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Original Article

Ovarian histology and reproductive health of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851), as threatened marine fish in Thailand

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Abstract

The ovarian structure and reproductive health of short mackerel, *Rastrelliger brachysoma* in the upper Gulf of Thailand from non-breeding (October to November 2013) and breeding seasons (December 2013 to February 2014), were examined by histological and histochemical techniques. The results revealed that the ovary in this species was enclosed by the tunica albuginea. It was considered as an asynchronous type, which could be classified into seven stages based on its histological characteristics. These seven stages were oogonium, chromatin nucleolar, perinucleolar, oil droplet and cortical alveolar, early, late and post vitellogenic stages. During the oogenic processes, the change and characterization of inclusions including lipids, cortical alveoli and yolk granules were detected by histolochemical techniques. The ovarian histopathology in *R brachysoma* was detected. This includes ovarian degeneration, ovarian atrophy and atresia in different stages (oogonia, previtellogenic stages and vitellogenic stages) as well as melano-magcrophage centers. Atretic follicles and ovarian atrophy were also observed and could negatively affect the reproductive health of the species, which could lead to population declines in *R. brachysoma* as well as threaten other marine fishes connected through the food web in Thailand.

Keywords: ovary, histopathology, *Rastrelliger brachysoma*, reproductive failure

1. Introduction

The short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851), is the principal resource supporting a large fishery industry in Thailand. Currently hypothesized reproductive scheme of the *R. brachysoma* in the Gulf of Thailand included three periods. The first period extends from January to March and the second period is from June to August. Both periods are in the vicinity of the spawning ground of the Prachuap Khiri Khan and *Surat Thani Province*s. The third reproductive season extends from October to November. The spawning ground in this period is in the vicinity of Samut Sakhon and Samut Songkram Provinces (Menaveta, 1980). This hypothesized reproductive scheme was based on the gonado-somatic index (GSI), the gross morphology and coloration of the ovarian tissue (Sritakon *et al*., 2006; Sutthakorn, 1998). Nonetheless, histological aspects of the ovarian tissue in *R. brachysoma* have not yet been identified. Using such techniques will give more accurate results and would increase the baseline knowledge of their reproductive development and reproductive cycle.

To date, this species is listed as a threatened marine fish in Thailand (Senarat *et al*., 2015) due to the occasional declines in the natural population. The declines were likely caused by over fishing and the deterioration of their natural habitat. Previous studies suggested that the Upper Gulf of

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Thailand, which is the prime habitat of *R. brachysoma*, has environmental stressors and high pollutants which include heavy metals and petroleum hydrocarbon (Wattayakorn, 2012). Previous studies on health assessment of *R. brachysoma* showed severe sign of histopathology such as various lesions and parasites in the liver, brain and testis (Senarat *et al*., 2015; Senarat *et al*. unpublished data). However, comprehensive study on the reproductive health of female *R. brachysoma* is still lacking.

In this article, we primarily attempted to identify the ovarian structure and reproductive mode of *R. brachysoma* during its annual reproductive season using histological and histochemical analyses. In addition to reproductive health, we assessed the *R. brachysoma* ovarian tissue using histopathological biomarker. This information is vital for the provision of scientific advice to aquaculture development. For example, the study of the oocyte quality and the functions/effects of neurotransmitter and other neurohormones, which are important in the maturation of the ovary can increase the success rate in aquaculture of this species which would ultimately help increase the natural population of this species.

2. Materials and Methods

Adult short mackerel, *Rastrelliger brachysoma* with a standard body length about 15-19 cm (average 16.5±0.49 cm) and total weight about (68.68±0.67 g) were considered as late – development stage of the ovarian histology. They were obtained by bamboo strake trap during non-breeding (October to November 2013, n = 30) and breeding seasons (January to February 2014, n = 30) from the area of Samut Songkram Province in the Upper Gulf of Thailand (13°16’ 18.4" N, 100°02’13.4" E). Identification of all fish was made according to the taxonomic key of FAO (Food and Agriculture Organization [FAO], 2010). Fish were then euthanized by the rapid cooling shock method (Wilson *et al*., 2009).

The light microscopic observation, the ovarian tissue of fish was removed from the body cavity and fixed in Davidson’s fixative (48 hrs). Then, they were processed using standard histological techniques (Bancroft & Gamble, 2002). The gonadal paraffin block was cut at 5 µm thickness and stained with Harris’s hematoxylin and eosin (H&E) and Masson’s trichrome (MT), periodic acid-schiff (PAS), Grocott methenamine silver (GMS), alcian blue pH 2.5 (AB) and reticulin (Rt) methods (Puchtler and Waldrop, 1978; Vidal, 1988; Bancroft and Gamble, 2002). Additionally, frozen tissues were cut at 10 µm thicknesses and stained with oil red O (ORO) (Culling, 1963). The ovarian structure oogenesis with size measurement was assessed to detail according to the guidelines of Dietrich and Krieger (2009) and Brown-Peterson *et al.* (2011) under light microscopy. The numbers of atretic follicles were quantified (50-100 oocytes/fish per section) in middle area of three ovarian sections, and followed the guidelines under light microscopy (10x and 40x) of Blazer (2002).

For ultrastructural observation, small pieces of ovarian atrophy were cut and rapidly pre-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 at 4°C and washed in the buffered fixative solution. Thereafter, they were post-fixed with 1% osmium tetraoxide (OsO_4) and then processed using standard ultrastructural techniques (Rowden & Lewis, 1974). The thin sections were cut by ultramicrotome about 90 nm thicknesses, stained with uranyl acetate and lead citrate. Lastly, the ultrathin sections were observed and photographed using a JEM-2100 at 200kV.

Histopathological analysis, the histopathological alterations of ovarian tissue were observed and recorded as a mean prevalence for each period.

