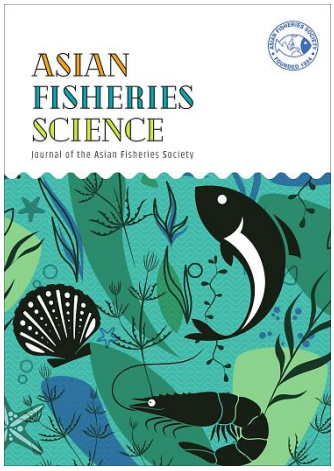

ISSN: 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2020.33.3.009

Oogenesis and Ovarian Health Problems in Economically Important Fishes from Different Habitats Potentially Affected by Pollution in Thailand

SINLAPACHAI SENARAT[1], JES KETTRATAD[2,3], WATTASIT SIRIWONG[4], SURATTA BUNSOMBOONSAKUL[2], ANAN KENTHAO[5], GEN KANEKO[6], ANEK SOPON[3], CHANYUT SUDTONGKONG[1], WANNEE JIRAUNGKOORSKUL[7,*]

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

[2]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[3]Marine Ecology and Marine Resources Utilization Research Unit, Aquatic Resources Research Institute, Chulalongkorn University, Bangkok 10330, Thailand

[4]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand

[5]Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand

[6]School of Arts and Sciences, University of Houston-Victoria, Texas 77901, USA

[7]Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

*E-mail: wannee.jir@mahidol.ac.th | Received: 09/04/2020; Accepted: 14/09/2020

Abstract

It is well-known that pollutants affect aquatic ecosystems; however, there is little information on fish reproductive health as an indicator of aquatic pollution. This study reports the oogenesis and ovarian health problems in important fishes from different habitats potentially affected by pollution. Nine fish species caught in 2016 to 2018 were studied: *Atherinomorus pinguis* (Lacépède, 1803), *Alepocephalus bicolor* Alcock, 1891 and *Neoscopelus microchir* Matsubara, 1943 from the mesopelagic habitats; *Monacanthus chinensis* (Osbeck, 1765) from the coastal habitat; and *Nuchequula gerreoides* (Bleeker, 1851), *Eubleekeria splendens* (Cuvier, 1829), *Pisodonophis boro* (Hamilton, 1822) and *Allenbatrachus grunniens* (Linnaeus, 1758) from the estuarine habitat. *Hippocampus barbouri* Jordan & Richardson, 1908 under captive condition was used as a control. The oogenetic processes were similar in all species examined and classified into two phases according to the nuclear and ooplasmic characteristics: primary growth (PG) and secondary growth (SG) phases. The PG and SG phases were further divided into two and three substages, respectively. The occurrence of the ovotestis (6.66 %) in *A. grunniens*, suggested the environmental endocrine disruption in its habitat. Atretic oocytes (AO), characterised by the abnormal shape and degeneration of yolk granules and follicular complexes, in both PG and SG phases were observed. The AOs were found in all fishes, but the ratio was significantly higher in mesopelagic and estuarine fishes compared to other fishes. It is plausible that the mesopelagic and estuarine fishes have poor reproductive health. The results of the study warrant further investigations on water quality associated with the long-term conservation efforts on the marine and estuarine ecosystems of Thailand.

Keywords: atretic oocyte, histology, mesopelagic fish, ovotestis, ovarian health

Introduction

Histopathology has gained increasing interest as an endpoint biomarker to assess fish health affected by a wide variety of environmental stressors. Often this biomarker is the integration of a large number of interactive physiological processes including the stress response to pollution and endocrine disruption by chemicals (Dietrich and Krieger, 2008) and is therefore highly sensitive. Several reviews summarised that gonadal tissues are especially a good target of histopathology because of their high sensitivity to environmental stresses and important functions as the primary organ of reproduction, which, once impaired, will cause a significant decline of the population (Spano et al., 2004; Dietrich and Krieger, 2008; Tillitt et al., 2010). The gonadal histopathology has been used not only as a biomarker for adverse health effects caused by human activities but also as a useful tool for assessing an unsuccessful reproductive status associated with environmental conditions in natural habitats (Blazer, 2002; Spano et al., 2004;

Tillitt et al., 2010; Senarat et al., 2015).

Estuarine and marine environments in Thailand have been recorded as an example of productive ecosystems, but they suffer from industrial discharge and heavily loaded nutrients from domestic wastes (ARRI, 2003). These situations have resulted in eutrophication (Suvapepun, 1991; ARRI, 2003), oxygen deficiency, and pollution, affecting urban, industrial, and agricultural activities (Suvapepun, 1991). These situations also increase the risk of reproductive failures in aquatic organisms (Senarat et al., 2015; 2017). For example, Senarat et al. (2015) demonstrated that the short mackerel *Rastrelliger brachysoma* Bleeker, 1851 from the Upper Gulf of Thailand showed reproductive disorders with a wide variety of histopathological alterations including ovarian atrophy and prominent atresia. Unfortunately, however, still, little information is available on the reproductive health status of aquatic organism in Thailand.

The present study conducted detailed histopathological analyses on nine economically important fishes from different habitats to increase the information regarding the female reproductive health in fishes from estuarine and marine environments in Thailand. The fish species and the habitat they were collected from were as follows; one species from the captive habitat, *Hippocampus barbouri* Jordan & Richardson, 1908, three from the mesopelagic habitat, *Atherinomorus pinguis* (Lacépède, 1803), *Alepocephalus bicolor* Alcock, 1891, and *Neoscopelus microchir* Matsubara, 1943) one from the coastal habitat, *Monacanthus chinensis* (Osbeck, 1765), and four from the estuarine habitat, *Nuchequula gerreoides* (Bleeker, 1851), *Eubleekeria splendens* (Cuvier, 1829), *Pisodonophis boro* (Hamilton, 1822) and *Allenbatrachus grunniens* (Linnaeus, 1758). This study provides normal oogenetic processes in the fish along with their histopathological alterations seen under light microscopy. The findings further summarise the ratio of histopathological alterations for each species. This work will provide an insight into the reproductive health of fishes in their habitat, with emphasis on the importance of standardising the monitoring for the reproductive health.

Materials and Methods

Fish sampling and study areas

A total of 163 fish from nine species were collected from different estuarine and marine habitats in Thailand from January to December in 2016-2018. The details of fish species and sampling sites are shown in Table 1, whereas the locations of each sampling site in Figure 1. The fish were preserved in Davidson's fixative. All fish samples were then stored as voucher specimens at the Fish Biology and Aquatic Health Assessment Laboratory (FBA-LAB), Department of Marine Science, Faculty of Science, Chulalongkorn University, Thailand.

Histological evaluations and histopathology

The gonadal tissues were dissected out from the abdominal cavities of the fixed fish specimens to assess their morphology and developmental stages using a stereoscopic microscope (Nikon SMZ800N, Japan). The ovary was then cut into small pieces of about 2-3 cm^2, fixed overnight in Davison's fixative, and processed using the standard histological technique with slight modifications (Presnell and Schreibman, 1997; Suvarna et al., 2018). Histological sections with 4 µm thickness were stained with the conventional haematoxylin and eosin (H & E) staining.

Table 1. Samples of fishes and sampling sites for study on the reproductive health status of fish from 2016 to 2018.

Habitats	Fishes	Sampling sites	Number
Captive	*Hippocampus barbouri* Jordan & Richardson, 1908	The Phuket Marine Biological Centre	3
Mesopelagic	*Atherinomorus pinguis* (Lacépède, 1803) *Alepocephalus bicolor* Alcock, 1891 *Neoscopelus microchir* Matsubara, 1943	Andaman Sea at the depths ranging 600-800 m Station 1: 7°50'20.4"N, 96°14'58.2"E Station 2: 7°49'54.1"N, 96°43'15.6"E Station 3: 7°32'22.9"N, 96°59'20.4"E Station 4: 7°11'24.7"N, 97°13'57.7"E	30
Coastal	*Monacanthus chinensis* Osbeck, 1765	Koh Srichang, the Upper Gulf of Thailand	10
Estuarine	*Nuchequula gerreoides* (Bleeker, 1851) *Eubleekeria splendens* (Cuvier, 1829) *Pisodonophis boro* (Hamilton, 1822) *Allenbatrachus grunniens* (Linnaeus, 1758)	Pranburi River estuary 12°24.314'N, 99°58.597'E	120

