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Full Length Article

Sperm storage dynamics in the blue swimming crab, *Portunus pelagicus*: Reduction and depletion of spermathecae during ovarian maturation

Ananda Adya [a], Yushinta Fujaya [a,b,*], Sharifuddin Bin Andy Omar [a], Siti Aslamyah [a,b], Andi Aliah Hidayani [a,b], Dwi Kesuma Sari [c], Tutik Wresdiyati [d], Matthew B. Ogburn [b,e], Khor Waiho [b,f]

[a] *Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia*
[b] *Tropical Crab Research Group, Hasanuddin University, Makassar, Indonesia*
[c] *Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[d] *Division of Anatomy, Histology, and Embryology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia*
[e] *Smithsonian Environmental Research Center, Edgewater, MD 21037, USA*
[f] *Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

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ABSTRACT

Understanding the relationship between spermathecae fullness and ovarian maturation is essential for elucidating the reproductive strategy of the blue swimming crab (*Portunus pelagicus*). This study examined the association between ovarian maturation stages (OMS) and spermathecae weight, sperm count, and related reproductive traits. A total of 240 female crabs were collected from Pare-Pare Bay, South Sulawesi, between January and June 2024. Carapace width and body weight were measured, OMS and spermathecae fullness were classified, and spermathecae weight and sperm counts were quantified, supported by histological analyses. Crabs at OMS I predominantly had full spermathecae, whereas those at OMS IV–V were mostly empty; notably, 21.43% of OMS I individuals had empty spermathecae. Histology revealed reduced seminal fluid in empty spermathecae, although spermatophores remained present. Sperm counts declined with decreasing spermathecae fullness, averaging $548 \times 10^8 \pm 91 \times 10^8$ (full), $242 \times 10^8 \pm 126 \times 10^8$ (partially full), and $158 \times 10^8 \pm 50 \times 10^8$ (empty). These findings indicate that reductions in spermathecae size and sperm count are primarily associated with seminal fluid depletion, while sperm storage persists.

Introduction

Reproductive efficiency is a critical determinant in the population dynamics of crustaceans, particularly in commercially significant species such as the blue swimming crab (*Portunus pelagicus*). The spermathecae, commonly known as seminal receptacles, are specialized, paired vaginas that enlarge and store sperm for fertilization across multiple spawning cycles, playing a central role in the reproductive process of female crabs. This adaptive feature facilitates successful reproduction even when mating opportunities are scarce (Pretterebner et al., 2022; Rodgers et al., 2011). However, the functionality of the spermathecae is dynamic, undergoing morphological and physiological changes influenced by ovarian maturation, environmental conditions, and potentially stress from repeated spawning (Alberts-Hubatsch et al., 2016; Elner & Beninger, 1995; Jivoff et al., 2007). Fertilization occurs internally in *P. pelagicus* and other Eubrachyura (e.g., Becker et al., 2011). Sperm are stored in the spermathecae until the oocytes mature. The mature oocytes are transported via oviducts to the spermathecae, where fertilization occurs, and are subsequently extruded onto the external pleopods (Becker et al., 2011; McLay & López Greco, 2011).

Evidence from several Eubrachyura indicates that spermathecae change during ovarian development. Spermathecae enlarge due to the transfer and seminal fluid during mating and formation of a sperm plug after mating (Zara et al., 2014). The sperm plug persists until the ovary reaches the developmental stage, but disappears during the transitional stage, causing the spermathecae to soften (Zara et al., 2014). The phenomenon of spermathecae shrinkage, characterized by a reduction in seminal fluid and overall spermathecae weight, has been documented in

* Corresponding author at: Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia.
E-mail address: yushinta.fujaya@unhas.ac.id (Y. Fujaya).

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the blue crab (*Callinectes sapidus*) (Wolcott et al., 2005), particularly in relation to spawning cycles and seasonal variations in the Chesapeake Bay (Ogburn, 2019; Ogburn et al., 2014). These findings have prompted inquiries into the underlying causes and implications of this phenomenon in other crab species, including *P. pelagicus*, which is of critical importance to tropical and subtropical fisheries. Despite its ecological and economic significance, research on the dynamics of spermathecae morphology in *P. pelagicus* remains limited, especially in regions such as Indonesia's Pare-Pare Bay. Unlike *C. sapidus* females that mate only once in their lifetime following a functional terminal molt (Hines et al., 2003; Jivoff, 2003), *P. pelagicus* females engage in multiple mating and spawning events (Kumar et al., 2003). This difference in reproductive strategies complicates the determination of whether mature female *P. pelagicus* with apparently empty spermathecae possess additional spawning potential.

Understanding the interaction between ovarian maturation and spermathecae dynamics is crucial for sustainable fisheries management. A reduction in spermathecae size may impact sperm storage capacity and fertility (Wang et al., 2023). This condition could have significant implications for population sustainability amid increasing fishing pressures and environmental stressors (Jivoff et al., 2007; Ogburn, 2019; Ogburn et al., 2014). This study aims to address this knowledge gap by examining the morphological and functional changes of the spermathecae during ovarian maturation in *P. pelagicus*. Specifically, it investigates the relationship between ovarian maturity levels, spermathecae weight, sperm counts, and environmental factors, thereby providing insights into the reproductive biology of this species.

Materials and methods

Female *Portunus pelagicus* specimens were procured alive from local fishermen operating in Pare-Pare Bay, Suppa, Pinrang Regency, South Sulawesi, Indonesia, during the period from January to June 2024. The specimens were caught by small-scale fisheries, using traps and gill nets operating in the study area with a mesh size of 3 in.. Sampling was conducted in collaboration with these fishers to ensure representation of the actively exploited population (Fig. 1). Pare-Pare Bay is one of the primary crab fishing centers in South Sulawesi.

Measurements of carapace width (CW) and body weight (wet weight) were conducted for all collected individuals. The females were dissected to examine the ovarian maturation stages (OMS). The ovarian maturation stage (OMS) was recorded following Ravi et al. (2012). Based on ovarian morphology, they were divided into five phases (Fig. 2). The spermathecae were weighed, and their fullness was assessed visually. Spermathecae fullness was categorized into three classifications: full, for spermathecae that were round, had raised walls, and were filled; partially full, for spermathecae that were flat, round, had empty walls, and were less filled; and empty, for spermathecae that were small, round, flabby, and appeared empty (Wolcott et al., 2005) (Fig. 3).

