Environ. Contam. Toxicol.*, **1988**, *41*, 902-909.
32. US Environmental Protection Agency, "Risks of paraquat use to federally threatened california red-legged frog (*Rana aurora draytonii*)", Office of Pesticide Programs, Washington, D.C., **2009**, 58-62
33. A. Spacie, L. S. McCarty and G. M. Rand, "Bioaccumulation and bioavailability in multiphase systems", in "Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment" (Ed. G. M. Rand), Taylor and Francis, New York, **1995**, Ch.16.
34. A. Annabi, K. Said and I. Messaoudi, "Heavy metal levels in gonad and liver tissues – Effects on the reproductive parameters of natural populations of *Aphanius facsiatus*", *Environ. Sci. Pollut. Res. Int.*, **2013**, *20*, 7309-7319.
35. D. Adjei-Boateng, K. A. Obirikorang, S. Amisah, H. A. Madkour and F. A. Otchere, "Relationship between gonad maturation and heavy metal accumulation in the clam, *Galatea paradoxa* (Born 1778) from the Volta estuary, Ghana", *Bull. Environ. Contam. Toxicol.,* **2011**, *87*, 626-632.
36. R. M. Pedlar, M. D. Ptashynski, R. Evans and J. F. Klaverkamp, "Toxicological effects of dietary arsenic exposure in Lake Whitefish (*Coregeonus clupeaformis*)", *Aquat. Toxicol.*, **2002**, *57*, 167-189.
37. M. I. Hanna, I. B. Shaheed and N. S. Elias, "A contribution on chromium and lead toxicity in cultured *Oreochromis niloticus*", *Egypt J. Aquat. Biol. Fish*., **2005**, *9*, 177-209.
38. F. G. T. Holliday, "The effects of salinity on the eggs and larvae of teleosts", in "Fish Physiology" (Ed. W. S. Hoar and D. J. Randall), Academic Press, London, **1969**, pp.293-311.
39. D. F. Alderdice, "Osmotic and ionic regulation in teleost eggs and larvae", in "Fish Physiology" (Ed. W. S. Hoar and D. J. Randall), Academic Press, London, **1988**, pp.163-251.
40. P. R. Hart and G. J. Purser, "Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Günther, 1862)", *Aquacult*., **1995**, *136*, 221-230.
41. M. S. Diniz, I. Peres, I. Magalhães-Antoine, J. Falla and J. C. Pihan, "Estrogenic effects in crucian carp (*Carassius carassius*) exposed to treated sewage effluent", *Ecotoxicol. Environ. Saf*., **2005**, *62*, 427-435.