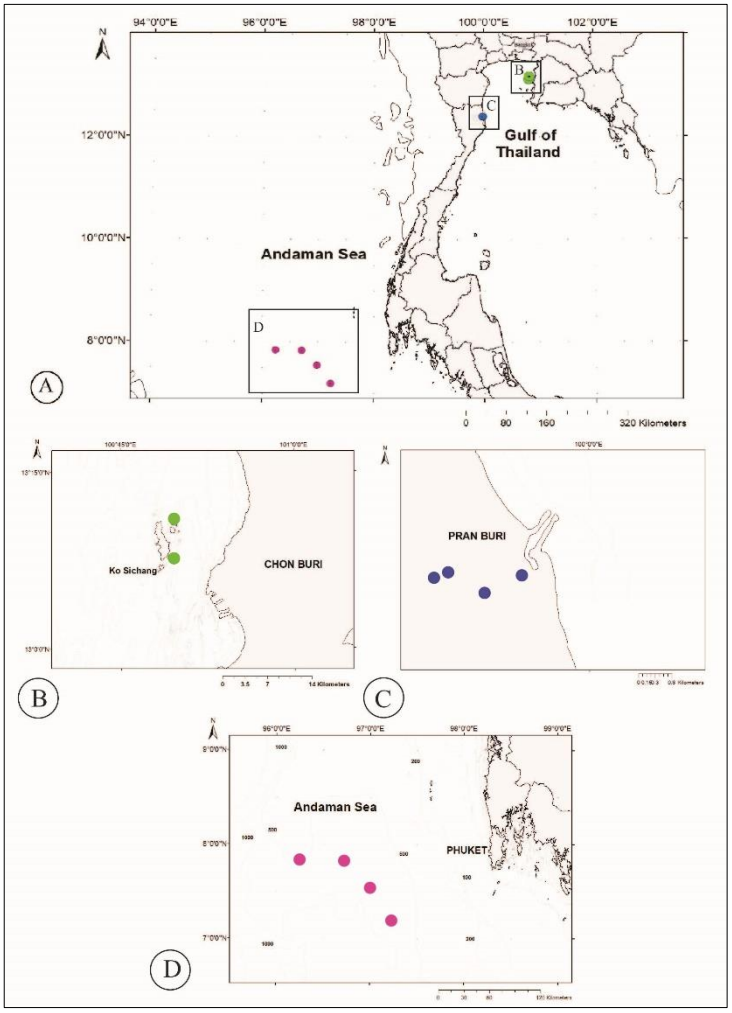

Fig. 1. Map on the overall sampling sites to study the reproductive health status of fish in Thailand (A). High magnification maps of the sampling sites (B) Koh Srichang, (C), Pranburi River estuary, (D) and the Andaman Sea are also shown using the same colour symbols.

The sex of each fish was first determined macroscopically and then confirmed by histology. The observation of the gonadal structure and gametogenesis was performed using a light microscope with the Lucia™ screen measurement system (Leica digital 750, Wetzlar, Germany) following the descriptions of Uribe et al. (2012).

Prevalence in histopathological changes of gonadal tissues was determined following Dietrich and Krieger (2008). Three ovarian sections were observed under the light microscope with the magnification of 10x and 40x at each developmental stage for each fish specimen (n = 50 cells per section). The number of atretic oocytes was then calculated and reported as percentages.

Semithin section

Three adults *H. barbouri* were chosen as the representative specimens to investigate the ultrastructure of atretic oocytes. Small fragments of ovarian tissue (3 × 3 mm^2 in size) were dissected and fixed in a fixative containing 2.5 % glutaraldehyde in 0.1M phosphate buffer, pH 7.4, at 4 °C for 24 h. After repeated washings with the phosphate buffer, tissues were post-fixed in 1 % osmium tetroxide and then processed following Rowden and Lewis (1974). Semithin plastic sections (500 nm) were stained with toluidine blue and analysed by light microscopy to investigate the accurate localisation and characterisation of atresia.

Statistical analysis

To evaluate differences in the prevalence of atretic oocytes between species, one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test was applied separately to the primary and secondary atretic oocytes.

Results

Histological determination of sex

The sex of fish specimens was determined based on macro-morphological and histology; i.e., gamete proportion in the gonadal tissues. Male and females were identified except for *A. grunniens* where only female were identified.

Comparative oogenesis

Most of the fish exhibited an asynchronous type of ovarian development, in which oocytes of several developmental stages were found in the ovary. *Pisodonophis boro* was the only species that represented a synchronous type of ovarian development as reported previously (Na Lampang et al., 2021). The developing oocytes in all species could be classified into three, including primary growth (PG) phase oocytes, secondary growth (SG) phase oocytes, and atretic oocytes (AO) (Figs. 2–4). Note that the details of AO are summarised in the section of ovarian histopathological observations.

Primary growth (PG) phase

Similar oogenetic processes were seen in all fish examined. In the PG phase, oocytes could be further classified into two substages, the perinucleolar (Pn) and oil droplets and cortical alveolar (Oc) stages. The Pn stage oocytes had a large, centrally located nucleus with deeply stained nucleoli lying along the nuclear membrane (Figs. 2A–2B). The ooplasm was highly basophilic and contained slightly acidophilic Balbiani bodies (Fig. 2B). A single layer of flattened follicle cells surrounded the Pn stage oocytes (Figs. 2A–2B).

The Pn stage oocytes showed a dramatic increase in size during the development into Oc stage oocytes (Figs. 2C–2D). In this substage, the onset of the formation of two important structures, oil droplets and cortical alveoli, in the slightly basophilic ooplasm was observed. A few tiny oil droplets were observed near the zona pellucida (Fig. 2D) as empty vacuolar structures. The cortical alveoli were observed as conspicuous structures with slightly purple-coloured and ultimately dispersed near the nuclear membrane (Figs. 2C–2D). The folliculogenesis occurred in this stage, resulting in the formation of three concentric layers surrounding the oocyte surface — zona pellucida, follicle cells, and theca cells (Figs. 2C–2D).

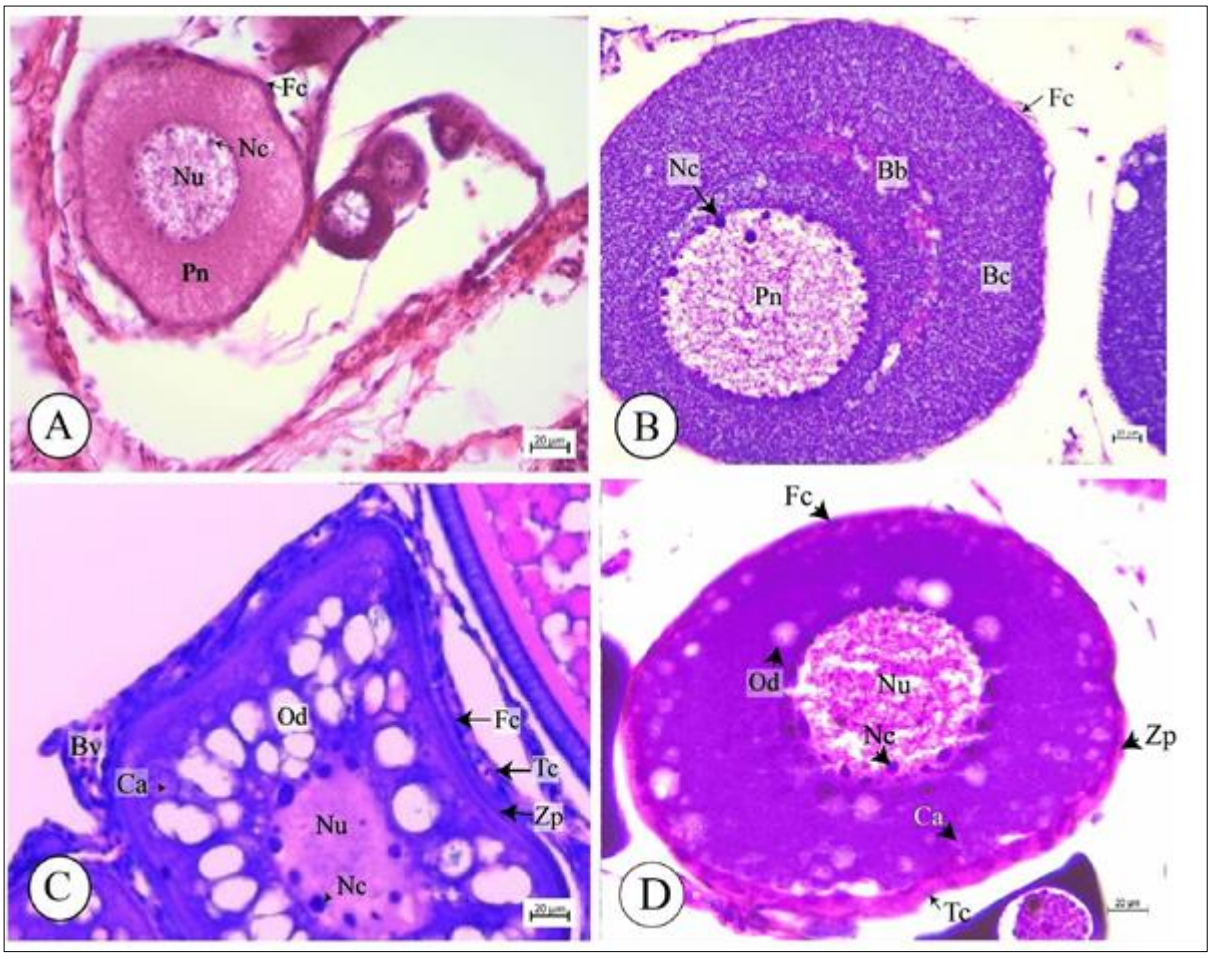

Fig. 2. Histology sections showing oocytes in the perinucleolar stage (Pn) [A-B] and the oil droplets and cortical alveolar stage [C-D] during the primary growth phase. Panel [C] is showing the degeneration of yolk granules. Abbreviations: Bb = Balbiani body, Bc = basophilic ooplasm, Bv = blood vessel, Ca = cortical alveoli, Fc = follicle cell, Nc = nucleolus, Nu = nucleus, Od = oil droplet, Pn = perinucleolar stage, Tc = theca cell, Zp = zona pellucida. Note: A = *Hippocampus barbouri*, B, D = *Alepocephalus bicolor*, C = *Monacanthus chinensis*.

