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Research article

Comparative stages of atretic oocyte between sesarmid crabs *Episesarma singaporense* and *E. versicolor* from Thailand: Implications on reproductive success

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Abstract

The oocyte atresia is the primary mechanism responsible for the degeneration and resorptive process as a common groundwork for aquatic animals, but it has rarely been reported in crabs. The present study focused on the characteristics and stages of the atretic oocyte from two important female sesarmid crabs (Singapore vinegar crab, *Episesarma singaporense* and violet vinegar crab, *E. versicolor*) during ovarian maturation. A total of 30 female samples from each crab species was collected from the Palian mangrove area of Trang Province, Thailand. The results shared among those crab samples from the primary growth (PG) and secondary growth (SG) phases underwent a degenerative atresia process. The latter atresia stage during SG was also divided into five stages (I, II, III, IV, and V). The degeneration of follicular cell and yolk architecture identified in the SG was highlighted. The number of atresias was compared between *E. singaporense* and *E. versicolor*, indicating that there were no significant differences. Observations suggested that the low number of atresia could be associated with the increased reproductive success of two female sesarmid crabs living in natural habitats.

Keywords: Bioindicator, Female reproductive histopathology, Sesarmid crabs, Thailand

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INTRODUCTION

It is well recognized that the process of ovarian atresia is a degenerative and eventually resorptive mechanism (Guraya, 1986). This character is commonly found in the ovary (Guraya, 1986). Previous research indicated that atretic oocyte occurrence could reduce the ovary egg production/formation and fecundity during its development or spawning season (Ganias et al., 2003; Guraya, 1986). Several significant factors are revealed in which a physiological condition governs the atresia due to external factors (Boyle and Chevis, 1992), marine pollution, or reduced food supply (Cabrera-Páez et al., 2009; Ortiz-Zarragoitia et al., 2011). In the same manner, various documents have reported the intensity and appearance of the atretic oocytes that have occurred, which is useful in the prediction of stocks through the spawning components of the animal population (Ganias et al., 2003; Kurita et al., 2003; Valdebenito et al., 2011). Yet the knowledge of other mangrove crabs is still little known.

Two significant sesarmid crabs— Singapore vinegar crab, *Episesarma singaporense* and Violet vinegar crab, *E. versicolor* (Decapoda: Sesarmidae)— from Thailand are of great importance for crabs and also highly favored by seafood consumers. They are mainly found in mangrove and estuarine areas, where critical services are located in high productivity ecological systems. Recently, the two aforementioned crab species have been identified to have great potential for aquaculture production in Thailand, and have been under developmental studies by the Marine Crab Laboratory, Department of Marine Science and Environment, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Campus (Sudtongkong et al., 2020; Jitnarong and Sudtongkong, 2014). Several aspects concerning reproductive biology in sesarmid crabs have been confirmed (Kyomo, 1986; Lima et al., 2006; Sudtongkong et al., 2021), but the stage of atretic oocytes in this crab group is still missing and requires clarification. The present study is particularly interested in collecting comparative information of atretic oocyte between the two important mangrove crabs mentioned above and makes use of histology and histochemistry from samples collected in the mangrove area at Palian District, Trang Province, Thailand.

MATERIALS and METHODS

Female crab collections

All female crabs, *Episesarma singaporense* and *E. versicolor* (n=30 individual samples from each species) were collected from the mangrove area of Palian District, Trang Province, Thailand (Figure 1) as the critical natural habitats and the fishing zones for crab fisheries in Thailand (7º 08' 58.12" N, 99º 40' 10.11" E), during October 2018 to February 2019 (Table 1). An identification guide of crab species followed the decapod taxonomic studies of Lee, et al. (2015). A practical method of euthanasia, a rapid cooling method, was utilized (Wilson et al., 2009). For ovarian morphology observation, the carapace's dorsal part of the individual crab was opened and then washed with Ringer's solution. The ovarian morphology was observed using a digital camera (Canon EOS 550D). They were finally preserved overnight in Davidson's fixative (Dietrich and Krieger, 2009) at an ambient temperature. The experiment was approved by the Animal Care and Use Committee of Rajamagala University of Technology Srivijaya (ID#IAC 02-01-62).

