

Biological aspects of slipper lobster (*Thenus orientalis*) on West Coast of Sabah, Malaysia

CHUI-FEN TEOH¹, ANNITA SEOK-KIAN YONG¹, YUDI NURUL IHSAN²,
NUR SYAFWANAH BINTI SAHARI¹, ALVIN ALVICIUS¹, IHSAN HANI BINTI RADZI¹,
AUDREY DANING TUZAN^{1,✉}

¹Borneo Marine Research Institute, Universiti Malaysia Sabah, Jl. UMS, Kinabalu 88400, Sabah, Malaysia. Tel.: +60-88-320000, Fax.: +60-88-320261,
✉email: audrey@ums.edu.my

²Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Jl. Raya Bandung-Sumendang KM.21, Jatinangor, Sumedang 45363, West Java, Indonesia

Manuscript received: 4 December 2024. Revision accepted: 18 March 2025.

Abstract. Teoh C-F, Yong AS-K, Ihsan YN, Sahari NSB, Alvicius A, Radzi IHB, Tuzan AD. 2025. Biological aspects of slipper lobster (*Thenus orientalis*) on West Coast of Sabah, Malaysia. *Biodiversitas* 26: 1443-1452. *Thenus orientalis*, a highly sought-after Scyllaridae species, is a promising aquaculture candidate. The acquisition of reliable fishery statistics is critical to reducing overfishing risks and understanding its biological aspects, ensuring long-term viability and sustainable practices. This study analyzed the biological characteristics of sex composition, monthly sex ratio, and size frequency distribution of 922 *T. orientalis* (473 males and 449 females; 47.24-99.02 mm Carapace Length; CL) collected at bimonthly intervals of 10 months from April 2019 to January 2020 from the West Coast of Sabah, Malaysia and the reproductive biology of ovarian maturation stages, Gonadosomatic Index (GSI) and size at first maturity of 179 females (46.24-82.66 mm CL), has practical implications for aquaculture. The overall sex ratio was found to be 1:0.95 (χ^2 : 0.54, $P=0.46$), with the highest abundance of males sampled between November and December. *Thenus orientalis* is distributed with an average size of approximately 64.14 mm CL, with males dominating smaller size classes (<65 mm CL) and females dominating the larger size classes. The ovarian development of *T. orientalis* was observed to progress through five stages: immature, early maturing, late maturing, mature, and spent, correlating with changes in the GSI ranging from 0.1256 (immature) to 2.8942 (mature). The peak of GSI coincided with lobster maturity and was accompanied by conspicuous color changes from transparent immature to dark orange mature ovaries. The monthly distribution of the different maturity stages of ovaries and ovigerous females indicated the spawning season of *T. orientalis* is definitely between October and May, with a major peak occurring in January and a minor peak in May. Female *T. orientalis* was found to reach Sexual Maturity (SM_{y-50}) at 51.9 cm CL. This understanding of the reproductive biology of *T. orientalis* has practical implications for sustainable aquaculture, enhancing breeding programs and hatchery productivity and contributing to ecological studies by evaluating population resilience and environmental impact on reproduction.

Keywords: Ovarian development, reproductive biology, slipper lobster, *Thenus orientalis*, West Coast of Sabah

INTRODUCTION

Thenus orientalis (Lund, 1793) has become a very valuable species in the fisheries sector due mainly to its abundance in natural habitats and increasing market demand (Mirzaei et al. 2023). This species, distributed in the Indo-West Pacific region (Saher et al. 2018; Rabaoui et al. 2022) and frequently found on soft sand or muddy substrates (Radhakrishnan et al. 2019), can reach a maximum body length of 25 cm (Kizhakudan 2017). Hatchery-raised *T. unimaculatus* (Burton & Davie, 2007) becomes sexually mature 340 days after hatching (Radhakrishnan et al. 2019). Slipper lobster is a non-aggressive species and tends to be gregarious (Alborés et al. 2019; Lavalli et al. 2019). Compared to spiny lobsters, slipper lobsters have a shorter larval stage (Mirzaei et al. 2023) but lower market value, with live *Thenus* sp. priced at USD 30/kg compared to USD 89/kg for live *Panulirus* sp. (Teoh et al. 2023). Despite this, the abundance of this species in the wild, combined with increasing demand, makes it a valuable target in the fisheries sector throughout