Secondary growth (SG) phase

The SG phase oocytes could be clearly classified into three steps: early secondary growth (Esg), late secondary growth (Lsg) and full-grown oocyte (Fgo) steps (Fig. 3A). However, the SG phase was absent in *N. gerreoides*, *P. boro,* and *E. splendens* specimens tested in this study.

The Esg stage oocytes dramatically increased its size due to the accumulation of yolk granules (Fig. 3B). The yolk granules at this stage were small, spherical in structure, and deeply acidophilic (Figs. 3B–3C). The oil droplets and cortical alveoli remained in oocytes, but they were fused and progressively increased in number and size (Fig. 3C), enclosed in the yolk granules (Fig. 3C). The zona pellucida became extremely thick (~16 µm), and the thickness of the single layer of follicle cells also increased to 9 µm (Fig. 3B).

The Lsg stage oocytes further increased in diameter due to the high accumulation of yolk granules in the basophilic ooplasm (Figs. 3C–3E). It was considered to be an acidophilic oocyte. A well-structured zona pellucida and the follicle cells continuously developed (Fig. 3C). The follicle cells formed a monolayer of high epithelium (Fig. 3C).

The Fgo stage oocytes reached the maximum diameter of up to 430 µm (Figs. 3F–3G). Large yolk granules were observed throughout the ooplasm in this step (Figs. 3A, 3G). The yolk granules were completely fused in some fish species, constituting the yolk platelets (Figs. 3F, 3H). The nucleus was absent because of the germinal vesicle breakdown (GVB), but the remained follicular complex continuously developed, as similarly seen in the Lsg stage.

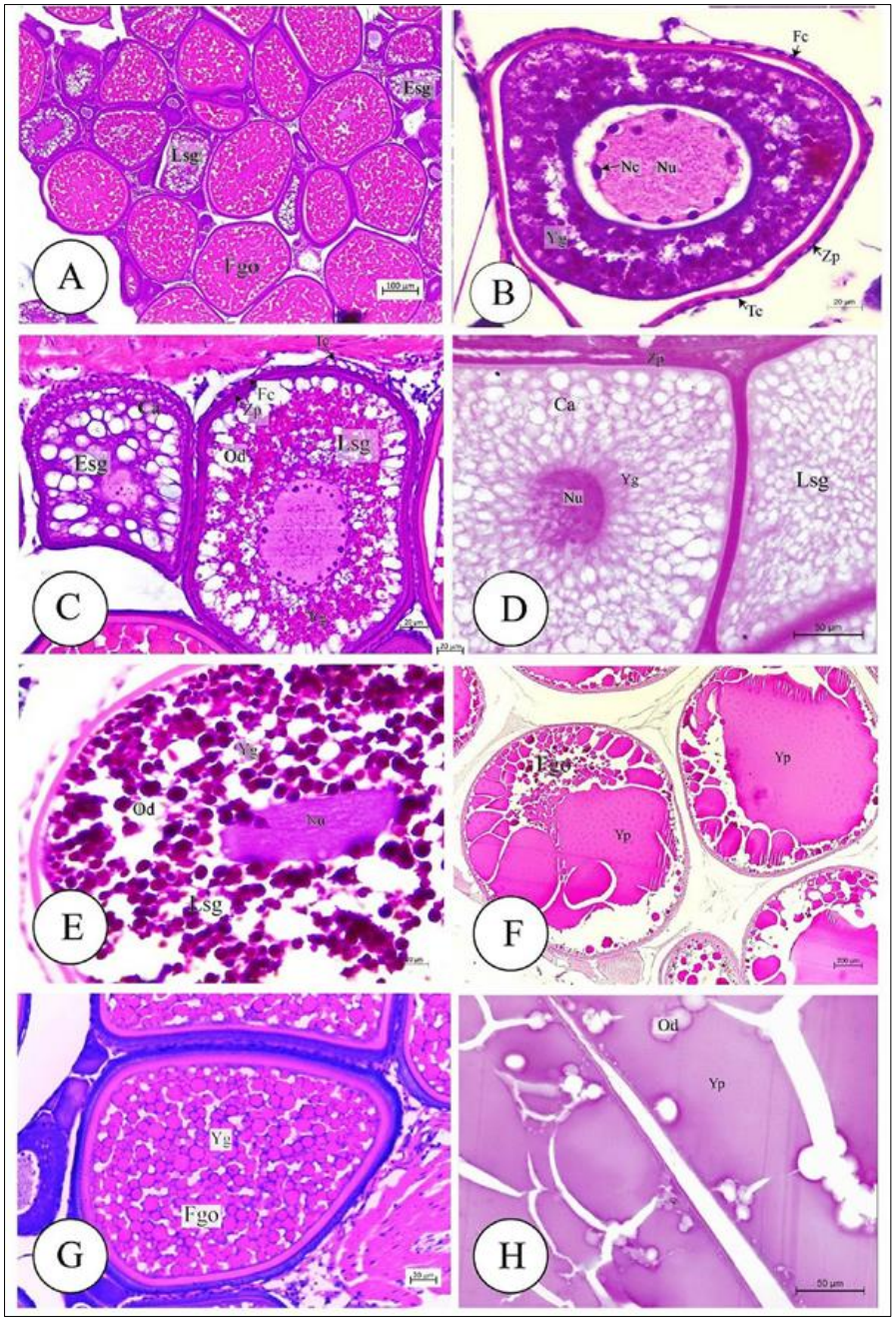

Fig. 3. Histology sections showing the early secondary growth step (Esg), late secondary growth step (Lsg) and full-grown oocyte step (Fgo) during the secondary growth phase [A]. [B-C]: Early secondary growth stage oocytes. [D-E]: late secondary growth stage oocytes. [F-H]: full-grown oocyte stage oocytes. Abbreviations: Ca = cortical alveoli, Fc = follicle cell, Nc = nucleolus, Nu = nucleus, Od = oil droplet, Tc = theca cell, Yg = yolk granules, Yp = yolk plates, Zp = zona pellucida. Note: A, C, G = *Monacanthus chinensis*, B = *Alepocephalus bicolor*, D, H = *Hippocampus barbouri*, F = *Allenbatrachus grunniens*.

Ovarian histopathological observations

The prevalence of ovarian histopathological alterations is presented in Table 1 and Figures 4–7. As mentioned above, testicular tissues were found only in *A. grunniens*. Normal testicular parenchyma was identified in the testicular tissue of most *A. grunniens* specimens (Fig. 4A); however, 6.66 % of them showed serious histopathological changes with the appearance of ovotestis (Fig. 4B). Abnormal erythrocytes were also observed near oocytes in the ovotestis (Fig. 4C).

Atresia of oocytes was the most prevalent histopathological change seen in both PG and SG phases of ovarian development. The atretic PG phase oocytes had irregular shape coupled with the degeneration of ooplasm (Fig. 4D). During the SG phase, the atretic oocytes showed the irregular shape with loss/degeneration of their follicles (Figs. 4E–4F). The disintegration and resorption of the yolk granule were identified along with the disintegration of follicular complex (Figs. 4G–4H and 5A–5F). The semithin sections clearly showed the irregular shape and degradation of vacuoles in follicle cells (Fig. 4F).