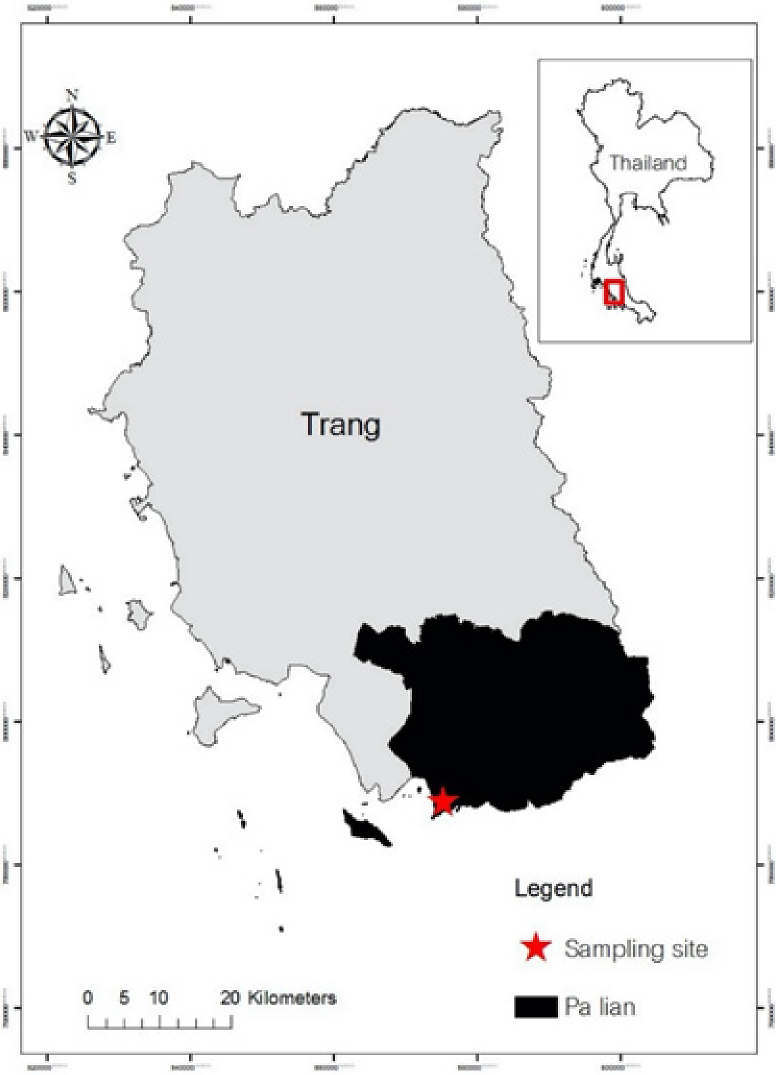

Figure 1 Map of sampling site for *Episesarma singaporense* and *E. versicolor* in Palian District, Trang Province, Thailand.

Histological observation

Fixed whole ovarian tissue of all crabs was dissected and then processed by standardization of the histological technique (Presnell and Schreibman, 1997; Suvarna et al., 2013; Na Lampang et al., 2020; Senarat et al., 2020). The typical paraffin blocks sequence for the specimens were sectioned at a thickness of 4 µm and stained with Harris's hematoxylin and eosin (H&E) and Masson's trichrome (MT). Identification of the ovarian histology/development and the stage of atretic oocyte was captured with a camera [DM750, Leica, Heidelberg, Germany).