3. Results

Based on anatomical analysis, the asymmetrical elongation of *R. brachysoma* ovaries were paired organs located in the abdominal cavity. Cross sections of the immature ovary in non-breeding season were histologically surrounded by tunica albuginea including a thick layer of connective tissue and numerous blood vessels (Figures 1a, 1b, 1c, 2r). They were suspended by mesovarium from the peritoneal dorsal

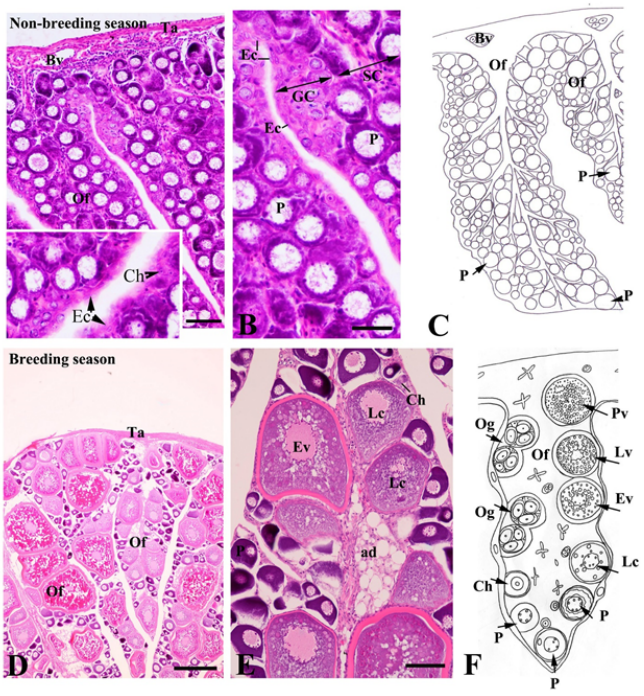

Figure 1. Light photomicrograph (a, b, c, e, f) and schematic summary of ovarian structure (d, g). adipose tissue (ad), blood vessel (Bv), chromatin nucleolar stage (Ch), epithelium cells (Ec), early vitellogenic stage (Ev), germinal compartment (GC), lipid and cortical alveoli stage (Lc), late vitellogenic stage (Lv), ovigerous fold (Of), oognia (Og), perinucleolar stage (P), post vitellogenic stage (Pv), stromal compartment (SC), tunica albuginea (Ta). Scale bar a, c, f = 100 µm, e = 200 µm.

wall. Overall histological sections in both immature and mature ovaries revealed that the ovigerous fold protruded into a central lumen where it could be distinguished into two compartments: (i) a germinal compartment which was covered with the ovigerous fold. The ovigerous fold were composed of epithelial cells and oogonia. (ii) A stromal compartment which contained the different of oogenic stages (Figure 1a and schematic diagram 1d). The ovary in this species was described as an asynchronous developmental type, which was distinctly classified into seven stages based on the cell size, histological features of each developing oocyte and staining properties are given as follows (Figures 1a-1g and 2a-2v).

Oogonia, the smallest among oogenic cells, were located and scattered within the somatic epithelial cells in the germinal epithelium. The oogonium was an oval-rounded cell

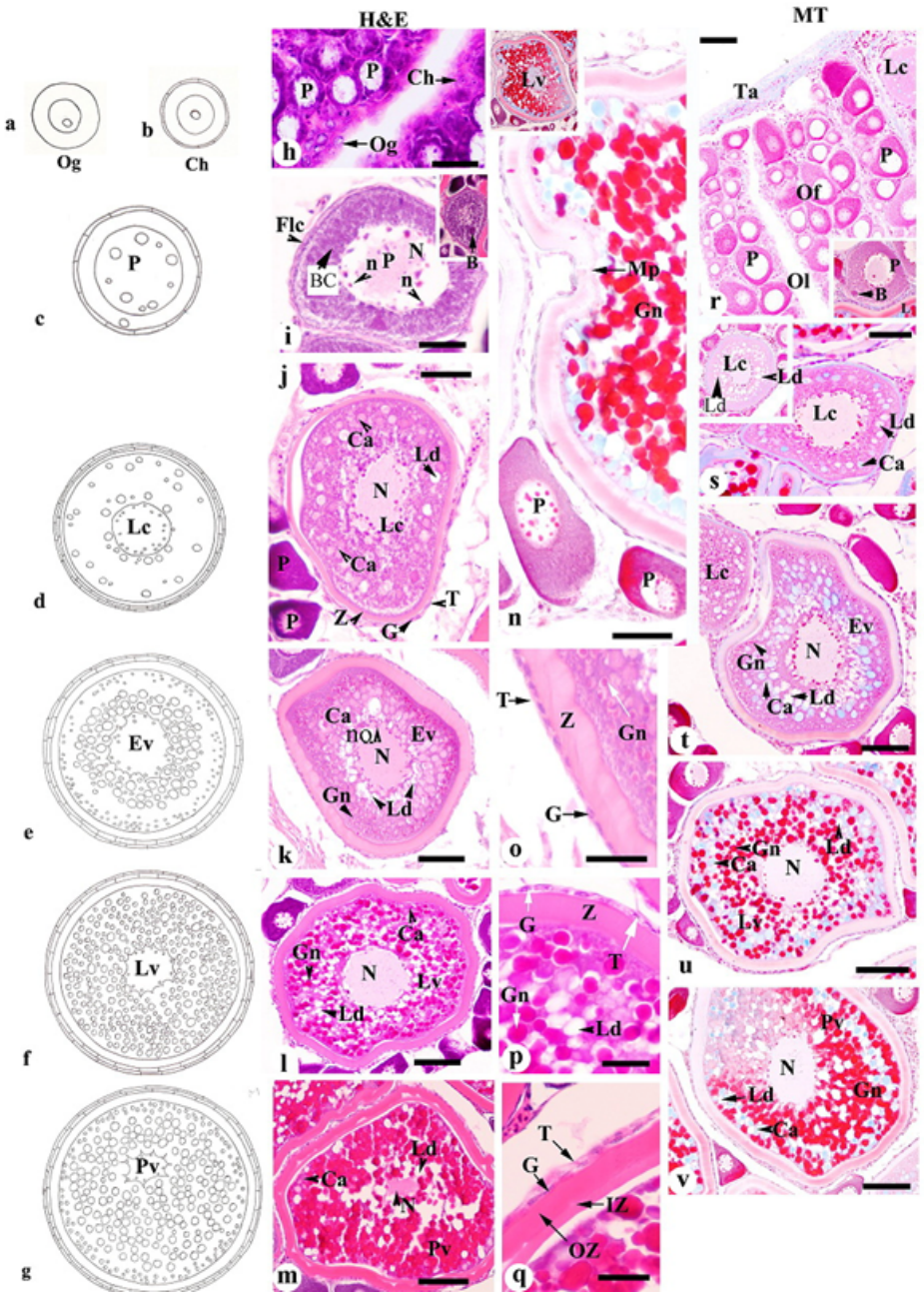

Figure 2. Schematic summary of oogenesis (a-g) and light photomicrograph (h-v); h-m, o-q based on H&E and n, r-v based on MT. Balbiani's bodies (B), circumnuclear ring (Bc), cortical alveoli (Ca), chromatin nucleolar stage (Ch), early vitellogenic stage (Ev), follicular complex (Flc), granulosa cells (G), yolk granules (Gn), inner zona pellucida (IZ), lipid and cortical alveoli stage (Lc), lipid droplets (Ld), late vitellogenic stage (Lv), micropyle (Mp), nucleus (N), nucleolus (no), ovigerous fold (Of), oognia (Og), ovigerous lamellae (OI), outer zona pellucida (OZ), perinucleolar stage (P), post vitellogenic stage (Pv), theca cell (T), tunica albuginea (Ta), zona pellucida (Z). Scale bar i-v = 100 µm, h = 20 µm.