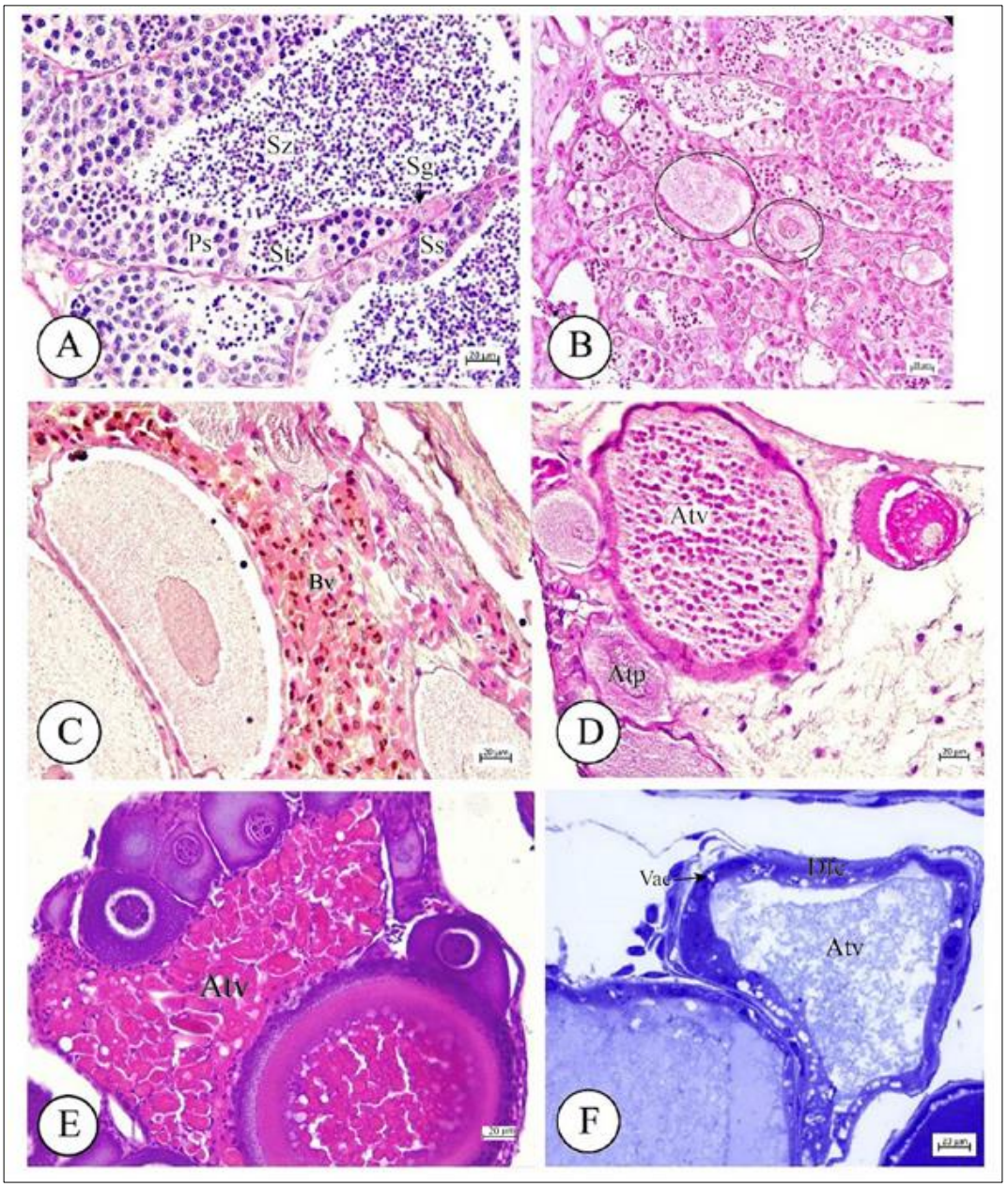

Fig. 4. Histology sections showing alteration in gonadal tissues. [A]: Normal *Allenbatrachus grunniens* testicular parenchyma containing sperms of different stages including spermatogonia (Sg), primary spermatocyte (Ps), secondary spermatocyte (Ss), spermatid (St) and spermatozoa (Sz). [B]: Several oocytes (circles) were found in the testis of *A. grunniens*. [C]: Abnormal erythrocytes within the blood vessel (Bv) of *A. grunniens* ovotestis. [D-F]: Atretic oocytes in two developmental stages: previtellogenic stage (Atp) and vitellogenic stage (Atv) from *A. grunniens* [D], *Monacanthus chinensis* [E] and *Hippocampus barbouri* [F]. Abbreviations: Dfc = degeneration of follicle cell, Vac = vacuoles.

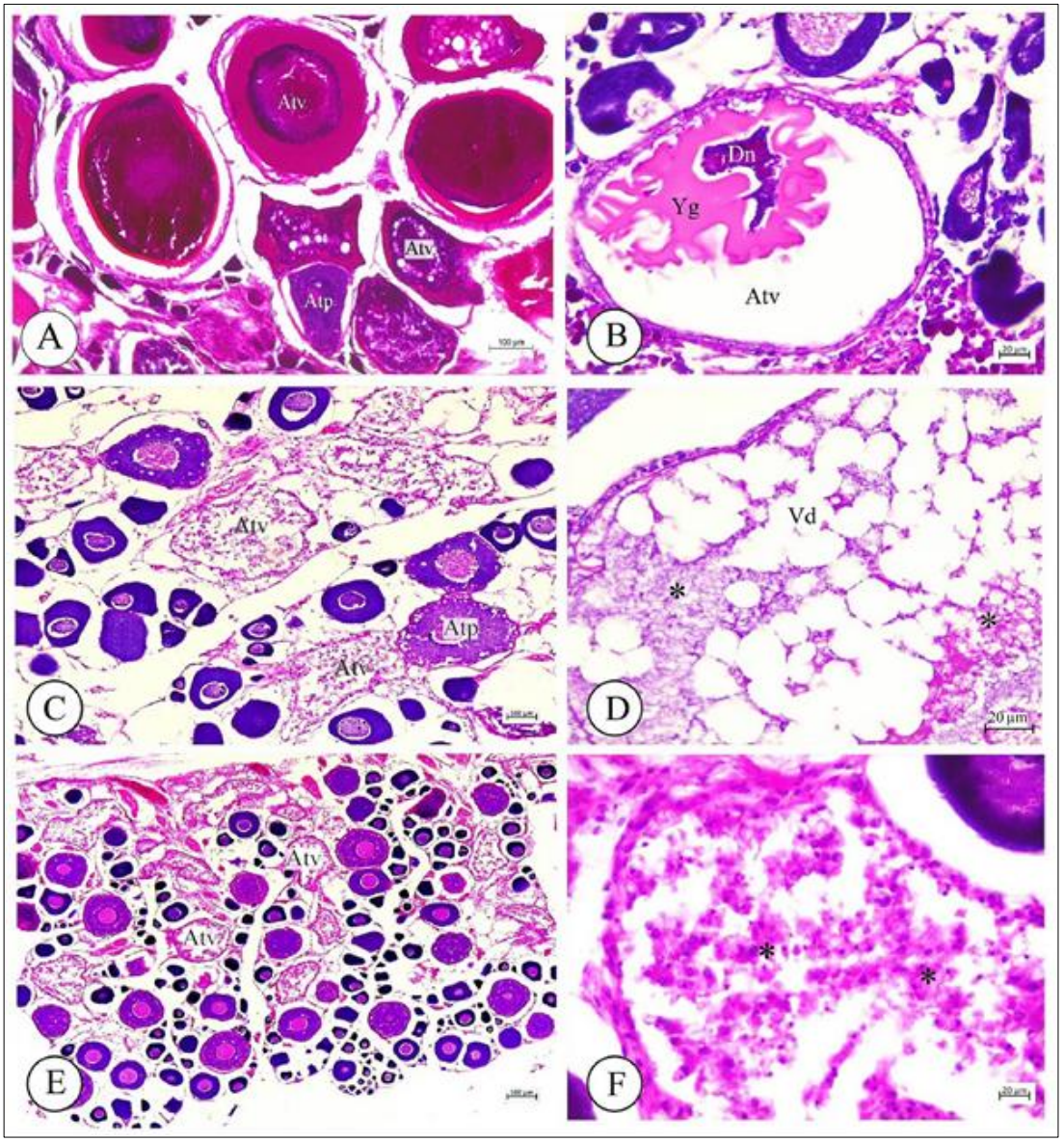

Fig. 5. Histology sections showing the atresia in the mesopelagic fishes, e.g., *Neoscopelus microchir* [A-B] and *Alepocephalus bicolor* [C-F]. Abbreviations: Atp = atresia of previtellogenic stage from primary growth phase, Atv = atresia of vitellogenic stages from secondary growth phase, Dn = degeneration of nucleus, Vd = vacuolar degeneration, Yg = yolk granule, * = degeneration of yolk granules.

Melanomacrophage centres (MMCs) were observed in ovarian connective tissue. MMCs were found only in atretic PG oocytes and in the connective tissue of the ovarian parenchyma (Figs. 6A-6B) in the specimens from estuarine habitats (Table 2).