Counting the number of atretic oocytes and statistical analysis

In qualitative analyses of the atretic oocyte stage, the numbers of these unusual occurrences were counted from the ovarian development. Three representative permanent slides (100 oocyte stages/ovary per slide) of the ovarian development were selected and observed under light microscopy (10x and 40x). The mean and standard error values of the number of atresias were then determined. The Kolmogorov-Smirnov Test (K-S Test) was typically performed to test for data distribution. The student t-test was employed for comparative data above between species at a significance level of $P < 0.05$ using SPSS software Version 25 (IBM Corp, 2017).

RESULT

Ovarian morphology, stage, and histological characterization of ovarian atresia

The ovarian development of all the crabs had a deep yellow to orange color (Figure 2). Microscopically, healthy primary growth (PG) and secondary growth (SG) (Figures 3-4A) were present. The stage of the atretic oocyte in all crabs was shared and could be further classified into two main phases, namely designed the atretic oocytes during primary growth and secondary growth stages, based on histological structure, and staining properties, as follows (Figures 4-6).

Phase I: Classification of atretic follicle during the primary growth stage (PG)

The atretic oocyte's main features noticeably agreed with the normal PG (Figure 4A), but it had an irregular shape and surrounded the degenerated basophilic cytoplasm (Figure 4A).

Phase II: Classification of the atretic oocytes during the secondary growth stage (SG)

Routine observation of the secondary growth stage showed abundant/large yolk granules (Figure 4B). A layer of squamous follicular cells surrounded it. During this phase, many types of atretic oocyte were observed, which could be subsequently classified into four sub-steps. These sub-stages were divided by a particular complex of several criteria, including shape, characterizations of the yolk granules, and follicle cell (Figures 4B-6).

Sub-stage I:

In this stage, most of the cells are in the normal secondary growth stage, and only a few cells present some abnormality with irregular shapes and the degeneration of yolk granules were visible in some areas of ooplasm (Figures 4B and 6).

Sub-stage II:

An irregular shape of this stage was observed. It was continuously found in parallel to the precocious degradation and regression of yolk compared to the previous stage (Figure 4C). The fragmented follicle cell layer was initially found (Figures 4C and 6).

Sub-stage III:

The permanent disorganizations and shrinkage of sub-stage III were identifiable compared with the previous sub-stage (Figure 4C). Surprisingly, the identified follicle cell was separated and exceptionally reliable for this sub-stage (Figures 4C and 6).

Sub-stage IV:

A considerable decrease in cell size was found. It is recognized that the cell structure was not easily identified from the observation (Figures 4D-5B). Unlike the previous stage, the liquefaction of yolk granules within the ooplasm was significant in providing structural support for this situation (Figures 4D-5A). The follicular cells began to phagocytize degenerating materials (Figures 4B, 5B, and 6).

Sub-stage V:

This stage typically appeared as an amoeboid-shape and decreased in size. The complete digestion of the yolk granules and follicle cell layer (Figure 5C) was noted to contain some apoptosis (Figure 6). Some phagocytize degenerating materials were also found (Figure 5C).

Figure 2 Representative figure on the external morphology of the ovarian development (stage IV) in *Episesarma versicolor*. Scale bar: 1 cm.