about 25-30 μm in diameter. This cell contained a spherical nucleus (18-20 μm in diameter) with a prominent nucleolus about 3-4 μm in diameter. Its cytoplasm was stained slight blue by H&E. It was noted that the oogonium was attached within clusters or oogonial cysts inside the ovigerous fold. Each oogonium was surrounded by a few squamous-shaped prefollicular cells (Figures 2a, 2h).

Chromatin nucleolar stage oocyte significantly increased in size (30-40 μm). The nucleus of oocytes (15-20 μm) had a weakly basophilic nucleoplasm showing a spherical form and enlarged size. Single and large spherical nucleoli were present. The ooplasm had a thin layer of progressively basophilic cytoplasm. Their follicle cells formed a squamous cell layer, which surrounded the oocyte (Figures 2b, 2h).

Perinucleolar stage oocyte was 80-100 μm in diameter. The nucleus, about 50 μm in diameter was in the central area. Multiple nucleoli of 5-8 μm in diameter had spherical shape and were arranged around the nuclear membrane. Balbiani's bodies were first present around the periphery. There was a slight decrease in basophilic property of the ooplasm. Then, the circumnuclear ring was seen during the oocyte under light microscope. The follicle cells were elongated and flat around the oocyte (Figures 2c, 2i).

Oil droplets and cortical alveolar stage oocyte were large. They attained a size of 150-180 μm in diameter. Multiple nucleoli at the periphery of the nuclear membrane progressively decreased in size (about 3-4 μm). At first appearance, spherical oil droplets were seen primarily encircling the nucleus. Their sizes were 10 μm in diameter. Later, oval cortical alveoli appeared with clear content as vesicles. At the end, oil droplets and cortical alveoli increased in both numbers and size throughout the ooplasm at the basophilic area. During this stage, the zona pellucida as an acidophilic acellular layer was first detected. The layer of simple granulosa cells and theca cells was similar to that seen in the previous stage (Figures 2d, 2j).

Early vitellogenesis stage oocyte was characterized by an increase in cell size to about 250-320 μm in diameter and a decrease of cell basophilia. The beginning of ooplasm of the oocyte was filled with numerous small yolk granules. The oil droplets increased in size and began to migrate throughout of the ooplasm. Numerous cortical alveoli were still detected and seemed to increase in number. The follicular complex of this oocyte consisted of three well-developed layers: zona pellucida, granulosa cell and theca cell. The zona pellucida was 20 μm thick. It was thicker than the previous stage. It was distinctly striated and strongly positive with eosin. In this stage, the layer of granulosa cell underwent morphological change in shape from squamous to low columnar epithelium. Basement membrane and theca cells were seen (Figures 2e, 2k, 2o).

Late vitellogenesis stage oocyte was 280-350 μm in diameter. It displayed reddish-stain with acidophilia according to the most accumulation of yolk deposition (10-15 μm). The periphery of the ooplasm was filled by lipid globules and a few cortical alveoli (around 15-18 μm in diameter). The irregular and eccentric nucleus appeared to move near the animal pole. Later, the micropyle in an oocyte was well-developed and occurred in the zona pellucida (Figure 2n). The zona pellucida was also present as a thick and striated layer (Figure 2g). In addition, the zona pellucida could be separated into two layers; a thick internal layer and a thin external layer. The layers of granulosa and theca cells were similar to those described in the previous stage (Figures 2f, 2l, 2p).

The post vitellogenesis oocyte stage or maturating oocyte was the largest oocyte (320-370 μm diameter) among oogenic stage because the yolk granules of the oocyte were not completely fused as a homogenous material. Enlarged yolk granules were also present at this stage. The nucleus was not visible at this stage. A few lipid droplets and cortical alveoli were rarely observed at the periphery of the ooplasm. The follicular complex was slightly flattened under histological changes, but the zona pellicida could be distinctly classified into two layers: the outer layer was located near the granulosa cells, whereas the inner layer was exhibited near to the oocyte surface (Figures 2g, 2m, 2q)

Histochemistry of the ovarian tissue revealed that numerous cortical alveoli were positively stained with MT as bluish and with PAS as pinkish. This indicated the presence of a glycoprotein. Also, it slightly stained with AB as bluish, indicating the presence of carbohydrate or acid mucopolysaccharide (Figure 2). Another inclusion, yolk granules in the vitellogeneic stage also strongly stained with MT as reddish and slightly stained with PAS reactions (Figures. 2n, 2p, 3b, 3e). The zona pellucida also strongly showed slight PAS reaction during lipid droplet and cortical alveolar stage throughout the post vitellogenic stage, indicating the presence of the protein (Figures. 3b and 3e). Surprisingly, the inner zona pellucida of the post vitellogenic stage was exclusively seen than the outer zona pellucida (Figure 3e).

Among lipids, lipid droplet and cortical alveolar stage, the most intense ORO reaction appeared in the lipid droplets. It first appeared near nuclear membrane, and then it distributed along the mid and peripheral regions. This distribution pattern could be found throughout the post vitellogenic stage (Figures 2f, 2g, 2h, 2i). Furthermore, MT, PAS and AB negative staining indicated empty vesicles (Figures 2 and 3).

MT staining was positive at the connective tissue with the covering epithelial cells that separated the two compartments of the ovary (Figure 2k). Additionally, they were positively reticulin stained, which located at the basement membrane of the active germinal epithelium. This germinal epithelium included the separation between the germinal epithelium and the ovigerous fold from the stromal compartment (Figure 4a), and the separation between the granulosa cells and the thecal cell layers (Figure 4e, 4f). Moreover, oogonia within the cell nest were also separated from the ovigerous fold by the same basement membrane (Figure 4b, 4c). Based on the Masson's trichrome (MT) and reticulin (Rt) stain, the reticulated fiber was found in the connective tissue that formed compartmentalization.