Histological preparation

An examination of the reproductive tract, extending from the vulva through the vaginal canal, spermathecae, and the majority of the ovaries, was conducted visually after dissecting frozen specimens. A transverse incision was made just above the vulva at the sixth thoracic segment (Oh & Hankin, 2004). For histological analysis (Fig. 4), the spermathecae were initially fixed in a 4% formaldehyde solution for a duration of two weeks. Following this, they were transferred to 70% ethanol for long-term preservation until analysis. After fixation, the samples underwent a dehydration process using graded alcohol (ranging from 70% to 100%) and were subsequently embedded in paraffin (Surgipath Paraplast).

The embedded blocks were sectioned using a microtome to a thickness of 5 μm, mounted on slides, and stained with hematoxylin for six minutes and eosin for two minutes, as per Kiernan (1990). Morphological observations were conducted using a CX23 light microscope (Olympus) equipped with a camera (Indomicro) and connected to a computer.

Sperm count

Sperm quantification was conducted in accordance with the methodology outlined by Ogburn et al. (2019). Spermatophores were manually extracted from the spermatheca membrane in a petri dish. The disintegration of spermatophores into individual sperm cells was achieved utilizing a PYREX Dounce glass homogenizer. Following

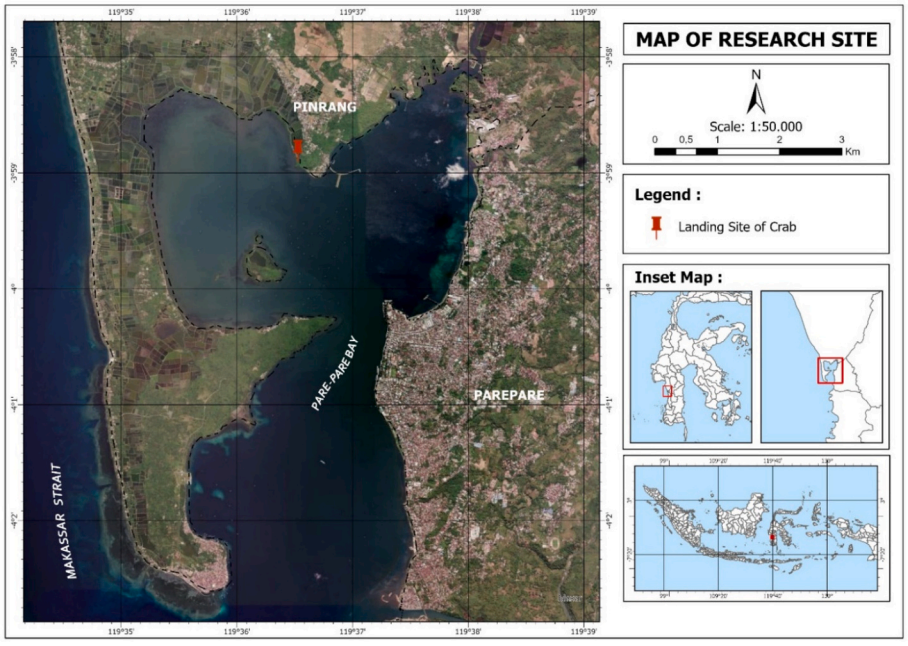


Fig. 1. Blue swimming crab sampling site (marked with a red pin) located at Pare-Pare Bay, South Sulawesi, Indonesia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

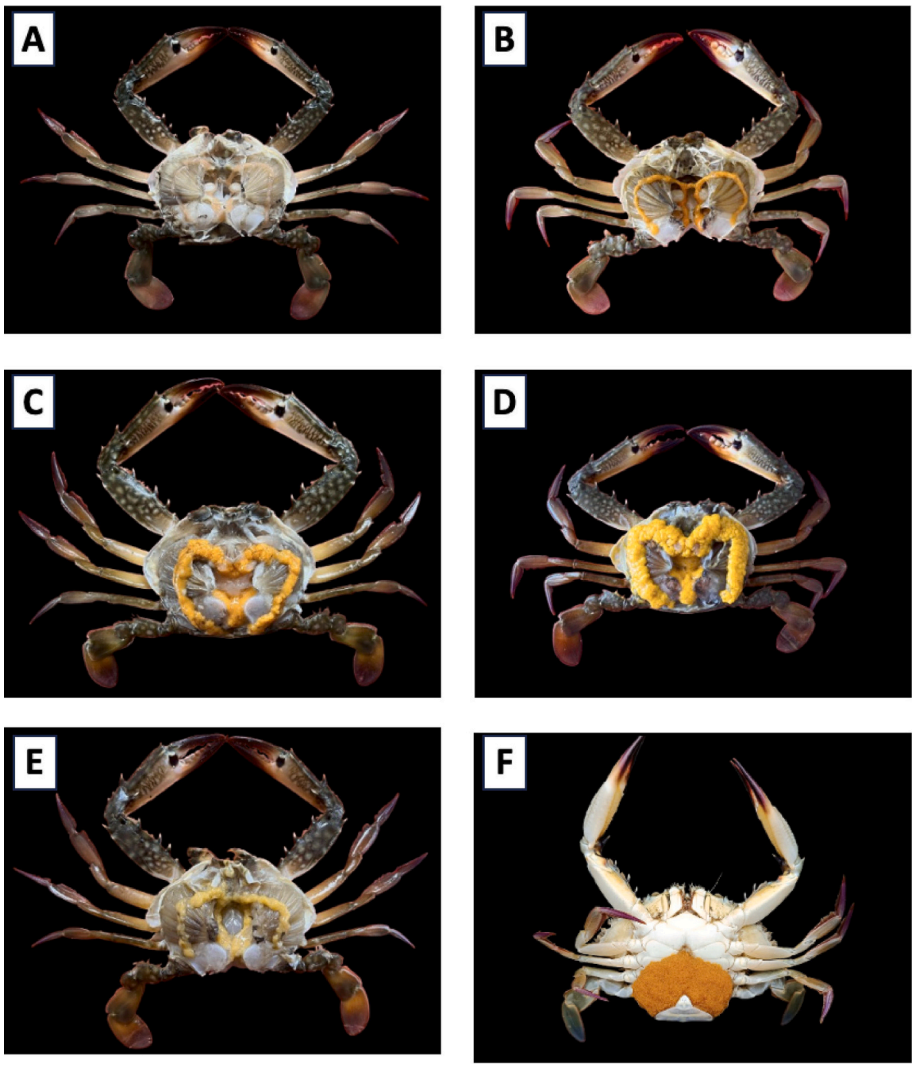


Fig. 2. The Ovarian Maturation Stages (OMS). A, OMS 1 (Immature), the ovarium is small, thread-like, and flattened; smooth in texture without pronounced lobulation; off-white to ivory. B, OMS II (Early Maturing), the ovarium is relatively larger than stage I; light yellow; posterior lobes are rather convoluted; the anterior part marginally displaces the hepatopancreas, and the middle covers the heart. C. OMS III (Late Maturing), the ovarium is large, yellow with rough texture because of nodulations; the anterior lobes are displaced by the hepatopancreas; the central and posterior lobes cover the gastric and intestinal cavities. D, OMS IV (Matured), the ovarium is very large and highly developed. E describes spent or recovery. F describes the spawning. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