its range (Mirzaei et al. 2023). As a significant fisheries resource, *T. orientalis* is ranked alongside the spiny lobster in export value (Mirzaei et al. 2023), making it a promising candidate for aquaculture in Malaysia and a source of pride for its significant economic contribution.

Often a bycatch in other fisheries (Gordula et al. 2022), slipper lobsters contribute to many of the demersal fisheries that operate along tropical coasts (Duarte et al. 2010). Continuous fishing of high-demand species, however, could jeopardize the sustainability of its resources (Taridala et al. 2019; Anna et al. 2020; Arieta et al. 2022; Mirzaei et al. 2023). In Malaysia, slipper lobsters are caught by artisanal fisheries, mostly in coastal waters, with wild catch production projected to decline by 49% from 2013 to 2023 (Department of Fisheries Malaysia 2013). Sabah contributed approximately 26% of the total landings of slipper lobster in Malaysia in 2013. However, in 2023, Sabah became the major contributor to wild catch production as total landings had increased by more than threefold (42%) (Department of Fisheries Malaysia 2013). Given the rising exploitation, reliable fisheries statistics

and stock assessments are essential for effective fisheries management to avoid overfishing (Wahle et al. 2020). Sustainable fisheries management depends on understanding the biological aspects of the species, including its reproductive biology, to inform and guide appropriate management measures (Kahar et al. 2022), which is essential for the establishment of sustainable fisheries and the conservation of current populations of slipper lobster species and their habitats (Alborés et al. 2019). Immediate action must be taken to restore populations or at least halt their decline when these are overfished and declining (Alborés et al. 2019).

Understanding the reproductive biology of *T. orientalis* is critical for effective brood-stock management and aquaculture development (Bertini and Baeza 2014; Kahar et al. 2022). While wild-caught brood-stock is commonly used in aquaculture, there is little information on the ecological and social factors that lead to spawning (Berlinsky et al. 2020). Studies on the reproductive biology of the slipper lobster have been conducted in various regions such as India (Kagwade and Kabli 1996; Kizhakudan et al. 2019), Indonesia (Tirtadanu et al. 2016), and Pakistan (Saher et al. 2018). Despite the growing interest in this species, biological information on *T. orientalis* in Malaysia remains scarce. Our study represents the first attempt to determine the population structure and female reproductive biology of *T. orientalis* on the West Coast of Sabah since no previous research had been published on this species. This current study provides information on the biological characteristics and reproductive biology of *T. orientalis*, which would be useful for the development of effective management and future conservation strategies for its specific habitat and the development of breeding populations of slipper lobsters in Malaysia. Distribution of size and sex composition, breeding season, ovarian development, and maturation stages of *T. orientalis* were studied throughout the sampling period.

MATERIALS AND METHODS

Sample collection

Thenus orientalis from the West Coast of Sabah, Malaysia were sourced from local fishermen at Sabah Fish Marketing Sdn. Bhd. (SAFMA) market in Kota Kinabalu, Sabah. Sampling was performed at bimonthly intervals of 10 months from April 2019 to January 2020 to assess reproductive patterns across different months. This sampling period provided insights into reproductive cycles, including potential reproductive peaks and fluctuations in reproductive activity over time. No samples were collected in February 2020 due to unavailability. In March 2020, sampling was halted due to the COVID-19-induced Movement Control Order (MCO) in Malaysia. All procedures for handling experimental animals were approved by Universiti Malaysia Sabah Animal Ethics Committee (AEC008/2022) while research permits to conduct research in Sabah [JKM/MBS.1000-2/2 JLD.13 (96)] were granted by the Sabah Biodiversity Council.