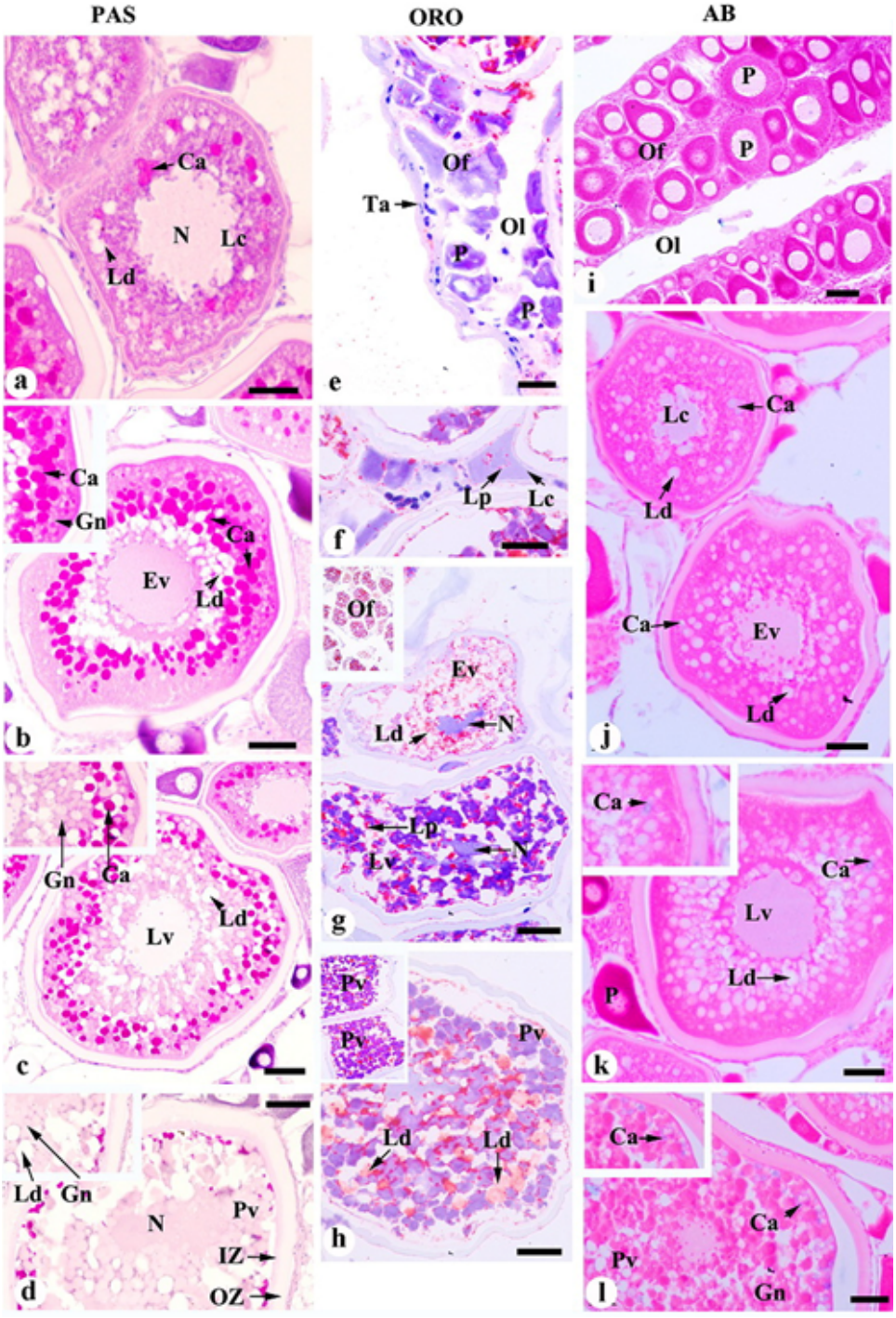

Figure 3. Light photomicrograph of oogenesis. a-d based on PAS, e-h based on ORO and i-l based on AB. cortical alveoli (Ca), early vitellogenic stage (Ev), yolk granules (Gn), inner zona pellucida (IZ), lipid and cortical alveoli stage (Lc), lipid droplets (Ld), late vitellogenic stage (Lv), nucleus (N), ovary (O), ovigerous fold (Of), oviferous lamellae (Ol), outer zona pellucida (OZ), perinucleolar stage (P), post vitellogenic stage (Pv), tunica albuginea (Ta). Scale bar a-d, f-h, j-l = 40 µm, e, i = 100 µm.

Histopathological alterations and prevalence of lesions in the ovarian parenchyma were found in both seasons (Figures 7-9 and Table 1). In contrast to the breeding season, when the ovarian atrophy was not found, the non-breeding season had 13.3% prevalence of fish with ovarian atrophy. All fish that showed ovarian atrophy and had asymmetrical ovaries. The smaller ovary exhibited histopathological damage. This included atrophy in some ovigerous folds,

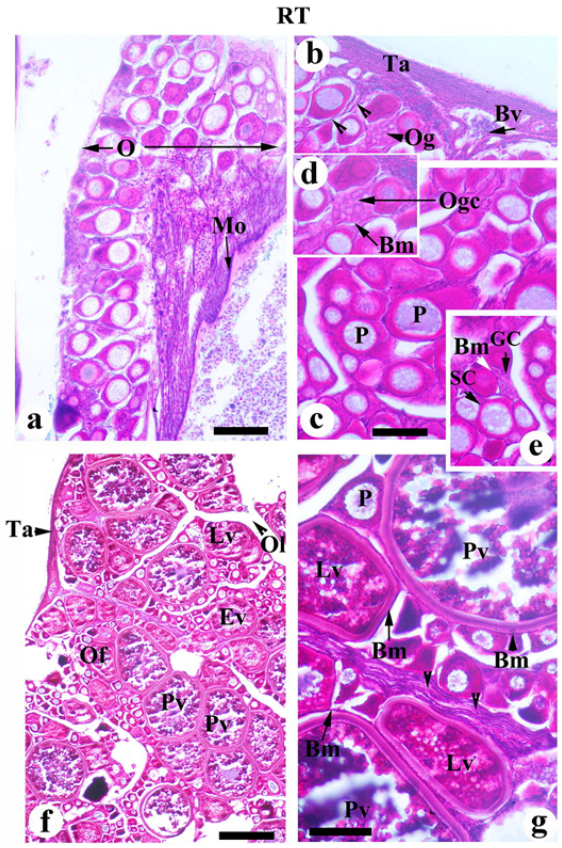

Figure 4. Light photomicrograph of ovarian structure (O) and oogenesis. a-g based on RT. basement membrane (Bm), Elastic fiber (head arrow), blood vessel (Bv), early vitellogenic stage (Ev), germinal compartment (GC), late vitellogenic stage (Lv), mesovarium (Mo), ovigerous fold (Of), oognia (Og), oogonia cyste (Ogc), oviferous lamellae (Ol), perinucleolar stage (P), post vitellogenic stage (Pv), stromal compartment (SC), tunica albuginea (Ta). Scale bar a, c, g = 100 µm, f = 200 µm.