homogenization, the combined volume of liquid and spermathecal material was supplemented with 70% ethanol to reach a total volume of 15 mL per vial. A single drop of the sample was placed in a Neubauer Improved Chamber (featuring a 5 × 5 grid) and examined under a phase contrast microscope at 400× magnification. All 25 squares within the grid were counted, and the number of sperm was documented. The total sperm count per crab was determined using the following equation:

$$Quantity\ of\ sperm = \frac{Number\ of\ sperm\ cells\ counted}{Volume\ of\ counting\ chamber} \times Dilution$$

The volume of the counting chamber is 0.004 μL (0.000004 mL), and the dilution is defined as the total volume (mL) of the homogenized fluid combined with the sperm content. This procedure was performed three times per sample, and the three measurements were averaged to determine the final sperm count per crab.

Data analysis

The data were analyzed using descriptive statistics in Microsoft Excel. Analysis of Variance (ANOVA) and the advanced Tukey test were conducted using SPSS version 23 software. A significance level of 0.05 was employed to assess statistical differences between the fullness and the number of sperm. The histological results were analyzed based on visual inspection of the images.

Results

A total of 240 female crabs were randomly collected from January to June 2024, and 152 of them were selected by evaluating their gonad maturity level, which were then processed to evaluate the dynamics of sperm storage. The body weight of selected crabs ranged between 20 and 182 g with an average range between 56.90 ± 22.16 g in January and 79.46 ± 26.43 g in February. The carapace width ranged between 35 and 138 mm, with an average range of 102.16 ± 9.51 mm in June and 107.72 ± 15.62 in February. No discernible trend was observed in either body weight or carapace width over the study period (Table 1). Table 2 shows the percentage of spermathecal fullness categories by months.

The degree of spermathecae fullness diminished as the Ovarian Maturation Stage (OMS) progressed (Fig. 5). Ovarian maturation stage I had the highest percentage of full spermathecae at 57.14%, decreasing



Fig. 3. Anatomical characterization of the spermathecae in female *Portunus pelagicus*. (A) The spermathecae (S) are located within the thoracic cavity and are connected to the ovaries (O); (B) spermathecae in a fully filled state; (C) spermathecae in a partially filled state; (D) spermathecae in an empty state.

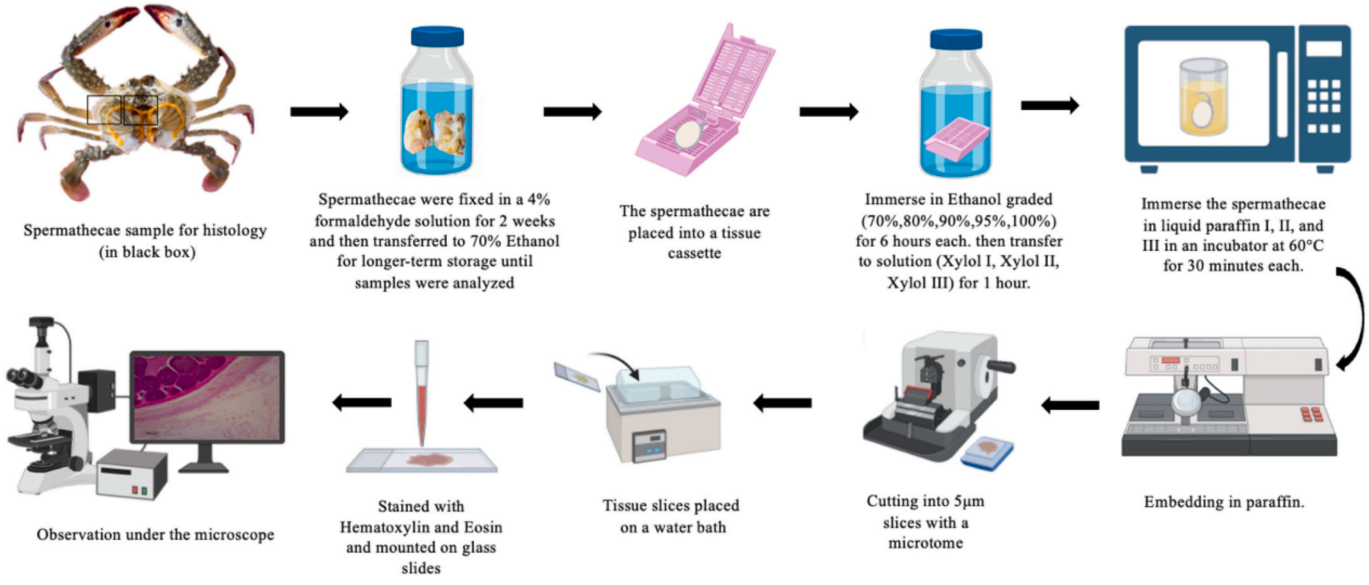


Fig. 4. Histology preparation process.

Table 1
Description of female crab samples from Pare-Pare Bay obtained from January to June 2024.