The eosinophilic granulocytes were identified in *N. gerreoides* and *E. splendens*. These cells had a diameter of 9-10 µm and large eosinophilic granules in the cytoplasm (Fig. 6C). These observations were associated with infiltration of lymphocytes (Fig. 6C). Lymphocytes had a large basophilic nucleus, which was surrounded by a relatively thin rim of cytoplasm.

Comparative analysis of atretic oocytes across species and habitats

Results of one-way ANOVA indicated that the number of follicular atresia were statistically different between species in both PG phase ($F_{(8,261)}$ = 923, p-value < 0.001) and SG phase ($F_{(8,261)}$ = 3405, p-value < 0.001) oocytes. The highest values of atresia of PG phase were observed in *N. microchir* (12.87 %) and *P. boro* (12.57 %), followed by a significantly different value in *A. grunniens* (11.03 %); the lowest value was observed in *H. barbouri* (0.17 %). For the prevalence of atresia of SG phase, *A. bicolor* and *A. grunniens* exhibited the highest value of 19.00 and 18.63 % respectively, followed by a significantly different value in *N. microchir* (12.77 %). Interestingly, several fishes including *E. splendens*, *N. gerrelodes* and *P. boro* did not show atresia in the SG phase (Fig. 7).

Table 2. Prevalence (%) of dominant histopathological lesions in the gonadal tissue of fishes caught from 2016 to 2018.

Histopathological changes	Ab (n = 10)	Ag (n = 30)	Ap (n = 10)	Es (n = 30)	Hb (n = 3)	Mc (n = 10)	Ng (n = 30)	Nm (n = 10)	Pb (n = 30)
Ovo-testis	0	6.66	0	0	0	0	0	0	0
Abnormal erythrocyte	0	50	0	0	0	0	0	0	0
Melanomacrophage centre	30	100	50	0	0	0	0	30	100
Eosinophilic granulocytes	0	0	0	100	0	0	100	0	0
Infiltration of lymphocytes	0	0	0	100	0	0	100	0	0

Ab = *Alepocephalus bicolor*, Ag = *Allenbatrachus grunniens*, Ap = *Atherinomorus pinguis*, Es = *Eubleekeria splendens*, Hb = *Hippocampus barbouri*, Mc = *Monacanthus chinensis*, Ng = *Nuchequula gerreoides*, Nm = *Neoscopelus microchir*, Pb = *Pisodonophis boro*.

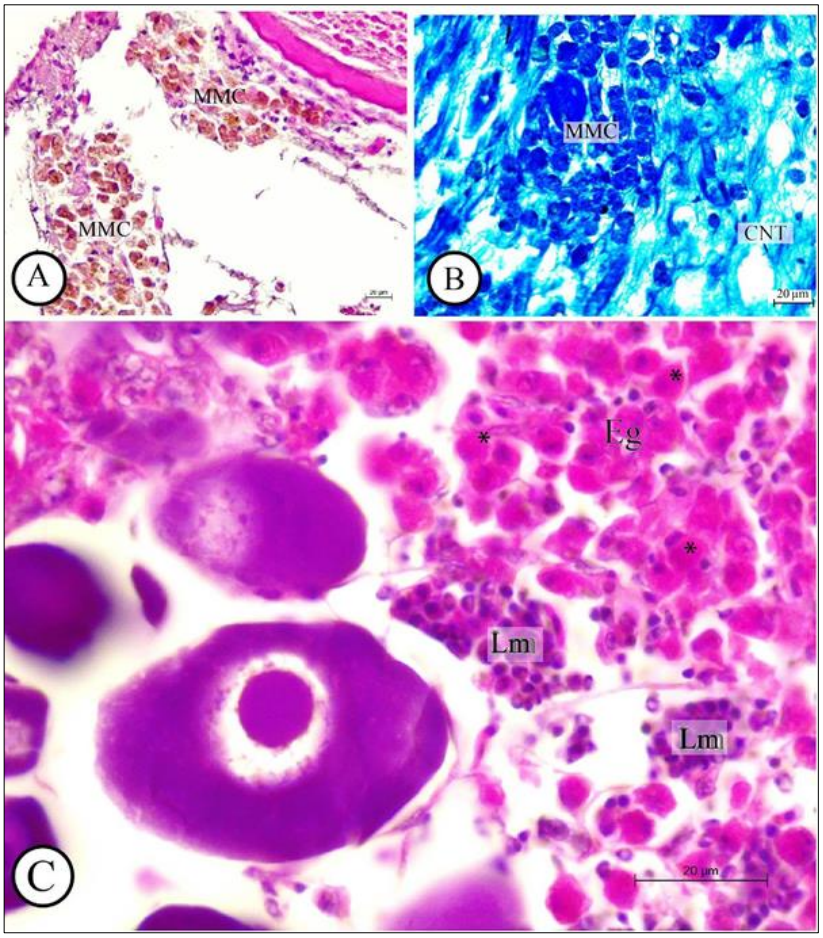

Fig. 6. Histology sections showing the melanomacrophage centres (MMCs) among the connective tissue (CNT) of *Allenbatrachus grunniens* [A-B]. [C]: Eosinophilic granulocytes (Eg) and lymphocyte infiltration (Lm) of *Eubleekeria splendens*. * = eosinophilic granule.

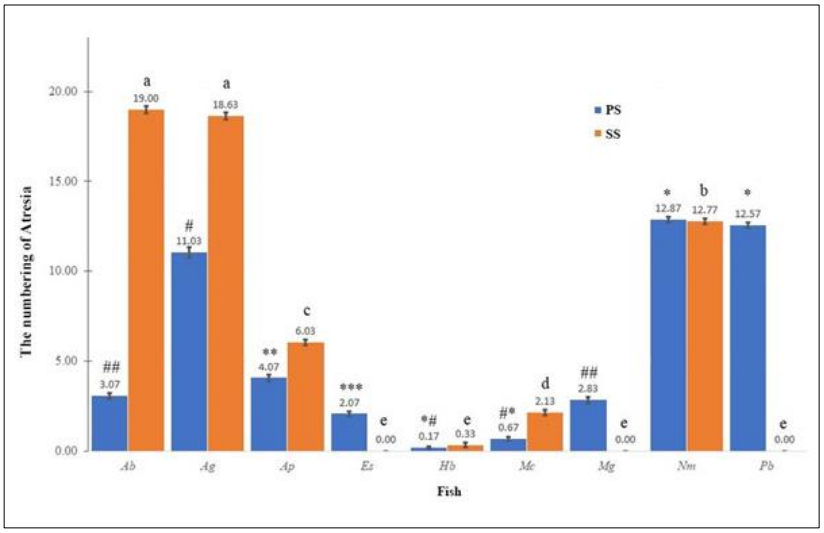

Fig. 7. Number of atresia of previtellogenic stage from primary growth phase (PS) and atresia of vitellogenic stages from secondary growth phase (SS). Values represent means ± SE; alphabet (a-e) and symbol (* and #) indicate significant differences at $P < 0.05$. Abbreviations: Ab = *Alepocephalus bicolor*, Ag = *Allenbatrachus grunniens*, Ap = *Atherinomorus pinguis*, Es = *Eubleekeria splendens*, Hb = *Hippocampus barbouri*, Mc = *Monacanthus chinensis*, Ng = *Nuchequula gerreoides*, Nm = *Neoscopelus microchir*, Pb = *Pisodonophis boro*.

Discussion

The histological analysis of the ovarian parenchyma revealed that most female fish in the present study have asynchronous ovarian development. This is possibly related to the fact that most of the analysed species have multiple spawning seasons and protracted spawning periods (Dietrich and Krieger, 2008). The synchronous development ovary was observed only in *P. boro*, suggesting that it is a single spawning species. This hypothesis requires further studies on the reproductive cycle and spawning season.

The absence of SG phase oocytes in *N. gerreoides*, *P. boro,* and *E. splendens* is possibly related to the fact that all of them are migratory fish (Blaber, 1997), for which the estuarine is considered to be a putative nursery area. Adults of these species may spawn in the coastal area, and larvae are passively transported toward the estuaries. This hypothesis can be verified by life history studies with broad sampling from the estuarine populations (Elliott and Hemingway, 2002; Potter et al., 2015).

