Field Observation the Female Reproductive Maturation of the Spotted Catfish, *Arius maculatus* that Inhabit in Estuarine Areas of Pranburi River, Thailand

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ABSTRACT

The *Arius maculatus* is one of the most economically important estuarine fish and a potentially viable estuarine fish for aquaculture in Thailand. However, an adequate understanding of its reproductive biology, in order to support the aquacultural development and fisheries management of this fish, is currently lacking. The goal of this study was to reveal the female reproductive maturation of *A. maculatus* inhabiting different water salinities (from lowest to highest salinities), using visual and histological observations. For the entire range of salinity, the female reproductive system evidenced similar morphological events, being paired organs of a creamy ova color before uniting into the short oviduct. Histological evaluation in all samples was of the ovarian maturation, which was contained in the differentiating stages of oocytes. It was considered to be an asynchronous oocyte pattern. The developing oocytes were classified into five distinct phases including the oogonial proliferation, the primary growth phase (2 sub–stages: perinucleolar and oil droplet and cortical alveolar stages), the secondary growth phase (3 sub–stages: early secondary growth, late secondary growth and full–grown oocyte stages), the atretic oocyte and the post–ovulatory follicle. Note that the pronounced atretic oocyte, especially in the secondary growth phase, was significantly different ($P < 0.05$) in the lowest salinity. In contrast to the evaluating such post–ovulatory follicles, naturally spawned eggs and some juvenile stages were only found in the highest salinity, implying that this situation was optimal for the spawning season and nursery ground for *A. maculatus*.

Keywords: Histology, oogenesis, reproductive tissue, spawning season, Thailand

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INTRODUCTION

The spotted catfish, *Arius maculatus* is an economically important estuarine fish due to its popularity as a food item in Thailand. This species is one of the most abundant species and lives in estuaries of the tropical and subtropical zones such as in China, Malaysia and Thailand (Carpenter and Niem, 1999). In Thailand, *A. maculatus* has been commercially exploited due to a great diversity and being widespread throughout both fresh and brackish waters (Carpenter and Niem, 1999). Several publications about the morphology and taxonomy (FAO, 1974), and feeding ecology of this species have been written (Mazlan *et al*., 2008; Manikandarajan *et al*., 2014); however accurate quantification about its reproductive biology has never been reported.

In the estuarine areas in Praburi River, Thailand, there exists a vital component of the transition zone between river and marine environments. In nature, the main important variable characterizations regarding estuarine environmental factors have been reported with relation to locality, tidal range, freshwater, etc. As the estuarine animals are likely to come into contact with various environmental/physical conditions and benthic pollution, it is essential that they alternate the organ/system/cell in osmoregulation in order to be successful survivors as well as to have reproductive success (Magwood *et al*., 2000). Among the various environmental factors, salinity can trigger or determine the reproductive features/ activity (Magwood *et al*., 2000).

To increase the information regarding reproductive aspects, we field–investigated quantitative data on the female reproductive structure of *A. maculatus* from the estuarine Pranburi River–using the visual and histological observations. Additionally, this research concentrated on comparing the characterization of the ovarian structure of fish inhabiting waters of different salinity levels, as measured by gonadosomatic index, and the amount of post–ovulatory follicle and atresia. Our observation provided the first detailed insights towards the prediction of the reproductive cycles/ characteristics of *A. maculatus*. Consequently, it can serve as a basis of standardized determination for future research to develop the aquaculture and fisheries management in this species.

MATERIALS AND METHODS

Fish Sampling and Study Area

Twenty death specimens of female *Arius maculatus* with average total length of 23.50 ± 0.43 cm (March 2017) and 23.12 ± 0.78 cm (November 2017) being donated by local fisherman, as voucher specimens on two occasions (n = 10) each time) based on different salinities–the highest salinity (27.02 PSU) in March 2017 and the lowest salinity (12.18 PSU) in November 2017 (Table 1) from the estuarine areas in Praburi river, Thailand (N12º 24.314' E099º 58.597'; Figure 1). Note that the fish referring to the process of releasing the eggs and juvenile stages were recorded and collected during our observation. Additionally, the other factors (water temperature, pH and DO) were measured using EC900 AMTAST Waterproof DO Kit 9–in–1 Meter (AMTAST, Lakeland, FL, USA) and present in Table 1. Because we only used dead specimens, this study did not require the protocol approval.