Month	n (240)	Body Weight (g) (mean ± SD)	Range Body Weight (g)	Outer Carapace width (mm) (mean ± SD)	Range Outer Carapace width (mm)	Ovarian Maturation Stage (OMS)				
						OMS I (%)	OMS II (%)	OMS III (%)	OMS IV (%)	OMS V (%)
January	21	56.90 ± 22.16	20–100	106.52 ± 9.53	79–122	19.05	4.76	9.52	19.05	47.62
February	39	79.46 ± 26.43	46–182	107.72 ± 15.62	35–131	25.64	15.38	20.51	5.13	33.33
March	48	70.46 ± 20.70	23–131	103.73 ± 9.50	83–124	16.67	6.25	18.75	14.58	43.75
April	50	71.94 ± 18.56	39–137	106.94 ± 10.05	83–138	4.00	12.00	24.00	20.00	40.00
May	45	73.76 ± 21.36	39–132	106.00 ± 10.59	85–128	6.67	15.56	13.33	20.00	44.44
June	37	69.57 ± 23.13	36–134	102.16 ± 9.51	78–124	2.70	10.81	32.43	16.22	37.84

in OMS II (33.33%), OMS III (10.20%), and OMS IV (5.36%). The percentage of partially full spermathecae also varied across OMS, with OMS I accounting for 21.43%, OMS II for 48.15%, OMS III for 48.98%, OMS IV for 18.42%, and OMS V for 10.00%. The highest percentage of empty spermathecae was found in OMS V at 80.00%, while OMS IV accounted for 76.32%, OMS III for 40.82%, OMS I for 21.43%, and OMS II for 18.52%. Notably, all levels of spermathecae fullness, ranging from full to empty, were observed across each OMS, and each OMS stage was documented in every month of the study, except that the fullness of OMS V was only recorded exclusively in January.

Table 2
Distribution of the number and percentage of crabs based on spermathecae fullness categories by month of observation.

Month	n (152)	Fullness		
		Full (n; (%))	Partially full (n; (%))	Empty (n; (%))
January	21	1; (4.76%)	2; (9.52%)	18; (85.71%)
February	26	7; (26.92%)	12; (46.15%)	7; (26.92%)
March	27	13; (48.15%)	7; (25.93%)	7; (25.93%)
April	30	2; (6.67%)	14; (46.67%)	14; (46.67%)
May	25	10; (40.00%)	10; (40.00%)	5; (20.00%)
June	23	0; (0%)	6; (26.09%)	17; (73.91%)

Macroscopically, the spermathecae of *P. pelagicus* were dorsally connected to the ovary and ventrally linked to the vulva (Fig. 6A). Histological analysis revealed morphological variation among different fullness categories, particularly in the width of the tissue surrounding the spermathecae and the presence of seminal fluid under fully filled conditions (Fig. 6C). Spermatophores were found at full, partially full, and empty levels of spermathecae fullness. However, structural differences in the spermathecae wall were clearly evident among conditions. In the fully filled condition (Fig. 6D, G, J), the wall appeared enlarged and compact, with the connective layer (CL) providing structural integrity. In the partially filled condition (Fig. 6E, H, K), the spermathecae wall appeared more relaxed in both the connective layer (CL) and the dense layer (DL), along with an increase in CL width. In contrast, in the empty condition (Fig. 6F, I, L), seminal fluid was absent, supporting tissue activity decreased, the CL became more relaxed, and the spermathecal wall width increased significantly.

A positive correlation was observed between the weight of the spermathecae and the sperm count. The minimum spermathecae weight when empty (0.07 ± 0.06 g) corresponded to the lowest sperm count recorded when empty ($158 \times 10^8 \pm 50 \times 10^8$). The weight at a partially full state (0.22 ± 0.11 g) and the sperm count ($242 \times 10^8 \pm 126 \times 10^8$) were intermediate. The maximum weight was observed when full (0.44 ± 0.18 g), with a corresponding sperm count of ($548 \times 10^8 \pm 91 \times 10^8$). Significant differences were found among the spermathecae weights ($P < 0.05$) and sperm counts ($P < 0.05$) across the categories of spermathecae fullness: full, partially full, and empty (Fig. 7). Overall, the range of sperm counts in *P. pelagicus* in Pare-Pare Bay was 73–685 × 10^8.

Discussion

The findings of this study offer significant insights into the dynamics of spermathecae morphology and sperm storage in *Portunus pelagicus*, elucidating the relationship between spermathecae fullness and ovarian maturation. These results uncover critical patterns essential for comprehending the reproductive biology of *P. pelagicus* and its spawning potential in Pare Bay, South Sulawesi, Indonesia.

Observations in this study confirmed that the spermathecae of *P. pelagicus* are located dorsally, as described by McLay and López Greco (2011). These spermathecae are directly connected to the oviduct and ventrally to the vulva. In this configuration, fertilization is likely to occur when a mature oocyte traverses the oviduct toward the spermathecae, where stored sperm are used for fertilization. Subsequently, the zygote is expelled through the vulva. This process aligns with prior findings for Eubrachyura that spermathecae function in both sperm storage (e.g. Monteiro et al., 2019) and internal fertilization Becker et al. (2011). The direct connection between the spermathecae and the ovaries via the oviduct ensures that sperm remains in optimal condition until oocyte maturation, as noted by (Pardo et al., 2017). Similar observations in *Ucides cordatus* indicate that oocytes enter the spermathecae through a dorsal post-pore, facilitating internal fertilization (Castilho-westphal et al., 2013).

There was no correlation between female body weight and spermathecae weight, indicating that factors such as reproductive stage and mating history are more important than body weight in determining spermathecae characteristics. Our findings reveal that spermathecae fullness, which is related to spermathecae weight, diminishes with the progression of ovarian maturation. The highest percentage of full spermathecae occurs at OMS I (57.14%), whereas fullness progressively declines at OMS IV and V. The presence of varying spermathecae fullness (full, partially full, and empty) across all OMSs indicates individual variation influenced by factors such as mating history, spawning