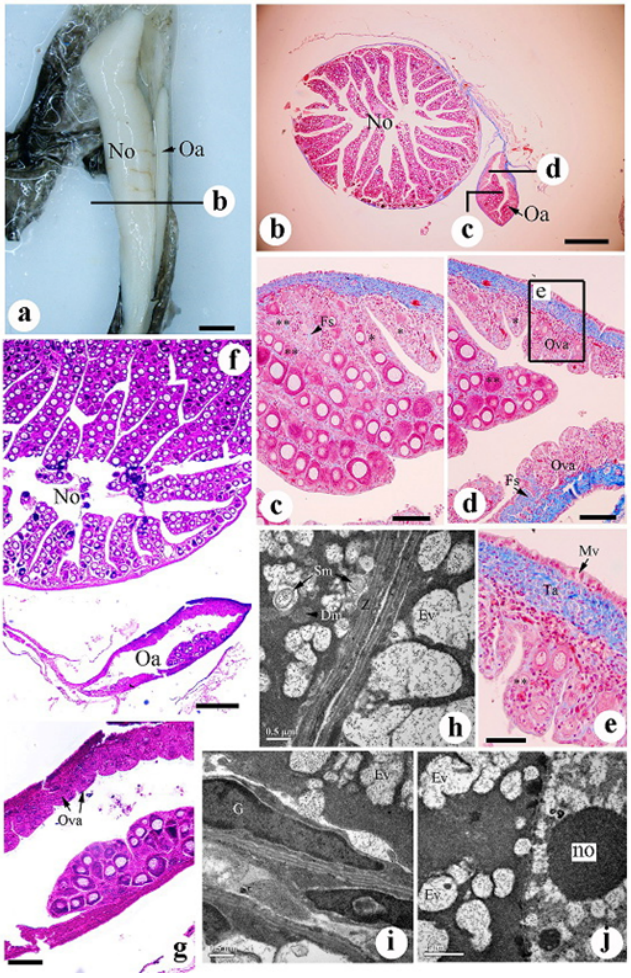

Figure 5. Gross anatomy (A) and light photomicrograph of histopathological alterations of the ovarian structures (B-J). oogonia degeneration (*), previtellogenic degeneration (**), mitochondrial degeneration (Dm), exocytotic vesicle (Ev), fibrosis (Fs), granulosa cell (G), ovarian atrophy (Oa*)*, ovigerous atrophy (*Ova)*, normal ovary (No), nucleolus (Nu), zona pellucida (Z). Scale bar a, b = 0.2 cm, f = 200 µm, c, d, g = 50 µm, e = 20 µm.

which contained degenerate oocytes in both oogonia and previtellogenic stages (chromatin nucleolar, perinucleolar, oil droplet and cortical alveolar stages). At high magnification, ovarian fibrosis was observed among oogenic stages. This was indicated by bluish when stained with MT method. Ultrastructural analysis showed that their previtellogenic stages exhibited the degeneration and swelling of mitochondria in the ooplasm. At the same time, the largest exocytotic vesicles with both irregular and oval shapes were detected near periphery of oocyte and nucleoplasm. Other important findings at the light microscopic level included the highest prevalence of atretic follicles, which was the ovarian histopathology symptom that exhibited the degeneration and disorganization in different stages of oocytes. The atresia in oogonia and the previtellogenic stages was shown in non-breeding season with 100% prevalence, whereas the breeding season fish showed atresia in many stages including oogonia (at 83.3% prevalence), previtellogenic stage (at 93.3% prevalence) and especially vitellogenic stage (early, late and post vitellogenic stages) (100% prevalence). Moreover, the atretic vitellogenic stage showed irregular, disorganized and disintegrated follicular cells. The atretic vitellogenic stage was found with 93.3% prevalence in breeding season. The amount of atretic vitellogenic stage in the breeding season was approximately 80.2 cells of atretic follicles per 100 oocytes (Table 2). Some ovarian parenchyma showed both blood dilation and congestion together with accumulating the melanomacrophage centers (as yellowish brown with GMS method). These lesions were slightly shown at 23.3% prevalence in the non-breeding season and at 33.3% prevalence in breeding season. Moreover, an unidentified parasite among the ovarian tissue based on GMS stain was detected in only non-breeding season at 10% prevalence.