Table 1 Environmental parameters from the estuarine areas of Pranburi River, Thailand between March 2017 and November 2017

Periods/parameters	Temp. (C°)	DO (mg/l)	pH	Salinity
March 2017	31.74	5.08	7.84	27.02
November 2017	29.04	4.48	8.31	12.18

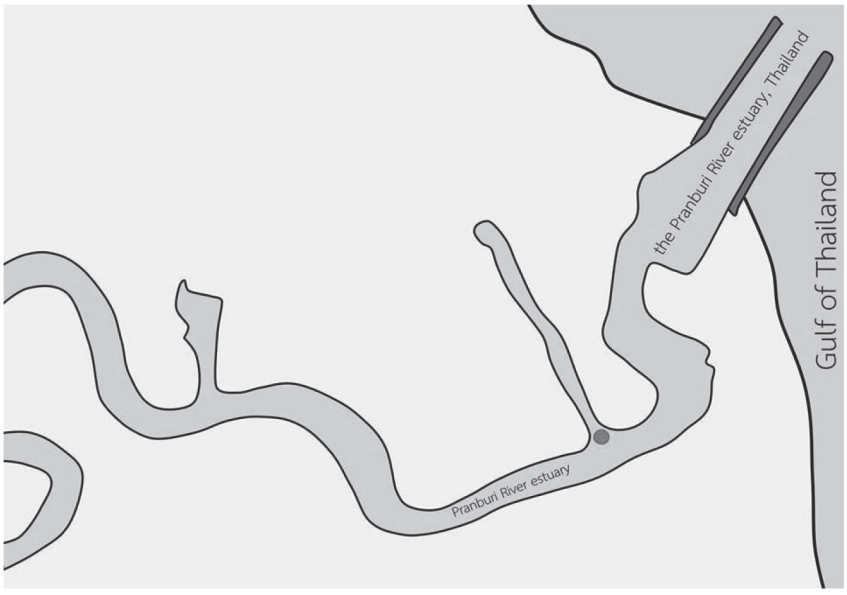

Figure 1 Sampling locality of the estuarine areas of Pranburi River, Thailand

Visual and Histological Evaluations

To determine ovary and oogenic characteristics, the abdominal cavity was opened and then the gonadal tissue was quickly dissected and assessed for its morphology and stages using a stereoscopic microscope. The ovarian weight and fish weight were recorded for calculation of the gonadosomatic indices (GSI) (GSI = [ovarian weight/total weight × 100]). After that, the sections of each ovary, spawned egg, and some juvenile stages were fixed overnight in Davison´s fixative and processed according to standard histological techniques (Presnell and Schreibman, 1997; Suvarna *et al*., 2013). Histological section of 4 µm thickness was performed and then stained with haematoxylin–eosin (H&E) (Presnell and Schreibman, 1997; Suvarna *et al*., 2013). To define the ovarian structure and oogenesis, observations was made using a light microscope (Leica digital 750) using the descriptions of Dietrich and Krieger (2009) and Uribe *et al*. (2012). The oocyte diameter (n = 30) and the atretic oocyte were counted and measured from three representative sections (n = 100).

Our observation was shown the relationship between proportion of secondary growth phase and different salinity. Three representative fish during the ovarian development were chosen as samples. The number of secondary growth phase from three sections (n = 50 cell per section) was courted and calculated as percentages under the light microscope (10x and 40x).

Statistical Analysis

Student t–tests were used to assess any significant difference ($P < 0.05$) of the gonadosomatic index, the amount of post–ovulatory follicle and atresia between lowest salinity and highest salinity. These statistics were calculated using the Statistical Package for the Social Sciences (SPSS) software (version 15.0).

RESULTS AND DISCUSSION

Female Reproductive Structure

The morphological analysis of *A. maculatus* individuals confirmed the data of the two sampling periods in that they were a shared morphology and

histology of reproductive maturation. There was a symmetrically saccular structure, aired organs (Figure 2A) and posterior location between the posterior kidney and intestine (Data not shown) before it joined the short oviduct. A creamy color was enclosed by the ovarian wall (Figures 2A–2C). Several arterioles were observed throughout the wall (Figure 2B). While undergoing morphological observation, the growing oocytes at different stages were clearly discriminated into primary growth phase, secondary growth phase, atretic oocyte, and post–ovulatory follicle based on the wet–mount (Figure 2C) and fixed tissue (Figure 2D). Histological observations indicated that the ovarian structure was surrounded by the ovarian wall, as also called "the tunica albuginea" (Figure 2E), likely as seen in other teleost (Selman and Walllace, 1986; Wallace and Selman, 1990; Selman *et al*., 1993). Four distinct layers including germinal epithelium, stroma, smooth muscle, and peritoneum (or serosa) were classified in the ovarian wall (Figure 2E). These layers extended into the ovarian lumen that could be separated into two compartments–germinal and stromal compartments (Figure 2F).