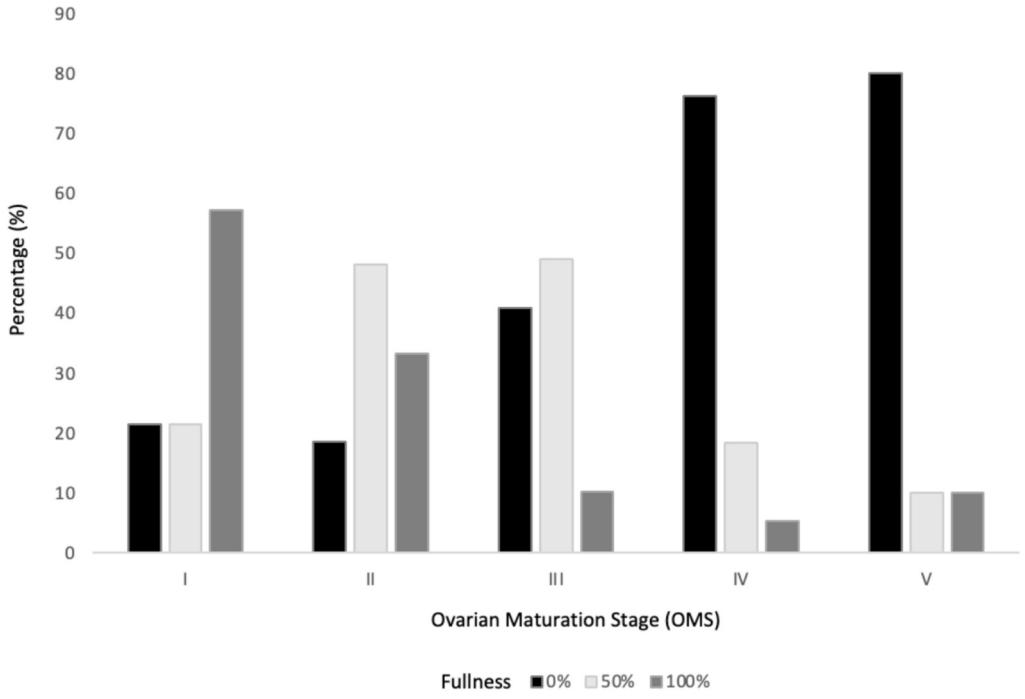


Fig. 5. Relationship between spermathecae fullness and ovarian maturation stages. Ovarian maturation state V was only assessed in January, whereas stages I-IV were assessed from January to June.

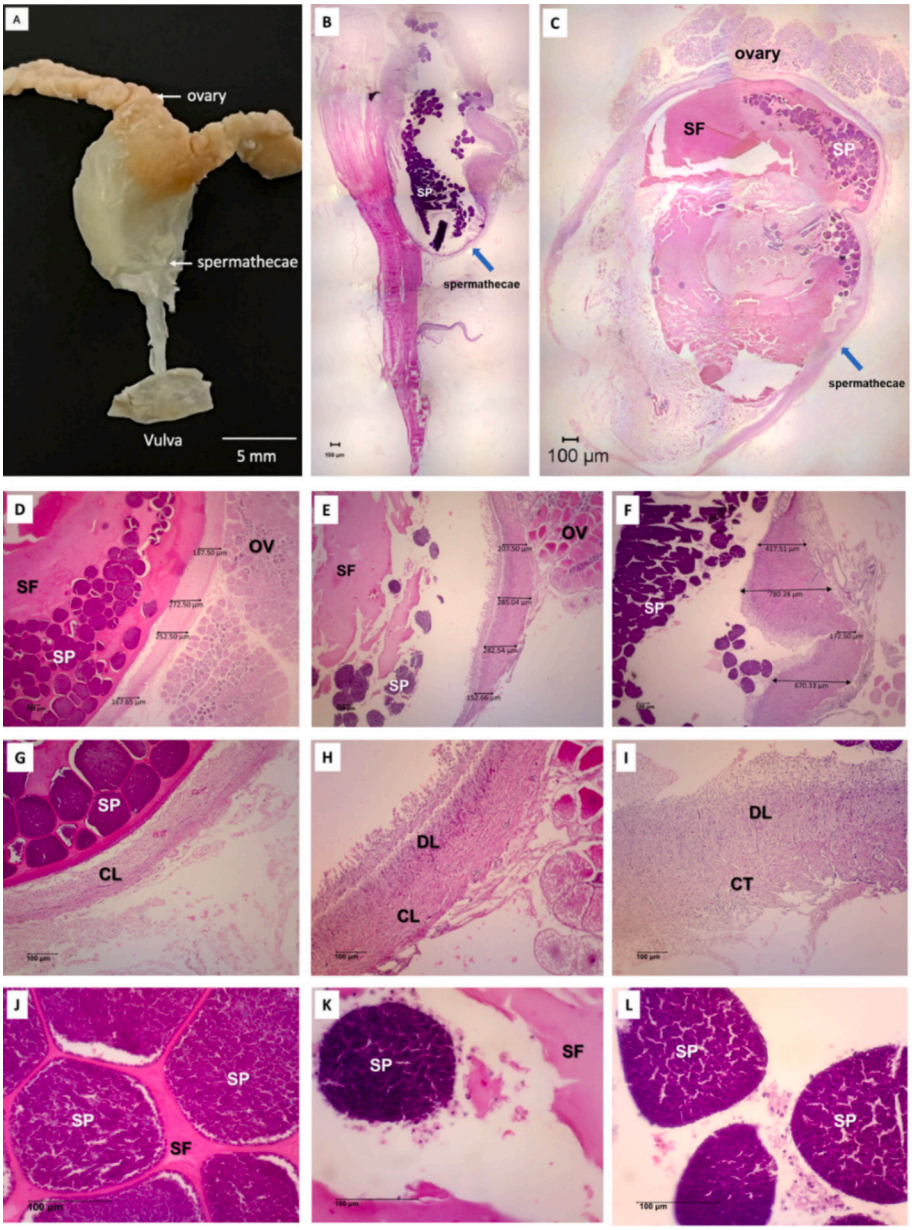


Fig. 6. Macroscopic and histological appearance of spermathecae of female blue swimming crab *Portunus pelagicus*. (A) Macroscopically, the spermathecae appear to be dorsally connected to each other and ventrally connected to the vulva. (B—C) Composite longitudinal sections of the spermathecae showing different storage conditions, namely (B) spermathecae in empty condition showing the region toward the vulva canal, and (C) spermathecae in full condition. (D, G, J) spermathecae in full condition derived from panel C; (E, H, K) spermathecae in partially full condition; and (F, I, L) spermathecae in empty condition derived from panel B. SF indicates seminal fluid, SP indicates spermatophore, OV indicates ovary, CL indicates connective layer, DL indicates dense layer, and CT indicates connective tissue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

frequency, and environmental conditions, which were beyond the scope of this study, as in Ogburn et al. (2014). The pattern observed here contrasts with findings in other species, such as the fiddler crab (*Uca triangularis* Bengali) and the blue land crab (*Cardisoma quantum*), where spermathecae size changes significantly across reproductive phases (Krishnapriya & Padmaja, 2014; Shinozaki-Mendes et al., 2011). These changes have been attributed to processes such as deutoplasmogenesis (Bawab & El-Sherief, 1988) and variations in sperm count (Pardo et al., 2013).

The observed positive correlation between spermathecae weight and sperm count underscores the functional relationship between spermathecal structure and reproductive potential. As spermathecae fullness declines, both spermathecae weight and sperm count decrease significantly. This pattern, also reported in other decapods such as *C. sapidus*, may reflect sperm utilization, degradation, or loss during reproductive processes (Ogburn et al., 2014; Rains et al., 2016; Wolcott et al., 2005). Despite the reduction in sperm count, the absence of seminal fluid (sperm plug) does not appear to compromise sperm quality. Seminal fluid primarily supports sperm viability during storage by providing nutrients and protection against oxidative stress (Pretterebner et al., 2022). Once encapsulated within spermatophores, sperm remain viable even without seminal fluid, thereby ensuring successful fertilization (Zara et al., 2014). This resilience allows species such as *P. pelagicus* and *C. sapidus* to maintain high fertilization success despite prolonged storage or seminal fluid loss.