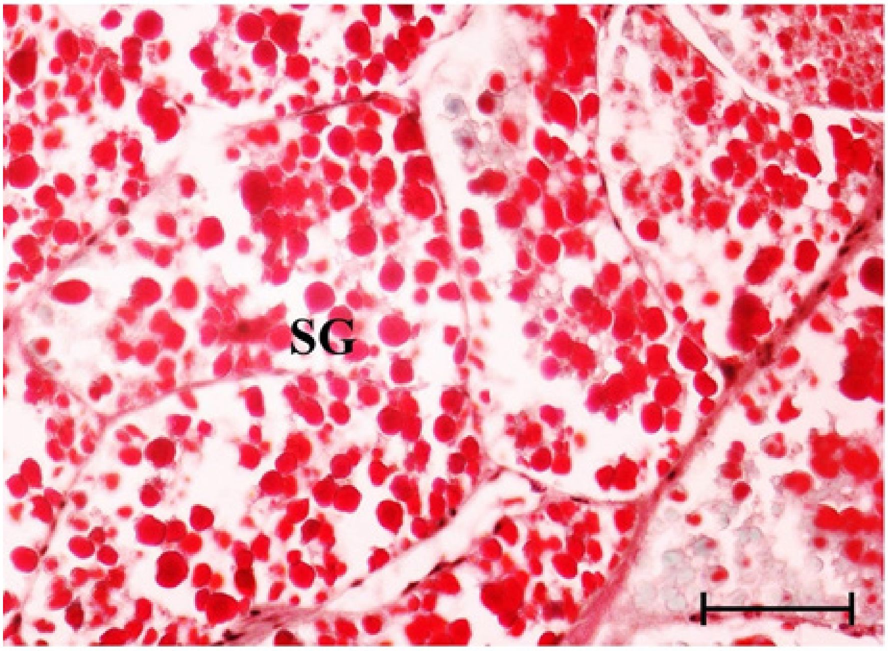

Figure 3 Light microscope showing the secondary growth stage (SG) of *Episesarma versicolor*, comprising several yolk granules (red color). Scale bar: 50 µm. Staining method: Masson's trichrome (MT).