Histological analysis revealed that the germinal compartment was constituted of both the active germinal epithelium (Figure 2E) and oogonial cysts (Figure 2F). It was as similarly observed in other teleost (Selman and Wallace, 1986; Wallace and Selman, 1990; Grier, 2000). The active germinal epithelium was covered the luminal surface of ovarian lamellae. It was composed of simple squamous cells. In the extension of the germinal compartment, a cluster of oogonia was observed in the cell cyst. Each cell was oval shape. A spherical nucleus was surrounded by a light acidophilic ooplasm (Figures 2F–2G). The pre–follicular cell was observed in this stage (Figure 2F).

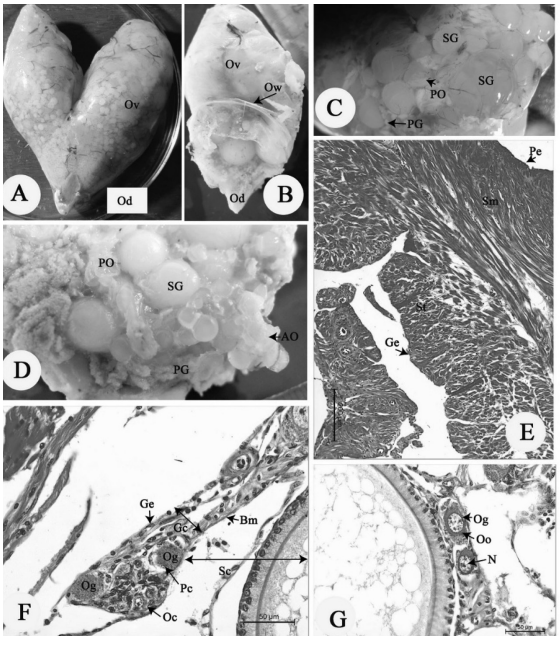

Figure 2 Morphology of the female reproductive system (A) with consisting of two regions (Ovary (Ov) and oviduct (Od)) of *Arius maculatus* was surrounded by the ovarian wall (Ow) (B). Wet–mount (C) and fixed tissue (D) of the ovarian tissue was consisted of differential stages of oocytes (primary growth stage (PG), secondary growth stage (SG), post–ovulatory follicle (PO) and atretic oocyte (AO)). Light photomicrographs of the tunica albuginea (E) with composing of four layers (peritoneal layer (Pe), smooth muscle (Sm), stroma (St) and germinal epithelium (Ge)) and ovarian tissue (F–G) of *Arius maculatus*. *Bm = basement membrane, Gc = germinal*

The stromal compartment was separated by basement membrane from the germinal compartment (Figure 2F). This compartment well–contained by various stages of oocyte differentiation, atretic oocyte and post–ovulatory follicle; therefore, the ovary of this species was of an asynchronous developmental

type (Figures 3A). This finding suggests that a particular aspect of these fish implied multiple spawning seasons and a protracted spawning period. To give a more accurate hypothesis of the reproductive cycle and spawning season will require further study.

Oogenesis

In histological section, the four phages of the developing oocyte, including primary growth phase, secondary growth phase, atretic phase, and post–ovulatory phases of *A. maculatus* were distinctly divided, based on staining of sections and histological aspects of sex cells, which are given below (Figures 3–5).