Notably, the observation that 21.43% of females at the earliest maturity stage (OMS I) exhibited empty or nearly empty spermathecae was surprising given that females at OMS I should have mated recently.

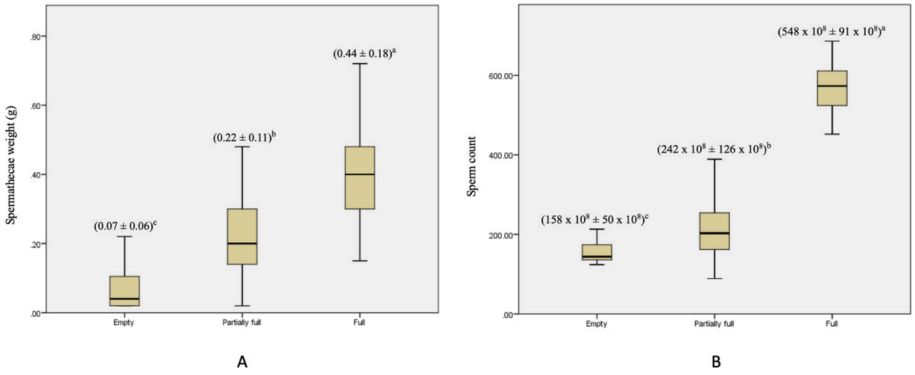


Fig. 7. *Portunus pelagicus* spermathecae weight (A) and sperm count (B) for each spermathecae fullness. Letter (a-c) shows statistically significantly different ($p < 0.05$).

This paradox may reflect the impact of male reproductive output and environmental stress on sperm storage dynamics, rather than indicating true reproductive immaturity. In certain instances, males may transfer only a minimal amount of seminal material, particularly if they are smaller in size or have recently mated with multiple females (Kendall et al., 2002). Ogburn et al. (2014) demonstrated that such limited sperm transfer can result in spermathecae that appear partially or empty despite recent mating. Consequently, the presence of empty or depleted spermathecae in some OMS I females is more likely related to post-mating sperm loss rather than the absence of mating.

Two drawbacks of this study are that crabs were only sampled from January to June and that there is limited information on the spermathecae fullness of female crabs with OMS V. Although this species is known to spawn continuously throughout the year in tropical waters (Ernawati et al., 2014), sampling in this study was limited to six months to obtain an overview of population conditions during the seasonal shift from the wet to dry season in South Sulawesi, which also includes the March peak in spawning activity (Fujaya et al., 2025). This timeframe is expected to capture potential variations in reproductive activity influenced by fluctuations in temperature and salinity, which may affect gonadal development and sperm storage dynamics. Conducting year-round sampling would facilitate a more comprehensive understanding of the dynamics of ovarian maturation and the condition of spermathecae, thereby providing more robust insights into the reproductive ecology of the blue swimming crab in South Sulawesi. While crabs at OMS V were present from January to June, spermathecae fullness checks for this stage were only conducted in January. Considering that *P. pelagicus* can undergo repeated spawning and has a long reproductive season in tropical waters (Ikhwanuddin et al., 2016; Zairion et al., 2015), the presence of OMS V in subsequent months indicates that reproductive activity continues from February to June. Further studies are needed to document the full annual pattern of reproductive dynamics of *P. pelagicus*.

Histological analyses supported the visual observations of structural modifications within the spermathecae across stages. When filled, the walls are taut and robust, signifying their function in long-term sperm storage. In a partially filled state, the loosening of connective layers indicates a transitional phase as sperm storage diminishes. In the empty state, the walls become notably wider and more relaxed, reflecting structural adaptation to an empty condition. Spermathecae that are partially full or empty exhibit a reduction in size due to the decrease in seminal fluid. The seminal fluid, situated between the spermatophores and stored in the spermathecae, comprises glycoproteins with neutral polysaccharides and acids (Assugeni et al., 2021). In *Metacarcinus edwardsii*, seminal fluid functions to facilitate reproduction, protect and nourish sperm, and form a sperm plug to prevent sperm mixing (Pretterebner et al., 2022). The presence of a sperm plug can also serve as an indicator of recent mating (Oh & Hankin, 2004). In *Callinectes sapidus,* young females contain acidic polysaccharides that persist until ovarian development. Once the ovaries enter the transitional stage, the blockage dissipates and becomes soft (Zara et al., 2014). These observations are consistent with studies emphasizing the dynamic nature of spermathecae in adapting to varying storage capacities (Farias et al., 2017; Kienbaum et al., 2017; Sant'Anna et al., 2007).

The observed variation in spermathecae fullness across different stages of ovarian maturity has important implications for the reproductive potential of *P. pelagicus* populations in Pare-Pare Bay. The presence of empty or partially filled spermathecae in both early (OMS I) and advanced (OMS IV–V) maturity stages suggests potential sperm limitation, where reproductive output is constrained by insufficient sperm availability rather than oocyte production (Ogburn, 2019). This condition may be exacerbated in exploited populations, where selective harvesting of large males reduces mating success and the quantity of sperm transferred to females (Hines et al., 2003). A similar pattern may occur in Pare-Pare Bay, where fishing pressure targeting larger individuals could contribute to reduced sperm reserves and potential demographic imbalances between males and females. However, in contrast to *C. sapidus* in temperate regions, which mates once and stores sperm for its lifetime, *P. pelagicus* in tropical environments may undergo multiple spawning events and experience partial sperm depletion across successive cycles. This suggests that "empty" spermathecae in mature females do not necessarily indicate reproductive senescence but rather reflect active sperm utilization followed by opportunities for remating, consistent with a multi-mating strategy. From a management perspective, this finding underscores the critical importance of maintaining adequate male abundance and size structure within the population to ensure sufficient mating opportunities and effective sperm transfer. Implementing measures such as size-based harvest restrictions, seasonal closures during peak mating periods, and the protection of berried females could be instrumental in safeguarding the reproductive capacity of the stock. By linking these biological patterns to management strategies, the present study contributes to the understanding of how reproductive processes directly influence the sustainability of the blue swimming crab fishery in South Sulawesi.