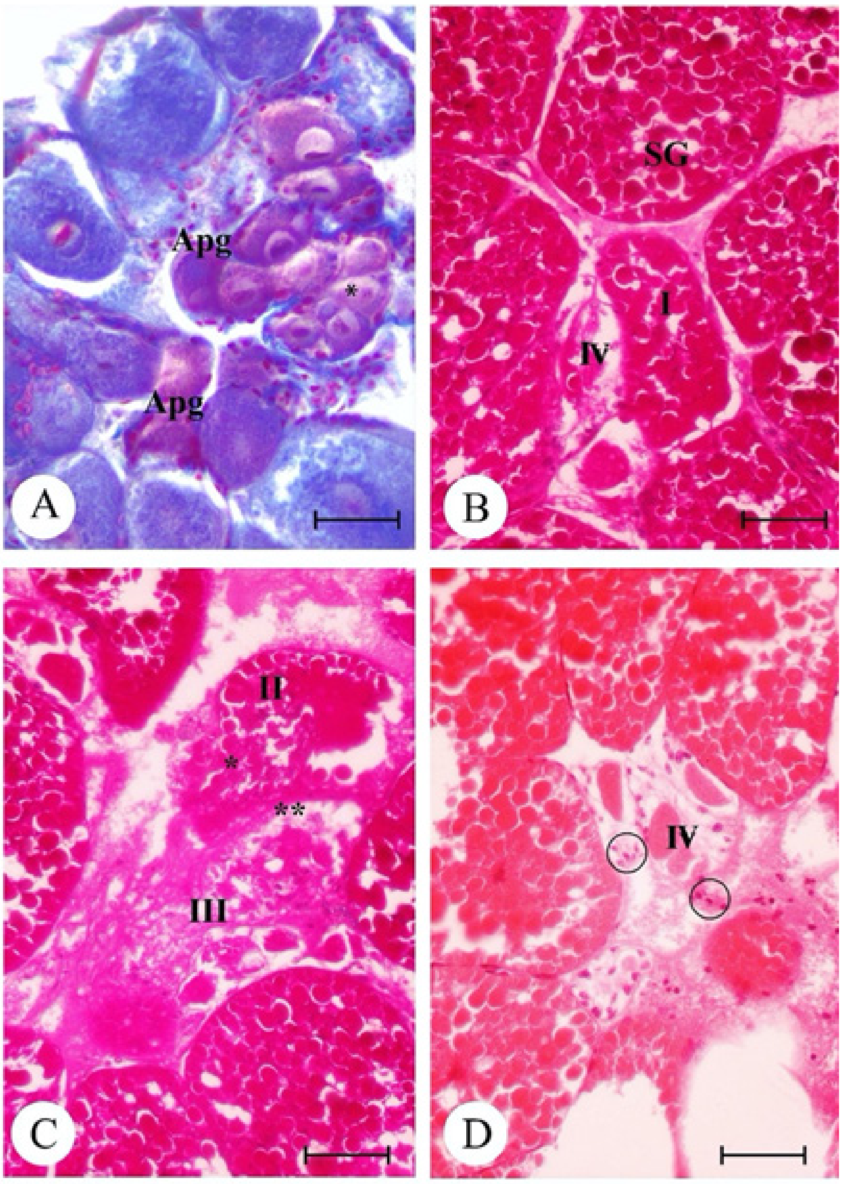

Figure 4 Light microscope showing the primary growth stage (*) and secondary growth stage (SG) of sesarmid crabs. A: The atresia of the primary growth stage (Apg) of *Episesarma versicolor*. B-C: Differentiating stages of stage I, stage II, stage III, and stage IV of *Episesarma singaporense* with degenerating yolk granules (**). Scale bar: A-D = 50 µm. Abbreviations: Yolk granules = *, phagocytize degenerating materials = cycles. Staining method: A = Masson's trichrome (MT); B-D = Harris's hematoxylin and eosin.

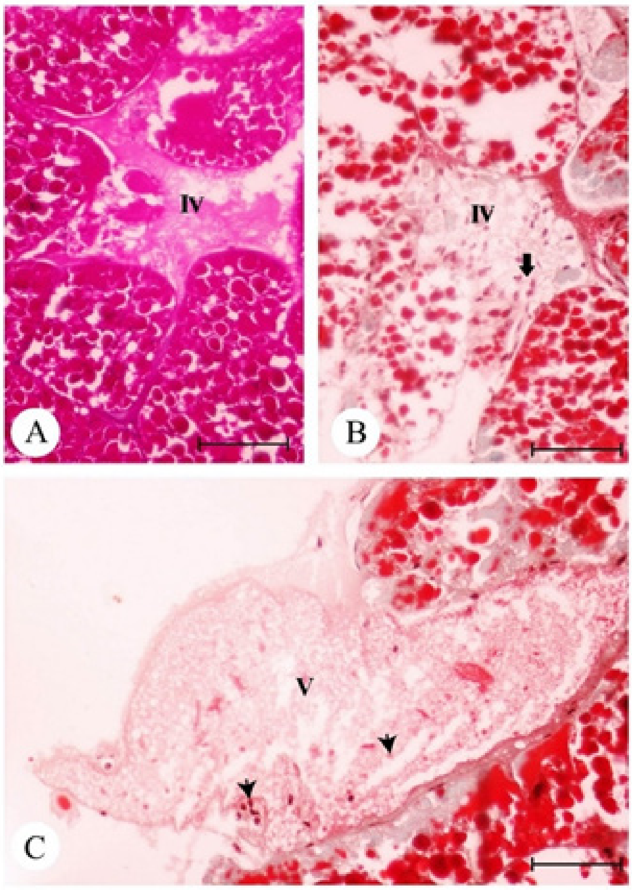

Figure 5 Light microscope showing the atresia of the secondary growth stage of *Episesarma versicolor* sesarmid crabs. A-B: Stage IV, C: stage V. Scale bar: A-C = 50 µm. Abbreviations: phagocytize degenerating materials = arrows. Staining method: A = Harris's hematoxylin and eosin; B-C = Masson's trichrome (MT).