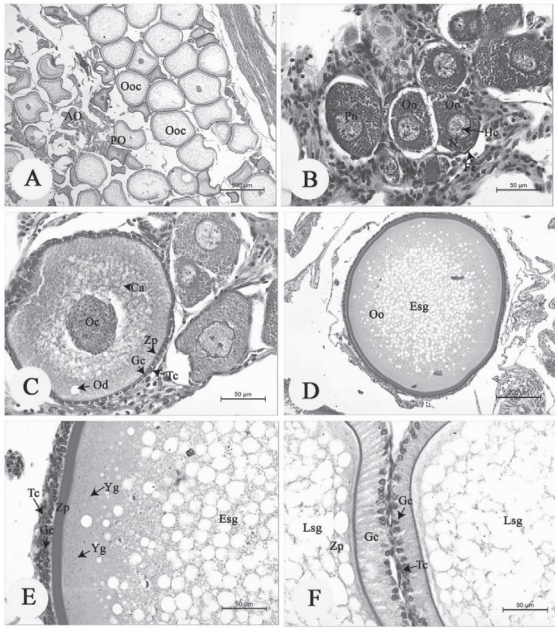

Figure 3 Light photomicrographs of the oocytes (Ooc) (A) was composed of perinucleolar (Pn) (B), oil droplets and cortical alveolar step (Oc) (C), early secondary growth step (Esg) (D–E) and late secondary growth step (Lsg) (F) of *Arius maculatus*. AO = atretic oocyte, Ca = cortical alveoli, Gc = granulosa cell, PO = post–ovulatory oocyte, Od = oil droplet, Oo = ooplasm, Tc = theca cell, Yg = yolk granule, Zp = zona pellucida

Primary Growth Phase (PG)

The PG was sub–divided into two steps of the oocyte: the perinucleolar (Pn) and oil droplets and cortical alveolar steps (Oc). The size of Pn measured 60 μm in diameter. A single and large central nucleus was observed. Heterochromatin prominently appeared near the nuclear membrane. This finding was similar to other teleost species (Selman and Wallace, 1986; Blazer, 2002; Patiño and Sullivan, 2002; Patiño *et al*., 2003). The ooplasm was strongly basophilic stained due to its high affinity for haematoxylin (Figure 3B), this is due to the synthesis of RNAs and the abundance of ribosomes and mitochondria in the cytoplasm (Wallace and Selman, 1990). Under folliculogenesis, the elongated follicle cells were formed into a single layer of squamous cells along the oocyte (Figure 3B).

The Oc had a dramatic increase to 180 μm in diameter. The spherical nucleus attained 50 μm in diameter, containing irregularly scattered nucleoli. During this stage, two inclusions (oil droplets and cortical alveoli) were the first prominent observation in the slight basophilic ooplasm (Figure 3C). A few small oil droplets were found adjacent to the follicular complex (Figure 2C). It was an empty–vacuolar structure. The cortical alveoli were dispersed near the nuclear membrane. Empirical evidence supports that the concise function of cortical alveoli involved relates to physiological response especially the prevention of polyspermy after ovulation (Nagahama,

1983; Selman and Wallace, 1986; Selman *et al*., 1988; Abascal and Medina, 2005). The folliculogenic process was completely defined, consisting of three layers–zona pellucida, granulosa cell, and theca cell–surrounding the ovarian surface (Figure 2C). The zona pellucida was an acidophilic striated and homogeneous layer, about 3–4 µm in diameter. A single layer of granulose cells and theca cells was still seen.

Secondary Growth Phase (SG)

A much more numerous population of oocytes was the SG in the ovarian sections, which was divided into three steps: early secondary growth step (Esg), late secondary growth step (Lsg) and full–grown oocyte step (Fgo).

The Esg reached an average diameter of 700 µm (Figure 3D). The change of the cytoplasm was observed and shifted from basophilic to acidophilic stains. This feature was related to the accumulation of several small yolk granules near the follicular complex (Figure 3E). The yolk granules were of a spherical shape and detected as a deeply acidophilic stain in the ooplasm. The oil droplets and cortical alveoli were still detected, but they were fused and progressively increased in both number and size. The zona pellucida was a continuous layer 10 µm thick. The granulosa cell changed from squamous epithelium to low cuboidal epithelium, about 5–6 µm diameter thickness. The theca cells exhibited similarly to the previous stage.

The Lsg increased up to 800 µm in diameter (Figure 3F). The irregular nucleus moved to the animal pole, as also referred to as germinal vesicle migration (GVM). The yolk granules merged into a mass of homogeneous feature. Interestingly, the zona pellucida was still visible, as similarly seen in Esg. The granulosa increasingly showed a monolayer of high columnar epithelium, attaining 15 µm in diameter. The theca cells remained similar to prior stage.

The Fgo reached its maximum diameter (1,100 µm) (Figure 4A). A large yolk granule was observed in this stage (Figure 4B). Germinal vesicle breakdown (GVB) was detectable. The granulosa cell was decreasingly observed as simple squamous epithelium, whereas the theca cell remained the same as previous stage (Figure 4A).