The common oogenic features identified in this study are generally inconsistent with those of other teleosts. A single and large central nucleus and nucleoli near the nuclear membrane in the Pn stage has been reported in other teleost species (Selman and Wallace, 1986; Blazer, 2002; Patino and Sullivan, 2002; Patino et al., 2003). The basophilic nature of ooplasm of PG phase oocytes is attributed to the synthesis of RNAs as well as the abundant ribosomes and mitochondria in the ooplasm (Wallace and Selman, 1990). And two important structures observed in SG phase oocytes, the lipid droplet and cortical alveoli, have been reported in other teleosts with their potential functions (Wallace and Selman, 1990; Senarat et al., 2017). Namely, the oil droplets support the metabolic activity for embryonic development additionally to the yolk granules (Chen et al., 2006), whereas cortical alveoli prevent polyspermy after ovulation (Nagahama, 1983; Selman and Wallace, 1986; Selman et al., 1988; Abascal and Medina, 2005). The migration of nucleus to the animal pole during the Lsg step (germinal vesicle migration; GVM) is observed in other species such as *Fundulus heteroclitus* (Linnaeus, 1766), (Kuchnow and Scott, 1977) and *Thunnus orientalis* (Temminck & Schlegel, 1844), (Chen et al., 2006). This aspect is common for all fishes. Finally, the coalescence of yolk globules is known as the indicator of oocyte maturation during the SG phase (Selman and Wallace, 1986; Selman et al., 1993). Since no nucleus is present at this point, the final oocyte maturation can be easily assessed in the teleost fish using this morphological marker (West, 1990).

The histological examination found that 6.66 % of *A. grunniens* specimens have ovotestis, which is the first report from Thailand. The presence of intersex in fish has been strongly associated with endocrine-disrupting chemicals (EDCs) in the environment (Allen et al., 1999; Vethaak et al. 2005; Vajda et al. 2008; Yoon et al., 2008; Muneeb, 2017), including estrogen in human wastewater effluent (Jobling et al., 1998) and industrial discharge (Van Aerle et al. 2001; Tetreault et al. 2011). For example, the occurrence of intersex in *Rutilus rutilus* (Linnaeus, 1758) captured at 45 sites in UK rivers was strongly associated with estrogen concentrations predicted from upstream estrogen inputs (Jobling et al., 2006). A similar tendency was reported by Antuofermo et al. (2017), they found excessive intersex gonads in wild fish populations: *Chelon labrosus* (Risso, 1827); *Liza aurata* (Risso, 1810) and *Mugil cephalus* Linnaeus, 1758, inhabiting xenoestrogen-polluted coastal and estuarine environments in Sardinia island. It is hence likely that the ovotestis of *A. grunniens* may be related to exposure to environmental pollutants with an endocrine disruption/estrogenic potential. Direct monitoring of estrogenic substances will be the next step to understand the effect of estrogen contamination on fish reproduction in these areas. Also, Harris et al. (2011) reported that the reproductive performance (fry production) of *R. rutilus* is reduced up to 76 % in intersex individuals. Assessment of *A. grunniens* reproductive ability is also warranted. In contrast, it is also possible that the presence of oocytes in the testis is a normal feature in this species. While we have a preliminary observation that this toadfish is a dioecious species (Mitparian et al., 2018), the prevalence and physiological significance of ovotestis should be carefully examined with comprehensive sampling.

The present study also found atretic oocytes with irregular shape and degenerated yolk granules in both PG and SG phases (Table 2 and Figures 4-5). This is the first description on atretic oocytes in these nine important fish species from Thailand. The histopathological characteristics of the atretic oocytes are similar to those reported for *Brycon orthotaenia* G Günther, 1864 (Goncalves et al., 2006), and several other fishes (Johnson et al., 1988; Blazer, 2002; Jamieson, 2009). Since we observed atretic oocytes in apparently healthy *M. chinensis* and *H. barbouri* (Table 2), this situation might be a normal physiological occurrence. However, atretic oocytes have also been associated to environmental stress like unfavourable temperature and pH as well as high anthropogenic pressure including environmental contaminants (Cross and Hose, 1988; Johnson et al., 1988; Kirubagaran and Joy, 1988; Blazer, 2002). In particular, EDCs are proposed as the major cause of increased atretic oocytes and follicular cell degeneration (Pedlar et al., 2002; Kinnberg and Toft, 2003; Diniz et al., 2005; Hanna et al., 2005). Specifically, atrazine (Spano et al., 2004; Tillitt et al., 2010), 17β-estradiol (E2) (Wood and van Der Kraak, 2002), and many other EDCs have been reported to increase atretic oocytes. The prevalence of atretic oocytes also justifies future studies on EDCs.

An important finding of the atretic oocytes in this study is that their highest prevalence was observed in mesopelagic (*N. microchir* and *A. bicolor at* SG phase) and some estuarine fishes (*P. boro* at PG phase and *A. grunniens*). Mesopelagic fishes live in the depth range from 200to 1,000 m (Gartner et al., 1997), typically at the edge of the continental slope where food resources are generally limited (Hopkins and Gartner, 1992; Close et al., 2013; Bode and Hernandez-Leon, 2018). Mesopelagic fish, therefore, exhibit the resource partitioning (Hopkins and Gartner, 1992), which often negatively affect their reproductive activity (Young, 2003; Brown-Peterson et al., 2011). It is therefore possible that the low feeding rate is responsible for the prevalence of atretic oocytes in *N. microchir* and *A. bicolor* as proposed by Billard (1992). The concentrations of anthropogenic pollutants such as mercury and other trace metals are not available in Andaman seas, although these are found in the deep-sea environment (Looser et al., 2000).

Several reports have documented that the Pranburi River estuary has been contaminated with anthropogenic wastes, especially lead and petroleum hydrocarbon in sediment (Cheevaporn and Menasveta, 2003; Wattayakorn, 2012). There are many previous observations that heavy metals exert adverse effects on fish reproduction (Ebrahimi and Taherianfard, 2011). Since heavy metals tend to bind to the soil particle (U.S. EPA, 2009), it is assumed that benthic species, *P. boro* and *A. grunniens*, were affected by heavy metals more strongly than pelagic fishes, *N. gerreoides* and *E. splendens*.

Melanomacrophage centres (MMCs) have been commonly used as environmental stress biomarkers in fishes (Agius and Roberts 2003, Robert, 2012). In this study, large MMCs were observed in ovarian parenchyma of mesopelagic and some estuarine fishes (Table 2 and Figure 6), suggesting that these fishes are living under certain environmental stress conditions. However, many previous studies claim that MMCs are formed by various factors including life history (i.e. sex, developmental stage and spawning seasons) or environmental changes (i.e. temperature and UV exposure) (Blazer et. al, 1997; Kumar and Singh, 2016; Natalie and Daniel, 2017). Parasites also cause the formation of MMCs (Alvarez-Pellitero et al., 2007). Continuous monitoring associated with environmental pollution levels will provide further provide insights into the empirical evidence for the use of MMCs as the pollutant marker.

The histopathological analysis also demonstrated that *N. gerreoides* and *E. splendens* have eosinophilic granulocytes (Ecs) in their ovarian tissues as reported in some fishes (Drury, 1915; DeMartini, 2017). The Ecs are sometimes referred to as mast cells, which are a type of immune cells (Drury, 1915). In addition, Besseau and Faliex (1994) proposed that the cytoplasmic granules of Ecs contain lytic enzymes, and thus Ecs correspond to phagocytic macrophages in gonads activated by environmental pollutants and diseases (Drury, 1915). The presence of Ecs in *N. gerreoides* and *E. splendens* collected form estuarine habitats might be related to the contamination from industrial and agricultural discharges in this area.

Conclusion

The present study showed ovarian structures and their histopathological alterations of nine important fishes in Thailand. The first observation of ovotestis in Thailand (*A. grunniens*) warrants the investigation of endocrine-disrupting chemicals in the Pranburi River estuary. Also, the high prevalence of atretic oocytes in the mesopelagic and estuarine fishes suggests a high level of pollution in these habitats and/or high susceptibility of these fishes to environmental stress. Further studies should be conducted to regularly monitor the pollutant flux, especially the endocrine-disrupting chemicals, in these areas. It is also important to evaluate the effect of the important heavy metals (iron, cadmium, zinc and manganese) on histopathological changes such as ovotestis and atretic oocytes identified in this study, the presence of which may be a normal feature to a certain extent.

Acknowledgements

Data for the mesopelagic fish samples were collected through the scientific surveys with the R/V Dr Fridtjof Nansen as part of the collaboration between the EAF-Nansen Programme and Thailand. The EAF-Nansen Programme is a partnership between the Food and Agriculture Organization of the United Nations (FAO), the Norwegian Agency for Development Cooperation (Norad) and the Institute of the Marine Research (IMR), Norway for sustainable management of the fisheries of partner countries.