Conclusions

This study offers novel insights into the reproductive biology of *Portunus pelagicus*, with a particular focus on understanding sperm storage dynamics across ovarian maturation stages (OMS). Spermathecae fullness diminished as ovarian development progressed, indicating a gradual use of sperm for fertilization during multiple spawning cycles. Some females at early OMS also had empty spermathecae, suggesting the potential for sperm limitation due to intensive male-biased fisheries. Our findings propose a model wherein two distinct pathways contribute to sperm depletion in the Pare-Pare Bay population: 1) a pre-maturation

pathway, potentially involving post-spawn ovarian reversion, which affects a subset of the population before the commencement of a new cycle, and 2) a maturation-dependent pathway where sperm is utilized for fertilization. This dual-pathway model reveals a more intricate reproductive dynamic than previously assumed and emphasizes the potential for sperm limitation to regulate population productivity. While this research advances the understanding of the reproductive strategies of *P. pelagicus*, its six-month duration constrains the ability to capture the complete annual reproductive cycle. Consequently, future studies conducted throughout the year are necessary to validate the proposed model and to offer a more comprehensive perspective on reproductive timing and sperm storage dynamics. These findings contribute to the broader context of tropical fisheries management, underscoring the importance of maintaining reproductive balance and sustainability within blue swimming crab populations.

CRediT authorship contribution statement

Ananda Adya: Writing – original draft, Investigation, Formal analysis, Data curation. **Yushinta Fujaya:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Sharifuddin Bin Andy Omar:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Siti Aslamyah:** Writing – review & editing, Supervision. **Andi Aliah Hidayani:** Writing – review & editing, Supervision. **Dwi Kesuma Sari:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Tutik Wresdiyati:** Writing – review & editing, Visualization, Validation, Supervision, Methodology. **Matthew B. Ogburn:** Writing – review & editing, Validation, Resources, Formal analysis. **Khor Waiho:** Writing – review & editing, Validation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alberts-Hubatsch, H., Lee, S. Y., Meynecke, J. O., Diele, K., Nordhaus, I., & Wolff, M. (2016). Life-history, movement, and habitat use of *Scylla serrata* (Decapoda, Portunidae): Current knowledge and future challenges. *Hydrobiologia, 763*(1), 5–21. https://doi.org/10.1007/s10750-015-2393-z

Assugeni, C. O., Toyama, M. H., & Zara, F. J. (2021). From sperm plug formation to ovulation: Morphological and ultrastructural modifications in the seminal receptacle of the blue crab *Callinectes danae*. *Zoologischer Anzeiger, 291*, 45–60. https://doi.org/10.1016/j.jcz.2021.02.002

Bawab, F. M., & El-Sherief, S. S. (1988). Stages of the reproductive cycle of the female crab *Portunus pelagicus* (L., 1758) based on the anatomical changes of the spermatheca (Decapoda Brachyura, Portunidae). *Crustaceana, 54*(2), 139–148. https://doi.org/10.1163/156854088X00041

Becker, C., Brandis, D., & Storch, V. (2011). Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Pinnotheridae). *Journal of Morphology, 272*(1), 12–26. https://doi.org/10.1002/jmor.10884

Castilho-westphal, G. G., Ostrensky, A., Pie, M. R., & Boeger, W. A. (2013). Morphology of the female reproductive system and reproductive cycle of the mangrove land crab *Ucides cordatus* (L.) in the Baı'a de Antonina, Parana', Brazil. *Acta Zoologica, 94*(1), 86–93. https://doi.org/10.1111/azo.12000

Elner, R. W., & Beninger, P. G. (1995). Multiple reproductive strategies in snow crab, *Chionoecetes opilio*: Physiological pathways and behavioral plasticity. *Journal of Experimental Marine Biology and Ecology, 193*, 93–112. https://doi.org/10.1016/0022-0981%2895%2900112-3

Ernawati, T., Boer, M., & Yonvitner, Y. (2014). Population biology of blue swimming crab (*Portunus pelagicus*) in surrounding Pati waters, Central Java. *BAWAL, 6*(1), 31–40. https://doi.org/10.15578/bawal.6.1.2014.31-40

Farias, N. E., Spivak, E. D., & Luppi, T. A. (2017). Functional morphology of the female reproductive system of a crab with highly extensible seminal receptacles and extreme sperm storage capacity. *Journal of Morphology, 278*(7), 1–17. https://doi.org/10.1002/jmor.20685

Fujaya, Y., Hidayani, A. A., Umar, M. T., Matthew, B., Ogburn, M. B., Aguilar, R., & Legett, H. D. (2025). Blue swimming crab *Portunus pelagicus*, Linnaeus, 1758 in pare Pare Bay, South Sulawesi, Indonesia: Study of reproduction and breeding potential. *Egyptian Journal of Aquatic Research, 51*, 578–584. https://doi.org/10.1016/j.ejar.2025.08.007

Hines, A. H., Jivoff, P. R., Bushmann, P. J., Van Montfrans, J., Reed, S. A., Wolcott, D. L., & Wolcott, T. G. (2003). Evidence for sperm limitation in the blue crab, *Callinectes sapidus*. *Bulletin of Marine Science, 72*(2), 287–310.

Ikhwanuddin, M., Azra, M. N., Noorulhudha, N. F., Aishah, A. S., & Abol-Munafi, A. B. (2016). Embryonic development and hatching rate of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) under different water salinities. *Turkish Journal of Fisheries and Aquatic Sciences, 16*, 669–677.

Jivoff, P. (2003). A review of male mating success in the blue crab, *Callinectes sapidus*, in reference to the potential for fisheries-induced sperm limitation. *Bulletin of Marine Science, 72*(2), 273–286.

Jivoff, P., Hines, a. H., & Quackenbush, L. S. (2007). Reproduction biology and embryonic development. In V. S. Kennedy, & L. E. Cronin (Eds.), *The Blue Crab: Callinectes Sapidus* (pp. 255–298).

Kendall, M. S., Wolcott, D. L., Wolcott, T. G., & Hines, A. H. (2002). Influence of male size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus*. *Marine Ecology Progress Series, 230*, 235–240. https://doi.org/10.3354/MEPS230235

Kienbaum, K., Scholtz, G., & Becker, C. (2017). The morphology of the male and female reproductive system in two species of spider crabs (Decapoda: Brachyura: Majoidea) and the issue of the velum in majoid reproduction. *Arthropod Systematics & Phylogeny, 75*(2), 245–260. https://doi.org/10.3897/asp.75.e31901

Kiernan, J. A. (1990). *Histological & Histochemical Methods*. Pergamon Press.