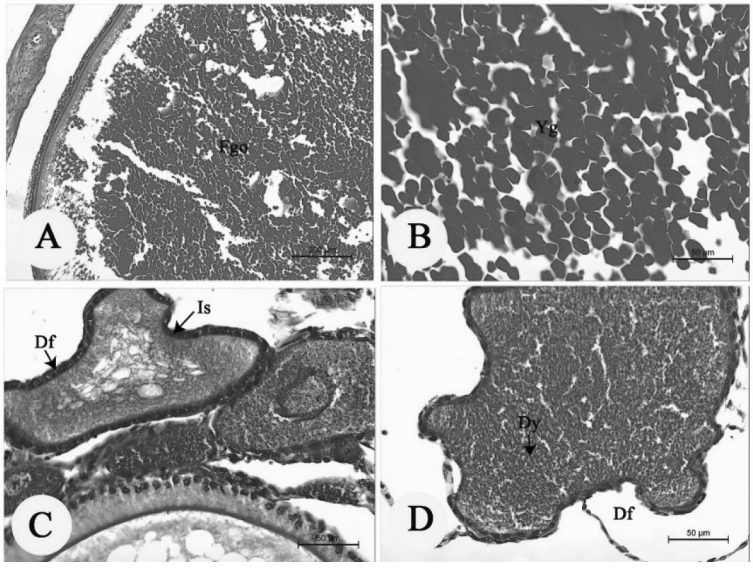

Figure 4 Light photomicrographs (B–D) of the full–grown oocyte step (Fgo) (A–B) and atretic oocytes (C–D) of *Arius maculatus*. Df = degeneration of the follicular complex, Dy = degeneration of the yolk granules, Is = irregular shape, Yg = yolk granule

Atretic Oocyte (AO)

The AO was especially notable in SG. The irregular shape and loss/degeneration of follicular complex was observed in the SG (Figure 4C). The gradual disintegration and resorption of the yolk granule and follicular complex was detected (Figure 4D).

Post–ovulatory Phase (PO)

The PO in *A. maculatus* was observed (Figure 5A). Histolgical observation showed that the PO was a compound structure including the irregular shape of the stratified follicle cell layer (Figure 5B) with containing in several blood capillaries. The presence of PO indicated that *A. maculatus* may be spawning during our observation. The precise reproductive cycle and spawning season will be determined by further study.

In this study, we also observed a large infiltration of the melanomacrophage center (MMC). The possible finding of the presence of MMC in *A. maculatus* may ensure the removal of the post–ovulatory follicles (Figures 5C–5D).

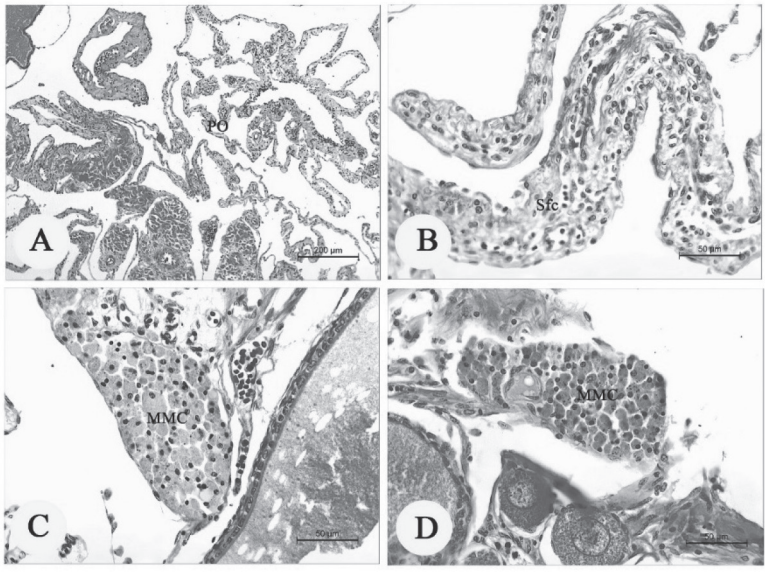

Figure 5 Light photomicrographs of the post–ovulatory phase (PO) and melanomacrophage center (MMC) of *Arius maculatus*. Stc = stratified follicular cell

Ovarian Comparative Structure in Different Salinity

The timing of gonadal maturation and reproductive seasons of fishes is typically determined by environmental factors such as water temperature, food availability Allen, 1975; Russell *et al*., 1977) and salinity (Haddy and Pankhurst, 2000; Magwood *et al*., 2000). However, only salinity was clearly differed during our observation (Table 1). This factor may be therefore obviously related to the reproductive activity of female *A. maculatus*. It was similarly reported in some fishes that the salinity is the main factor to control the reproductive activity (Haddy and Pankhurst, 2000; Magwood *et al*., 2000).