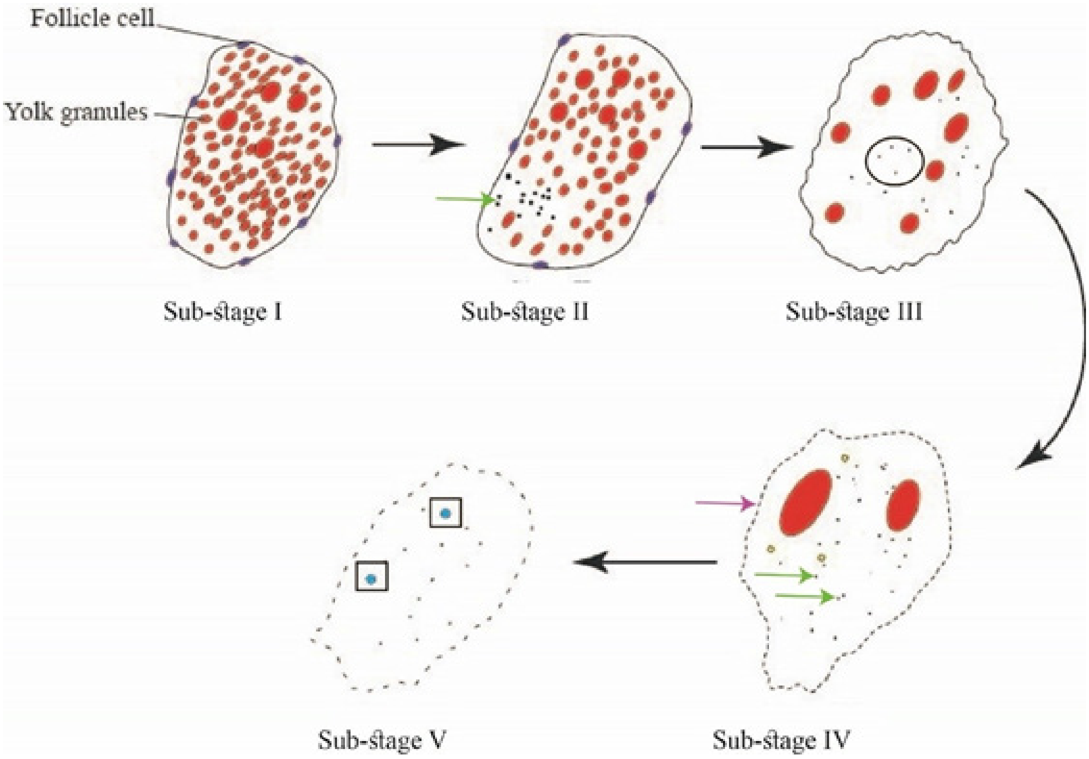

Figure 6 Schematic diagram of the atretic oocyte stage from stages I to V. Sub-stage I was an irregular in shape and there was degeneration of some yolk granules in certain ooplasm areas. Sub-stage II was still observed as an irregular shape, whereas the degradation and regression of yolk (green arrow) were visible. The disorganization and shrinkage (cycle) of the yolk granule in the sub-stage III was noted. Sub-stage IV was then observed. The liquefaction of yolk granules (green arrow) was reported within the ooplasm. The follicular cells began to phagocytize degenerating materials (pink arrow). The final stage was sub-stage V. Both yolk granules and follicle cell layer was wholly digested. Some phagocytize degenerating materials were also recorded (boxes).

Quantification of the atretic oocyte during final ovarian maturation

The mean of atretic oocytes was compared among the crabs (Table 1). The number of atretic oocyte during PG and SG was slightly different between the crabs, but this was not statistically significant (Table 2).

Table 1 The collected number of *Episesarma versicolor* and *E. singaporense* from October 2018 to February 2019.

Months/ sesarmid crabs	*E. singaporense*	*E. versicolor*
October 2018	5	5
November 2018	5	5
December 2018	5	5
January 2019	10	10
February 2019	5	5

Table 2 Qualitative data of the atretic oocytes between *Episesarma singaporense* and *E. versicolor* from Thailand.