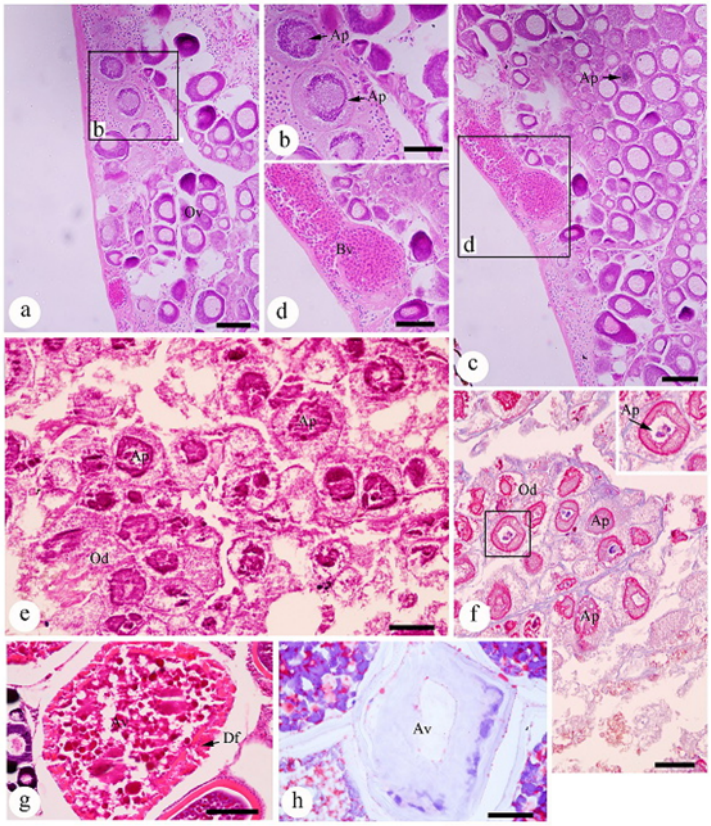

Figure 6. Light photomicrograph of histopathological alterations of the ovarian structures (a-h). atresia of previtellogenic stage (Ap), atresia in vitellogenic stage (Av), blood congestion (Bv), degeneration of follicular cell (Df), ovarian degeneration (Od). Scale bar a, d, e, f = 100 µm, b, c, g, h = 50 µm.

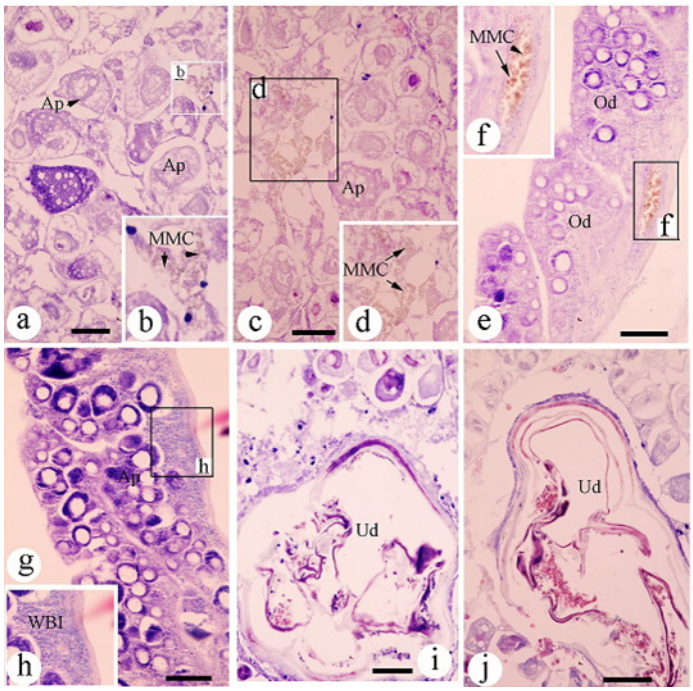

Figure 7. Light photomicrograph of histopathological alterations of the ovarian structures (a-j). atresia of previtellogenic stage (Ap), malanomacrophage center (MMC), ovarian degeneration (Od), white blood cell infiltration (WBI), unidentified parasites (Ud). Scale bar a, c, e, g, i, j = 50 µm, b, d = 20 µm.

Table 1. Alteration in prevalence (%) of dominant histopathological observations in the ovarian tissue of *Rastrelliger brachysoma* caught during non-breeding (October to November 2013, n = 30) and breeding seasons (January to February 2014, n = 30) from Samut Songkram Province, the Upper Gulf of Thailand

Ovarian lesions	Alteration prevalence (%)	
	Non-breeding	Breeding
Ovarian degeneration	100.0 (n = 30)	100.0 (n = 30)
Ovarian atrophy	13.3 (n = 4)	-
Ovarian fibrosis	10.0 (n = 3)	10.0 (n = 3)
Atresia in oogonia	100.0 (n = 30)	83.3 (n = 25)
Atresia in previtellogenic stage	100.0 (n = 30)	93.3 (n = 28)
Atresia in vitellogenic stage	-	100.0 (n = 30)
Irregular and disorganized with breakdown of follicular cells	-	93.3 (n = 28)
Accumulation of melanomacrophage centers	23.3 (n = 7)	33.3 (n = 10)
Unidentified parasites	10.0 (n = 3)	-

- : data not found

Table 2. Oocyte quality in different stages of the atretic follicles in *Rastrelliger brachysoma* caught from the Upper Gulf of Thailand

Atresia	Seasons	
	Non-breeding	Breeding
Oogonia	23.90±3.03 (50 oocytes)	19.60±4.42 (50 oocytes)
Pre-vitellogenic stage	63.50±3.83 (100 oocytes)	57.40±4.83 (100 oocytes)
Vitellogenic stage	-	80.20 ± 3.45 (100 oocytes)

4. Discussion

Based on the histological structure, the ovarian tissue of *R. brasochyma* was the asynchronous oocyte development type, which is similar to all other scombrids (Chellappa *et al*., 2010). This indicated a protracted spawning period with multiple spawning. At the initiation phase of oogenic stage, the oogonium was observed and they were the smallest among female germ cells. They was proliferated within cell nests. They were characterized as being enclosed by pre-follicular cells in the germinal compartment. This feature was commonly found in most teleost (Grier, 2000; Selman *et al*., 1993). Then, it progressed to the primary growth stage. In this stage, the primary growth stage is characterized by basophilic cell due to a period of intense RNA synthesis together with ribosome production to support the oocyte development (Wallace and Selman, 1990). Generally, this stage can be divided into two phases based on nuclear morphological characteristics: (i) the chromatin nucleolus and (ii) the peri-nucleolar stages, as in this species. The characterization of the chromatin nucleolus stages of this fish was seen but rarely observed, as shown in *Thunnus orientalis* (Chen *et al*., 2006) and other teleosts oocyte (Sarasquete *et al*., 2002). This probably was caused by the rapid transformation process of oogonia, turning into the chromatin nucleolus stage and then it turning into the perinucleolar stage (Sarasquete *et al*., 2002 and Mandich *et al*., 2002). The perinucleolar stage was first observed in the Balbiani's body as a non-homogeneous structure with oval shape and basophilic staining located near the nucleus membrane. A similar characteristic was also found in *Oryzias latipes* (Wallace & Selmann, 1981). The functional structure of Balbiani's body was uncertain, but it was believed to be a center for the formation of various organelles. It was region rich in nucleic acid (Hamaguchi, 1993), RNA and proteins (Selman & Wallace, 1989).