References

Abascal, F.J., Medina, A. 2005. Ultrastructure of oogenesis in the bluefin tuna, *Thunnus thynnus*. Journal of Morphology 264:149–160. https://doi.org/10.1002/jmor.10325

Agius, C., Roberts, J. 2003. Melano-macrophague centres and their role in fish pathology. Journal of Fish Diseases 26:499–509. https://doi.org/10.1046/j.1365-2761.2003. 00485.x

Allen, Y., Scott, A.P., Matthiessen, P., Haworth, S., Thain, J.E., Feist, S. 1999. Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. Environmental Toxicology and Chemistry 18:1791–1800. https://doi.org/10.1002/etc.5620180827

Alvarez-Pellitero, P., Palenzuela O., Sitjà-Bobadilla A. 2007. Histopathology and cellular response in *Enteromyxum leei* (Myxozoa) infections of *Diplodus puntazzo* (Teleostei). Parasitology International 57:110–120. https://doi.org/10.1016/j.parint.2007.09.004

Antuofermo, E., Ariu, R., Burrai, G.P., Polinas, M., Sanna, M.A., Esposito, G., Prearo, M. Pais, A. 2017. First evidence of intersex condition in extensively reared mullets from *Sardinian lagoons* (Central-Western Mediterranean, Italy). Italian Journal of Animal Science 16:283–291. https://doi.org/10.1007/s11356-013-1745-3

Aquatic Resources Research Institute (ARRI). 2003. Monitoring of red tides in Thailand. Technical paper, Aquatic Resources Research Institute, Chulalongkorn University. 213 pp. (in Thai).

Besseau, L., Faliex, E. 1994. Resorption of unemitted gametes in *Lithognathus mormyrus* (Sparidae, Teleostei): A possible synergic action of somatic and immune cells. Cell and Tissue Research 276:123–132. https://doi.org/10.1007/bf00354791

Billard, R. 1992. Reproduction in rainbow: Sex differentiation, dynamics of gametogenesis, biology and preservation of gametes. Aquaculture 100:263–298. https://doi.org/10.1016/0044-8486(92)90385-X

Blaber, S.J.M. 1997. Fish and fisheries in tropical estuaries. Springer Science and Business Media, Netherlands. 367 pp.

Blazer, V.S. 2002. Histopathological assessment of gonadal tissue in wild fishes. Fish Physiology and Biochemistry 26:85–101. https://doi.org/10.1023/A:1023332216713

Blazer, V.S., Fournie, J.W., Weeks-Perkins, B.A. 1997. Macrophage aggregates: Biomarker for immune function in fishes? In: Environmental toxicology and risk assessment: modeling and risk assessment, Dwyer, F.J., Doane, T.R., Hinman, M.L. (Eds.), Volume 6. American Society for Testing and Materials, Philadelphia, PA. pp. 360–375.

Bode, A., Hernandez-Leon, S. 2018. Trophic diversity of plankton in the epipelagic and mesopelagic layers of the tropical and equatorial Atlantic determined with stable isotopes. Diversity 10:48. https://doi.org/10.3390/d10020048

Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz. B.J., Lowerre-Barbieri, S.K. 2011. A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries 3:52–70. https://doi.org/10.1080/19425120. 2011.555724

Cheevaporn, V., Menasveta, P. 2003. Water pollution and habitats degradation in the Gulf of Thailand. Marine Pollution Bulletin 47:43–51. https://doi.org/10.1016/S0025-326X(03)00101-2

Chen, K.S., Crone, P., Hsu, C.C. 2006. Reproductive biology of female Pacific Bluefin tuna *Thunnus orientalis* from South-Western North Pacific Ocean. Fisheries Science 72: 985–994. https://doi.org/10.1111/j.1444-2906.2006.01247.x

Close, H.G., Shah, S.R., Ingalls, A.E., Diefendorf, A.F., Brodie, E.L., Hansman, R.L., Freeman, K.H., Aluwihare, L.I., Pearson, A. 2013. Export of submicron particulate organic matter to mesopelagic depth in an oligotrophic gyre. Proceedings of the National Academy of Sciences 110:12565–12570. https://doi.org/10.1073/pnas.1217514110

Cross, J.N., Hose, J.E. 1988. Evidence for impaired reproduction in white croaker *Genyonemus lineatus* from contaminated areas of Southern California. Marine Environmental Research 24:185–188. https://doi.org/10.1016/0141-1136(88)90295-4

DeMartini, E.E. 2017. Eosinophilic granulocytes: A new bio-marker of sexual maturity in fishes? Copeia 105:664–669. https://doi.org/10.1643/CI-17-758

Dietrich, D.R., Krieger, H.O. 2008. Histological analysis of endocrine disruptive effects in small laboratory fish. John Wiley and Sons, New Jersey. 341 pp. https://doi.org/10.1002/9780470431795

Diniz, M.S., Peres, I., Magalhães-Antoine, I., Falla, J., Pihan, J.C. 2005. Estrogenic effects in crucian carp (*Carassius carassius*) exposed to treated sewage effluent. Ecotoxicology and Environmental Safety 62:427–435. https://doi.org/10.1016/j.ecoenv.2004.11.004

Drury, A.N. 1915. The eosinophil cell of teleostean fish. Journal of Physiology 49:349–367. https://doi.org/10.1113/jphysiol.1915.sp001714

Ebrahimi, M., Taherianfard, M. 2011. The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. Iranian Journal of Fisheries Sciences 10:13–24. http://jifro.ir/article-1-121-en.html

Elliott, M., Hemingway, K. 2002. Fishes in estuaries. Blackwell Science, London. 636 pp. https://doi.org/10.1002/9780470995228

Gartner, J.V., Crabtree, R.E., Sulak, K.J. 1997. Feeding at depth. In: Deep-sea fishes, Randall, D.J., Farrell, A.P. (Eds.), Academic Press, London. pp. 115–194.

Goncalves, T.L., Bazzoli, N., Brito, M.F.G. 2006. Gametogenesis and reproduction of the matrinxa *Brycon orthotaenia* (Gunther, 1864) (Pisces: Characidae) in the Sao Francisco river, Minas Gerais, Brazil. Brazilian Journal of Biology 66:513–522. https://doi.org/10.1590/S1519-69842006000300018

Hanna, M.I., Shaheed, I.B., Elias, N.S. 2005. A contribution on chromium and lead toxicity in cultured *Oreochromis niloticus*. Egyptian Journal of Aquatic Biology and Fisheries 9:177–209.

Harris, C.A., Hamilton, P.B., Runnalls, T.J., Vinciotti, V., Henshaw, A., Hodgson, D., Coe, T.S., Jobling, S., Tyler, C.R., Sumpter, J.P. 2011. The consequences of feminization in breeding groups of wild fish. Environmental Health Perspectives 119:306–311. https://doi.org/10.1289/ehp.1002555.

Hopkins, T.L., Gartner, J.V. 1992. Resource-partitioning and predation impact of a low-latitude myctophid community. Marine Biology 114:185–197. https://doi.org/10.1007/BF00349518

Jamieson, B.G.M. 2009. Reproductive biology and phylogeny of fishes (Agnathans and bony fishes). CRC Press, Boca Raton. 802 pp. https://doi.org/10.1201/9781482280609

Jobling, S., Nolan, M., Tyler, C.R., Brightly, G., Sumpter, J.P. 1998. Widespread sexual disruption in wild fish. Environmental Science and Technology 32:2498–2506. https://doi.org/10.1021/es9710870

Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C.R., van Aerle, R., Santos, E., Brighty, G. 2006. Predicted exposures to steroid estrogens in UK Rivers correlate with widespread sexual disruption in wild fish populations. Environmental Health Perspectives 114 (Suppl.1):32–39. https://doi.org/10.1289/ehp.8050

Johnson, L.L., Casillas, E., Collier, T.K., McCain, B.B., Varanasi, U. 1988. Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Canadaian Journal of Fisheries and Aquatic Science 45:2133–2146. https://doi.org/10.1139/f88-248

Kinnberg, K., Toft, G. 2003. Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). Ecotoxicology and Environmental Safety 54:16–24. https://doi.org/10.1016/S0147-6513(02)00010-6

Kirubagaran, R., Joy, K.P. 1988. Toxic effects of mercuric chloride, methylmercuric chloride, and emisan 6 (an organic mercurial fungicide) on ovarian recrudescence in the catfish *Clarias batrachus* (L.). Bulletin of Environmental Contamination and Toxicology 41:902–909. https://doi.org/10.1007/bf02021053

Kuchnow, K.P., Scott, J. R. 1977. Ultrastructure of the chorion and its micropyle apparatus in the mature *Fundulus heteroclitus* (Walbaum) ovum. Journal of Fish Biology 10:197–201. https://doi.org/10.1111/j.1095-8649.1977.tb05125.x

Kumar, R., Joy, K.P., Singh, S.M. 2016. Morpho-histology of head kidney of female catfish *Heteropneustes fossilis*: Seasonal variations in melano-macrophage centers, melanin contents and effects of lipopolysaccharide and dexamethasone on melanins. Fish Physiology and Biochemistry 42:1287–1306. https://doi.org/10.1007/s10695-016-0218-2

Looser, R., Froescheis, O., Cailliet, G.M., Jarman, W.M., Ballschmiter, K. 2000. The deep-sea as a final global sink of semivolatile persistent organic pollutants? Part II: Organochlorine pesticides in surface and deep-sea dwelling fish of the North and South Atlantic and the

Monterey Bay Canyon (California). Chemosphere 40:661–670. https://doi.org/10.1016/S0045-6535(99)00462-2

Mitparian, T., Kettratad, J., Jiraungkoorskul, W. 2018. Gametogenic maturation of the *Allenbatrachus grunniens* (Linnaeus, 1758) from Pranburi Estuary. Proceedings of the 5th National Meeting on Biodiversity Management in Thailand. pp. 99–107.