Sub-stages	Species	Mean±SE	P-value
I	*E. singaporense*	1.70±0.14	0.46
	E. versicolor	1.80±0.17	
II	*E. singaporense*	1.66±0.14	0.11
	E. versicolor	1.40±0.11	
III	*E. singaporense*	1.56±0.11	0.10
	E. versicolor	1.56±0.92	
IV	*E. singaporense*	1.00±0.14	0.33
	E. versicolor	0.90±0.12	
V	*E. singaporense*	0.73±0.12	0.95
		0.46±0.12	

DISCUSSION

Morphological description of the ovaries in female sesarmid crab *E. singaporense* that occurred during the annual reproductive cycle could be classified into four stages, namely stages I (immature stage), stage II (developing stage), stage III (developed stage), and stage IV (mature stage), which were in agreement with former observations (Sudtongkong et al. 2021). Therefore, our ovarian histology from both sesarmid crabs is considered to be stage IV as the final maturation composing in most mature oocyte and secondary growth phases.

Along the reproductive cycle, atretic follicle characterization has been exten sively investigated in numerous invertebrates including fish under the regulation of many factors, such as reproductive hormone and reproductive physiology (Ganiaset et al., 2003; Nagahama, 1983). These features should be contemplated when applying to determine the fecundity and spawning season (Jans and van der Kraak, 1997) and reproductive health in aquatic animals (Blazer, 2002). Thorough knowledge of atresia contributes information regarding its structure and stages among several fish species (Kurita et al., 2003; Linares-Casenave et al., 2002; Senarat et al., 2017). In contrast to the different atretic follicles or atretic oocyte stages, they have been rarely found in crustaceans (Kelemec and Smith 1980; Senarat et al., 2020; Tan-Fermin

1991). In the pea crab *Pinnotheres cyclinus*, only atretic oocytes during the primary growth phase were visible, showing irregular nucleic shapes and the detachment of the thin follicular cell (Senarat et al., 2020), similar to the observations from the present study.

The reasons for atresia occurrence during the final ovarian maturation remains unknown in crustaceans (Kelemec and Smith, 1980). A strong oocyte atresia phenomenon in the aquatic animal has appeared to respond to environmental factors alteration and pollution such as heavy metals (Pierron et al., 2008). Moreover, endocrine-disrupting chemicals (Pollino et al., 2007) and inappropriate nutritional proportion (Hunter and Macewicz, 1985) could cause this abnormality. Meanwhile, former observations revealed that the increased proportion of ovarian atresia is related to temperature and feeding changes among certain animals (Bromley et al. 2000; Linares-Casenave et al., 2002). It was possible that the critical factors above could increase the number of atresia in our observations, but this hypothesis verification through further studies.

Unfortunately, the proportion of atresia in the crabs was not seen, but this was the first observation of these crabs. It is reasonable to suggest that the rare atretic oocytes with high-quality oocyte/fecundity reports might be associated with high hatching performance. This hypothesis is supported by Cherel and Beninger (2017) who reported the atretic oocyte level on bivalve reproduction, which is significantly related to oocyte mortality and leads to high mortality rates of early life stage bivalves.

CONCLUSION

It could be concluded that the atretic oocyte stage consisted of primary growth and secondary growth phases. These characteristics of atretic oocyte occurred in female sesarmid crabs, *E. singaporense* and *E. versicolor*. The number of atresias was documented during final ovarian maturation in these crabs, suggesting that the increased frequency of high-quality oocyte/fecundity and reproductive success of two female sesarmid crabs is recommended.

CONFLICT of INTEREST

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

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AUTHOR CONTRIBUTION

Conceptualization, C.S. and S.S; methodology, S.S.; investigation, S.S., S.K. and C.S.; resources, C.S.; data curation, C.S. and S.S.; writing — original draft preparation, S.S. and C.S.; writing—review and editing, C.S., S.S., S.K., P.P., K.W. and W.J; supervision, C.S.; project administration, C.S.; funding acquisition, C.S. All authors have read and agreed to the published version of the manuscript.

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