Afterwards, two types of inclusion occurred, lipid droplet and cortical alveoli. Lipid droplet inclusion, occurred prior to the cortical alveoli inclusion in the cortical alveolar stages. The sequence of these inclusions were used in the egg type postulation in most teleost fishes (Brown-Peterson *et al*., 1988; Selman & Wallace, 1989). The ovary pattern of *R. brachysoma* agreed with the some marine fishes including *Dicentrachus labrax* (Mayer *et al*., 1988) and *C. undecimalis* (Neidig *et al*., 2000). Our inclusion sequence confirmed that the egg in *R. brachyoma* is a pelagic type. Although the structural role of lipids is still unclear, it is concerned with energy sources for embryo development (Wiegand, 1996), whereas cortical alveoli were negatively stained with H&E and ORO, but were strongly positive to MT as greenish color, indicating the presence of the polysaccharide. The staining

pattern in *R. brachysoma* was similar to that of other fish species (Selman *et al*., 1988; Wallace and Selman, 1990). The role and function of this inclusion was concerned with physiological roles, especially the prevention of polyspermy after ovulation (Nagahama, 1983).

The early vitellogenic oocyte started either synthesis or accumulation of yolk granules Hence, it increased in size. Wallace and Selman (1990) reported that these yolk granules in the ooplasm were the material stored during the secondary growth stage. Their structural roles were usually related to the nutrition and metabolic activities in the embryonic development as described in most fish species (Chen *et al*., 2006). Then, entering into late vitellogenic stage, the oocyte showed several processes in both cytoplasm and nucleus. In the cytoplasm, the characterization of yolk granule in this species is similar to *Serra Spanish* (Chellappa *et al*., 2010). The yolk was not fused until it entered into the mature stage. This feature was suggested to be typical of pelagic egg (Wallace and Selman, 1981) which is different from demersal eggs, in which the beginning of yolk fusion was observed during the early vitellogenesis stage. This was also found in *Gasterosteus aculeatus* and *Apeltes quadracus* (Selman and Wallace, 1989). Then, the nucleus of the late vitellogenesis stage first migrates to the animal pole and the micropyle was observed. This pattern was also observed in other teleost fishes, including *Fundulus heteroclitus* (Kuchnow & Scott, 1977) and *T.orientalis* (Chen *et al*., 2006). The micropyle function is concerned with the entering location of the spermatozoa to the oocyte surface without acrosomal reaction (Bartsch and Britz, 1997). The final stage of oocyte in this species is the post-ovulatory stage or mature oocyte with processes involving the nucleus region. No nucleus with germinal vesicle breakdown was observed under the first meiotic cell division. This breakdown is widely used as an indicator of the final maturation in the teleost fish (West, 1990).

Our observation found that atretic follicles mainly appeared in vitellogenic stages. It is possible that the endocrinological activities in both decreasing of gonadotropin (GTH) and estrogen may be responsible for such atretic follicles. Similarly, Wood and Van Der Kraak (2002) found that atretic follicles were observed in the female *Oncorynchus mykiss* after exposed to low level of 17β-estradiol (E_2). Although it is weakly evidenced in this case, the atretic follicles seemed to be the results of the reduced oocyte number, which is due to impairment of the oogenic process as well as reproductive failure. The reduced ovarian size, which is caused by the ovarian atrophy was observed to be relatively low frequency in the non-breeding season. Unfortunately, the effects of estrogen mimics on ovarian atrophy was only known from laboratory work. None have been reported from filed experiment. For instance, Sridevi *et al*. (2015) who reported that the ovarian atrophy together with oocytes degeneration of the *Clarias gariepinus* was observed after the exposure in both estrogens, 17α-ethynylestradiol (EE_2) and diethylstilbesterol (DES). Both of these were classified as endocrine disruptive chemicals (EDCs). These two pollutants were also reported as chemicals that induce significant incidence of ovarian atrophy in *Dicentrarchus labrax* during early development (Blazquez *et al*., 1998). It strongly suggested that the reduction and the impairment of female reproductive tissue may be directly caused by these reagents and then disrupted via the endocrine system through the hypothalamus-pituitary-gonadal pathway (Sridevi *et al*., 2015). Therefore, we hypothesized that the Upper Gulf of Thailand may accumulate various pollutants, particularly EDCs. This should urgently be investigated in further research. However, based on this result, it should be expected that the ovarian impairment would have a significant impact on fecundity and fertility of *R. brachysoma* population. Also, it is should be note that the occurrence of the ovarian atrophy, referring to ovarian morphological abnormalities, is a case-effective and useful sensitive indicator of the exposure of the model estrogenic compounds.

The ovarian tissue was noticeably seen as melano-macrophage centers with evidence of chronic inflammation as well as an inflammatory response in some areas. It could be suggested the occurrence of melanomacrophages is the result of the long-term injury of this organ. This is similar to what was reported in *Danio rerio* which were exposed to 17α-ethinylestradiol (9.3 ng/L; 177 days) (Schãfers *et al*., 2007). We also observed unidentified parasites in ovarian tissue of the *R. brachysoma.* Even though the identity of the parasite could not be confirmed, it was suggested that the presence of parasite in the system could potentially be detrimental to the host (Barber *et al*., 2000; Hecker & Karbe, 2005)

An important conclusion from in this study was that the histological and histochemical characteristics of the ovarian tissue in *R. brachysoma* should be applied to use in aquacultural development. It is also suggested that *R. brachysoma* is a threatened marine fish in the Upper Gulf of Thailand, and there is still much research required to provide further information on the effects of the EDCs and some heavy metals on ovarian tissue in *R. brachysoma*. We emphasize again the importance of understating the effect of overall histopathological alterations, especially atretic follicles and ovarian atrophy on the reproductive health of *R. brachysoma* to the improvement of aquaculture of this species, which would ultimately help in ensuring the suitability and safety of food consumption by humans.

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References

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques*. Amsterdam, The Netherlands: Elsevier Health Sciences.

Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behavior: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, *10*, 131-165.

Bartsch, P., & Britz, R. (1997). A single micropyle in the eggs of the most basal living actinopterygian fish, Polypterus (Actinopterygii, Polypteriformes). *Journal of Zoology, 241*(3), 589-592.

Blazer, V. (2002). Histopathological assessment of gonadal tissue in wild fishes. *Fish Physiology and Biochemistry, 26*(1), 85-101.

Blazquez, M., Zanuy, S., Carrillo, M., & Piferrer, F. (1998). Structural and functional effects of early exposure to estradiol-17β and 17α-ethynylestradiol on the gonads of the gonochoristic teleost *Dicentrarchus labrax*. *Fish Physiology and Biochemistry*, *18*, 37-48.