Muneeb, U.R., Rayeesa, A., Sheikh, B., Gowher, G., Ishraq, H., Shahzada, M., Manzoor, R.M. 2017. Endocrine disrupting Chemicals (EDCs) and fish health - A brief review. International Journal of Livestock Research 7:45–54. https://doi.org/10.5455/ijlr.20170812034344

Na Lampang, P., Palasia, A., Senarat, S., Jiraungkoorskul, W., Kaneko, G., Kettratad, J. 2021. Body Size distribution and ovarian histology of *Pisodonophis boro* (Hamilton, 1822) (Anguillifomes: Ophichthidae) from Pranburi River Estuary, Thailand. Chiang Mai University Journal of Natural Sciences (in press).

Nagahama, Y. 1983. Chapter 6: The functional morphology of teleost gonads. In: Reproduction endocrine tissues and hormones, fish physiology. Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Vol. 9A. Academic Press, New York. pp. 223–275.

Natalie, C.S., Daniel, I.B. 2017. Melanomacrophage centers as a histological indicator of immune function in fish and other poikilotherms. Frontiers in Immunology 8:827. https://doi.org/10.3389/fimmu.2017.00827

Patino, R., Sullivan, C.V. 2002. Ovarian follicle growth, maturation, and ovulationin teleost fish. Fish Physiology and Biochemistry 26:57–70. https://doi.org/10.1023/A:1023311613987

Patino, R., Wainscott, M. R., Cruz-Li, E. I., Balakrishnan, S., McMurry, C., Blazer, V.S. Anderson, T.A. 2003. Effects of ammonium perchorate on the reproductive performance and thyroid follicle histology of zebra fish. Environmental Toxicology and Chemistry 22:1115–1121. https://doi.org/10.1002/etc.5620220520

Pedlar, R.M., Ptashynski, M.D., Evans, R., Klaverkamp, J.F. 2002. Toxicological effects of dietary arsenic exposure in Lake Whitefish (*Coregeonus clupeaformis*). Aquatic Toxicology 57:167–189. https://doi.org/10.1016/S0166-445X(01)00198-9

Potter, I., Tweedley, J., Elliott, M., Whitfield, A. 2015. The ways in which fish use estuaries: A refinement and expansion of the guild approach. Fish and Fisheries 16:230–239. https://doi.org/10.1111/faf.12050

Presnell, J.K., Schreibman, M.P. 1997. Humason's animal tissue techniques. 5th Edition. John Hopkins University Press. Baltimore. 600 pp.

Robert, R.J. 2012. Fish pathology. 4th Edition. Wiley-Blackwell, New Jersey. 590 pp.

Rowden, G., Lewis, M.G. 1974. Experience with a three-hours electron microscopy biopsy service. Journal of Clinical Pathology 27:505–510. https://doi.org/10.1136/jcp.27.6.505

Selman, K., Wallace, R.A., Barr, V. 1988. Oogenesis in *Fundulus heteroclitus*. V. The relationship of yolk vesicles and cortical alveoli. Journal of Experimental Zoology 246:42–56. https://doi.org/10.1002/jez.1402460107

Selman, K., Wallace, R.A., Sarka, A., Qi, X. 1993. Stages of oocyte development in the zebra fish, *Brachydanio rerio*. Journal of Morphology 218:203–224. https://doi.org/10.1002/jmor.1052180209

Selman, K., Wallace. R.A. 1986. Gametogenesis in *Fundulus heteroclitus*. American Zoologist 26:173–192. https://doi.org/10.1093/icb/26.1.173

Senarat, S., Kettretad, J., Jiraungkoorskul, W. 2015. Classification stages of novel atretic structure in short mackerel *Ratrilliger brachysoma* (Bleeker, 1851) from the Upper Gulf of Thailand. Songklanakarin Journal of Science and Technology 37:569–573. https://doi.org/10.14456/sjst-psu.2017.26

Senarat, S., Kettretad, J., Jiraungkoorskul, W. 2017. Ovarian histology and reproductive health of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851), as threatened marine fish in Thailand. Songklanakarin Journal of Science and Technology 39:225–235. https://doi.org/10.14456/sjst-psu.2017.26

Spano, L., Tyler, C.R., van Aerie, R., Devos, P., Mandiki, S.N., Silvestre, F., Thome, J.P., Kestemont, P. 2004. Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*). Aquatic Toxicology 66:369–379. https://doi.org/10.1016/j.aquatox.2003.10.009

Suvapepun, S. 1991. Long term ecological changes in the Gulf of Thailand. Marine Pollution Bulletin 23:213–217. https://doi.org/10.1016/0025-326X(91)90677-K

Suvarna, K.S., Layton, C. Bancroft, J.D. 2018. Bancroft's theory and practice of histological techniques. 8th Edition. Elsevier Health Sciences, Toronto. 672 pp. ISBN: 978-0702068867

Tetreault, G.R., Bennett, C.J., Shires, K., Knight, B., Servos, M., McMaster, M.E. 2011. Intersex and reproductive impairment of wild fish exposed to multiple municipal wastewater discharges. Aquatic Toxicology 104:278–290. https://doi.org/10.1016/j.aquatox.2011.05.008

Tillitt, D.E., Papoulias, D.M., Whyte, J.J., Richter, C.A. 2010. Atrazine reduces reproduction in fathead minnow (*Pimephales promelas*). Aquatic Toxicology 99:149–159. https://doi.org/10.1016/j.aquatox.2010.04.011

United States Environmental Protection Agency (U.S. EPA). 2009. Risks of paraquat use to federally threatened California red-legged frog (*Rana aurora draytonii*). Environmental Fate and Effects Division, Office of Pesticide Programs, Washington D.C. 293 pp.

Uribe, M.C., Grier, H.J., Parenti, L.R. 2012. Ovarian structure and oogenesis of the oviparous Goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (Teleostei, Cyprinodontiformes). Journal of Morphology 273:371–387. https://doi.org/10.1002/jmor.11028

Vajda, A.M., Barber, L.B., Gray, J.L., Lopez, E.M., Woodling, J.D., Norris, D.O. 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environmental Science and Technology 42:3407–3414. https://doi.org/10.1021/es0720661

van Aerle, R., Nolan, M., Jobling, S., Christiansen, L.B., Sumpter, J.P., Tyler, C.R. 2001. Sexual disruption in a second species of wild cyprinid fish (the gudgeon, *Gobio gobio*) in United Kingdom freshwaters. Environmental Toxicology and Chemistry 20:2841–2847. https://doi.org/10.1002/etc.5620201225

Vethaak, A.D., Lahr, J., Schrap, S.M., Belfroid, A.C., Rijs, G.B., Gerritsen, A., de Boer, J., Bulder, A.S., Grinwis, G.C., Kuiper, R.V., Legier, J., Murk, T.A., Peijnenburg, W., Verhaar, H.J., de Voogt, P. 2005. An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. Chemosphere 59:511–524. https://doi.org/10.1016/j.chemosphere.2004.12.053

Wallace, R.A., Selman, K. 1990. Ultrastructural aspects of oogenesis and oocyte growth in fish and amphibians. Journal of Electron Microscopy Technique 16:175–201. https://doi.org/10.1002/jemt.106016302

Wattayakorn, K. 2012. Petroleum pollution in the Gulf of Thailand: A historical review. Coastal Marine Science 35:234–245.

West, G. 1990. Methods of assessing ovarian development in fishes: A review. Australian Journal of Marine and Freshwater Research 41:199–222. https://doi.org/10.1071/MF9900199

Wood, A., van Der Kraak, G. 2002. Inhibition of apoptosis in vitellogenic ovarian follicles of rainbow trout (*Oncorhynchus mykiss*) by salmon

gonadotropin, epidermal growth factor and 17β-estradiol. Molecular Reproduction and Development 61:511–518. https://doi.org/10.1002/mrd.10108

Yoon, S.H., Itoh, Y., Kaneko, G., Nakaniwa, M., Ohta, M., Watabe, S. 2008. Molecular characterization of Japanese sillago vitellogenin and changes in its expression levels on exposure to 17β-estradiol and 4-tert-octylphenol. Marine Biotechnology 10:19–30. https://doi.org/10.1007/s10126-007-9055-8

Young, C.M. 2003. Reproduction, development and life history traits. In: Ecosystems of the deep oceans. Tyler, P.A. (Ed.). Elsevier Science, London. pp. 381–426.