Krishnapriya, R., & Padmaja, D. M. (2014). The role of spermatheca in ovarian maturation of the fiddler crab, *Uca tiangularis* bengali (crane, 1975). *International Journal of Scientific and Research Publications, 4*(3), 1–5.

Kumar, S., Zaidi, S. S. A., Gautam, A. K., Dave, L. M., & Saiyed, H. N. (2003). Semen quality and reproductive hormones among welders - A preliminary study. *Environmental Health and Preventive Medicine, 8*(2), 64–67. https://doi.org/10.1007/BF02897929

McLay, C. L., & López Greco, L. S. (2011). A hypothesis about the origin of sperm storage in the Eubrachyura, the effects of seminal receptacle structure on mating strategies and the evolution of crab diversity: How did a race to be first become a race to be last? *Zoologischer Anzeiger, 250*(4), 378–406. https://doi.org/10.1016/j.jcz.2011.06.002

Monteiro, M. F., Lisboa, L. C. O., Carvalho-Costa, T. M., Nevoa, J. C., Oliveira, C. J. F., Serrão, J. E., & Souza, E. A. (2019). Morphology of the spermatheca of *Triatoma lecticularia* (Hemiptera: Reduviidae) (Stal, 1859). *Brazilian Journal of Biology, 79*(1), 144–148. https://doi.org/10.1590/1519-6984.180932

Ogburn, M. B. (2019). The effects of sex-biased fisheries on crustacean sex ratios and reproductive output. *Invertebrate Reproduction and Development, 63*(3), 200–207. https://doi.org/10.1080/07924259.2019.1612787

Ogburn, M. B., Richie, K. D., Jones, M. A., & Hines, A. H. (2019). Sperm acquisition and storage dynamics facilitate sperm limitation in the selectively harvested blue crab *Callinectes sapidus*. *Marine Ecology Progress Series, 629*, 87–101. https://doi.org/10.3354/meps13104

Ogburn, M. B., Roberts, P. M., Richie, K. D., Johnson, E. G., & Hines, A. H. (2014). Temporal and spatial variation in sperm stores in mature female blue crabs *Callinectes sapidus* and potential effects on brood production in Chesapeake Bay. *Marine Ecology Progress Series, 507*, 249–262. https://doi.org/10.3354/meps10869

Oh, S. J., & Hankin, D. G. (2004). The sperm plug is a reliable indicator of mating success in female Dungeness crabs, *Cancer magister*. *Journal of Crustacean Biology, 24*(2), 314–326. https://doi.org/10.1651/C-2389

Pardo, L. M., Ceroni, C., Riveros, M. P., Ernst, B., & Pino, J. (2017). Morphology of seminal receptacle of the harvested golden crab *Chaceon chilensis* and its implication in the fertilization process. *Invertebrate Biology, 136*(2), 1–8. https://doi.org/10.1111/ivb.12170

Pardo, L. M., Riveros, M., Fuentes, J. P., & López-Greco, L. (2013). Functional morphology of the seminal receptacle in the crab *Metacarcinus edwardsii*. *Invertebrate Biology, 132*(4), 386–393. https://doi.org/10.1111/ivb.12038

Pretterebner, K., Pardo, L. M., Paschke, K., & Riveros, M. P. (2022). Influence of mating strategies on seminal material investment in crabs. *Scientific Reports, 12*(18376), 1–12. https://doi.org/10.1038/s41598-022-21116-4

Rains, S. A. M., Wilberg, M. J., & Miller, T. J. (2016). Sex ratios and average sperm per female blue crab *Callinectes sapidus* in six tributaries of Chesapeake Bay. *Marine and Coastal Fisheries, 8*(1), 492–501. https://doi.org/10.1080/19425120.2016.1208126

Ravi, R., Manisseri, M. K., & Sanil, N. K. (2012). Ovarian maturation and oogenesis in the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae). *Acta Zoologica, 94*(3), 1–10. https://doi.org/10.1111/j.1463-6395.2011.00555.x

Rodgers, P. J., Reaka, M. L., & Hines, A. H. (2011). A comparative analysis of sperm storage and mating strategies in five species of brachyuran crabs. *Marine Biology, 158*(8), 1733–1742. https://doi.org/10.1007/s00227-011-1687-6

Sant'Anna, B. S., Pinheiro, M. A. A., Mataqueiro, M., & Zara, F. J. (2007). Spermathecae of the mangrove crab *Ucides cordatus*: A histological and histochemical view. *Journal of the Marine Biological Association of the United Kingdom, 87*(4), 903–911. https://doi.org/10.1017/S0025315407056676

Shinozaki-Mendes, R. A., Silva, J. R. F., de Sousa, L. P., & Hazin, F. H. V. (2011). Histochemical study of the ovarian development of the blue land crab *Cardisoma guanhumi* (Crustacea: Gecarcinidae). *Invertebrate Reproduction and Development, 56* (3), 1–9. https://doi.org/10.1080/07924259.2011.587277

Wang, D., Yu, Y., Gao, W., Xiang, Z., Zhao, Z., Fazhan, H., Waiho, K., Ikhwanuddin, M., & Ma, H. (2023). Dynamic changes characteristics of the spermatozoon during the reproductive process of mud crab (*Scylla paramamosain*): From spermatophore formation, transportation to dispersion. *Aquaculture Reports, 33*(101866), 1–16. https://doi.org/10.1016/j.aqrep.2023.101866

Wolcott, D. L., Hopkins, C. W. B., & Wolcott, T. G. (2005). Early events in seminal fluid and sperm storage in the female blue crab *Callinectes sapidus* Rathbun: Effects of male mating history, male size, and season. *Journal of Experimental Marine Biology and Ecology, 319*(1–2), 43–55. https://doi.org/10.1016/j.jembe.2005.01.001

Zairion, Wardiatno, Y., Boer, M., & Fahrudin, A. (2015). Reproductive biology of the blue swimming crab *Portunus pelagicus* (Brachyura: Portunidae) in East Lampung waters, Indonesia: Facundity and reproductive potential. *Tropical Life Sciences Research, 26* (1), 67–85.

Zara, F. J., Pereira, G. R. R., & Sant'anna, B. S. (2014). Morphological changes in the seminal receptacle during ovarian development in the speckled swimming crab *Arenaeus cribrarius*. *Biological Bulletin, 227*(1), 19–32. https://doi.org/10.1086/BBLv227n1p19