During the fish sampling, the GSI of female *A. maculatus* was 10.12 ± 0.34% in lowest salinity and 10.94 ± 0.26% in highest salinity. It parallel to the number of SG [64.2% in proportion] was present in lowest salinity and was dramatically increased (70.4% in proportion) in the ovarian parenchyma. Comparative data above, these results were not different between was not different between two period time.

We only found spawned eggs and some juvenile stages in the highest salinity. Naturally spawned eggs of about 0.8 cm were field–observed in gross section (Figures 6A–6B), which fixed spawned egg showed in both animal and vegetal poles (Figures 6C–6D). Histologically, a large mass of homogeneous fluid yolk was observed in this stage (Figures 6E–6F). This is evidence of the proportions of ovulating fish, as well as their adaptation for suitable offspring service, which needs further study.

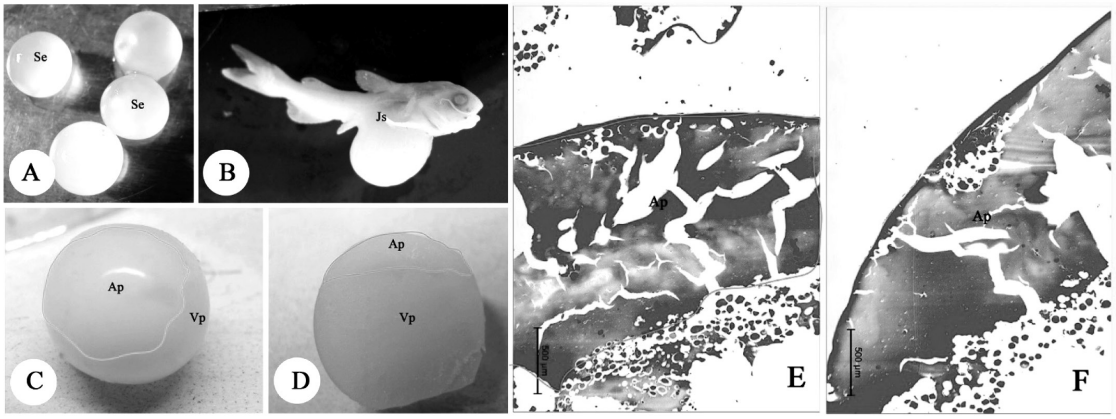

Figure 6 Morphology of the spawned eggs (Se) with separating into animal pole (Ap) and vegetal pole (Vp) and light photomicrographs showing the spawned eggs (E–F) of *Arius maculatus*

Results from the present study show that the post–ovulatory follicle was only found in highest salinity, whereas the number of pronounced atretic oocyte, especially secondary growth phase in the lowest salinity (n = 67 ± 0.34), was significantly different ($P < 0.05$) in the highest salinity (n = 12 ± 1.03). We assumed that highest salinity was adequate for oocyte maturation of *A. maculatus*; however, there is much unknown regarding the effects of salinity on reproductive activity/ pathology in fish. It was possible that the successful fertilization and larval hatching at highest salinity was appropriate for this fish. This hypothesis needs to be investigated further by studying the effects of salinity on the inducing ovarian histopathology of *A. maculatus* under laboratory conditions. However, in most cases the cause pronounced atresia is related to over–season and environmental stressors such as heavy metals (Pierron *et al*., 2008), endocrine disrupters (Pollino *et al*., 2007) and lipid–poor diets (Hunter and Macewicz, 1985; Sherwood *et al*., 2007).

CONCLUSION

In the results presented here, the mature ovarian structure of *A. maculatus* was identified for the first time. The present study could be used to support an understanding of the oogenic process of this fish, which will be applied to study the reproductive cycle and management. At the same time, it is worthy to note that spawned eggs and juvenile stage were only found in highest salinity. Results from our study suggested that it may be implied physiologically this was to prepare for the spawning season and nursery ground reproduction of estuarine areas of Pranburi River, Thailand.

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