Brown-Peterson, N., Thomas, P., & Arnold, C. (1988). Reproductive biology of the spotted seatrout, Cynoscion nebulosus, in south Texas. *Fishery Bulletin*, *86*, 373-388.

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.

Chellappa, S., Lima, J., Araújo, A., & Chellappa, N. (2010). Ovarian development and spawning of Serra Spanish mackerel in coastal waters of Northeastern Brazil. *Brazilian Journal of Biology, 70*, 451-456.

Chen, K., Crone, P., & Hsu, C. (2006). Reproductive biology of female Pacific Bluefin tuna *Thunnus orientalis* from South-Western North Pacific Ocean. *Fisheries Science*, *72*, 985-994.

Culling, C. F. A. (2013). *Handbook of histopathological and histochemical techniques: including museum techniques*. Oxford, England: Butterworth-Heinemann.

Dietrich, D., & Krieger, H. O. (2009). *Histological analysis of endocrine disruptive effects in small laboratory fish*. Hoboken, NJ: John Wiley and Sons.

Food and Agriculture Organization. (2010). *Report of the First Workshop on the Assessment of Fishery Stock Status in South and Southeast Asia* (FAO Fisheries and Aquaculture Report No.913). Retrieved from http://www.fao.org/docrep/012/i1555e/i1555e00.pdf

Grier, H. (2000). Ovarian germinal epithelium and folliculogenesis in the common snook, *Centropomus undecimalis* (Teleostei: Centropomidae). *Journal of Morphology, 243*, 265-281.

Hamaguchi, S. (1993). Alterations in the morphology of nuages in spermatogonia of the fish, *Oryzias latipes*, treated with puromycin or actinomycin D. *Reproduction Nutrition Development, 33*, 137-141.

Hecker, M., & Karbe, L. (2005). Parasitism in fish – an endocrine modulator of ecological relevance? *Aquatic Toxicology*, *72*, 195-207.

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.

Mandich, A., Massari, A., Bottero, S., & Marino, G. (2002). Histological and histochemical study of female germ cell development in the dusky grouper Epinephelus marginatus (Lowe, 1834). *European Journal of Histochemistry, 46*(1), 87-100.

Mayer, I., Shackley, S., & Ryland, J. (1988). Aspects of the reproductive biology of the bass, Dicentrarchus labrax LI A histological and histochemical study of oocyte development. *Journal of Fish Biology, 33*, 609-622.

Menasveta, D. (1980). *Resources and fisheries of the Gulf of Thailand*. Bangkok, Thailand: Training Department of the Southeast Asian Fisheries Development Center.

Nagahama, Y. (1983). The functional morphology of teleost gonads. In W.S. Hoar, D.J. Randall D.J. & E.M. Donaldson (Eds.), Fish Physiology Vol. 9A (pp. 223-275). New York, NY: Academic Press.

Neidig, C. L., Skapura, D. P., Grier, H. J., & Dennis, C. W. (2000). Techniques for spawning common snook: broodstock handling, oocyte staging, and egg quality. *North American Journal of Aquaculture, 62*, 103-113.

Puchtler, H., & Waldrop, F. S. (1978). Silver impregnation methods for reticulum fibers and reticulin: A re-investigation of their origins and specifity. *Histochemistry, 57*, 177-187.

Rowden, G., & Lewis, M.G. (1974). Experience with a tree hours electron microscopy service. *Journal of Clinical Pathology*, *27*, 505.

Sarasquete, C., Cardenas, S., de Gonzalez, C.M., & Pascual, E. (2002). Oogenesis in the bluefin tuna, *Thunnus thynnus* L.: A histological and histochemical study. *Histology and Histopathology, 17*, 775-788.

Schäfers, C., Teigeler M, Wenzel A, Maack G, Fenske M., & Segner H. 2007. Concentration and time-dependent effects of the synthetic estrogen, 17alpha-ethinylestradiol, on reproductive capabilities of the zebra fish, *Danio rerio*. *Journal of Toxicology and Environmental Health Sciences*, *9*, 768-779.

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.

Selman, K., & Wallace, R. A. (1989). Cellular aspects of oocyte growth in teleosts. *Zoological Science, 6*, 211-231.

Selman, K., Wallace, R. A., Sarka, A., & Qi, X. (1993). Stages of oocyte development in the zebrafish, Brachydanio rerio. *Journal of Morphology, 218*, 203-224.

Senarat, S., Kettratad, J., Poolprasert, P., Boonyoung, P., Kangwanrangsan, N., & Jiraungkoorskul, W. (2015). Hepatic histopathology in *Rastrelliger brachysoma* (Bleeker, 1851) from The Upper Gulf of Thailand. *Asia-Pacific Conference on Engineering and Applied Science* (pp. 369-374). Osaka, Japan.

Sridevi, P., Chaitanya, R.K., Prathibha, Y., Balakrishna, S.L., Dutta-Gupta, A., & Senthilkumaran, B. (2015). Early exposure of 17á-ethynylestradiol and diethylstilbestrol induces morphological changes and alters ovarian steroidogenic pathway enzyme gene expression in catfish, *Clarias gariepinus. Environtal Toxicology*, *30*, 439-451.

Sritakon. T., Songkaew. N., Chotithammo, S., & Vechprasit, S. (2006). *Reproductive biology of short mackerel Rastrelliger brachysoma (Bleeker, 1851) and Indian mackerel R. kanagurta (Cuvier, 1817) in the Southern Gulf of Thailand.* Bangkok, Thailand: Department of Fisheries.

Sutthakorn, P. (1998). *Biological aspects of short mackerel, Rastrelliger brachysoma (Bleeker, 1851) of the Andaman Sea Coast of Thailand*. Phuket, Thailand: Andaman Sea Fisheries Department Center, Department of Fisheries.

Vidal, B. (1988). Histochemical and anisotropical properties characteristics of silver impregnation: the differentiation of reticulin fibers from the other interstitial collagens. *Zoologische Jahrbücher*. *Abteilung* für *Anatomie* und *Ontogenie* der *Tiere*, 117, 485-494.

Wallace, R. A., & Selman, K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoologist, 21*, 325-343.

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.

Wattayakorn, K. (2012). Petroleum pollution in the Gulf of Thailand: A historical review. *Estuarine and 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.

Wiegand, M. (1996). Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries*, *6*, 259-286.

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

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.