Relationship between sex ratio and size-frequency distribution

A total of 922 dead *T. orientalis* (473 males and 449 females) with a diverse size range (47.24-99.02 mm Carapace Length; CL) were randomly selected to analyze reproductive cycles and breeding patterns. The CL of each lobster was measured using a vernier caliper with 0.01mm accuracy based on the morphometric analysis proposed by Burton and Davie (2007). CL is defined as the distance along the dorsal side of the carapace from the antennal plate sinus base to the posterior edge. Sexing and morphometric measurements were conducted on-site, with specimens randomly selected for examination. The Body Weight (BW) of each specimen was then weighed to the nearest 0.01 g using a digital scale. The lobsters were grouped in a size range of 5.0 mm CL interval to determine the size-frequency distribution. The sex of the lobsters was determined based on the location of the gonopore on the pereopods of *T. orientalis*, with the gonopore of male lobsters located at the base of the fifth pair of pereopods. At the same time, females presented their gonopore at the base of the third pair of pereopods (Kizhakudan et al. 2019). Ovigerous female lobsters sampled throughout the sampling period were also recorded by observing the presence of egg capsules attached to the ovigerous setae (Kizhakudan et al. 2019).

Determination of ovarian maturation

At the end of each sampling, up to ten female lobsters were randomly selected and transported to the Borneo Marine Research Institute (BMRI) laboratory at Universiti Malaysia Sabah. A total of 179 live female *T. orientalis* (46.24-82.66 mm CL) were transported alive in well-aerated seawater and immediately brought to the BMRI laboratory to study ovarian development and size at the time of sexual maturity based on external morphological and histological observations. Before collecting the ovaries, the lobsters were anesthetized by immersing them in ice water for 30 minutes prior to dissection. The specimens were dissected immediately on the same day. CL and BW of each specimen were measured and recorded. The carapace was dissected, and the color and appearance of the exposed ovaries were observed and photographed using Huawei P30 Pro. The ovaries were removed from the abdominal cavity and weighed to determine the Gonadosomatic Index (GSI). The developmental stages of the ovaries were categorized based on their morphological appearance and confirmed with standard histological processing of each ovary (Kizhakudan 2014).

Histological analysis

For histological analysis, a small part of the right anterior ovarian lobe was fixed in Davidson's solution (Kellee and Jocelyn 2021) for 24 hours, then transferred to 70% ethanol for preservation. The tissues were progressively dehydrated in increasing concentrations of ethanol (70-100%), cleared with xylene, and embedded in paraffin wax for impregnation. The solidified wax blocks were sectioned into 7 μ m thick ribbons using a rotary microtome (Shandon, model AS325) and mounted on clean

glass slides. These sections were expanded overnight on a slide warmer (Fisher Scientific) at 40–45°C, then subjected to a series of xylene and ethanol treatments (70%, 80%, 90%, 95%, 100%) followed by hematoxylin-eosin staining (Liu et al. 2019). The stained sections were covered with macroscopic coverslips (Matsunami Glass Industries, Ltd., Osaka, Japan) using Canada balsam (Merck, Germany). The developmental stages of the ovaries were observed and analyzed at 10x magnification using a light microscope (Leica, Germany). The diameter of 20 oocytes per sample ($n: 3$) was measured using Image J image processing software.

Data collection and statistical analysis

Differences in the overall sex ratio between males and females from the first batch of sampling were analyzed using the chi-square test (χ^2) (Alborés et al. 2019), while seasonal variations in the sex ratio in the sampling periods were analyzed using ANOVA. Significant differences were assumed when $P < 0.05$ using IBM SPSS Statistics version 26. The Gonadosomatic Index (GSI) was expressed as mean values \pm standard deviation. The GSI of each lobster was adopted from Kizhakudan (2014) and calculated using the following formulae: $GSI: (GW \times 10^5) / CL^3$ with GW being the weight of the gonad. Differences in GSI of the individual ovarian maturation stages were analyzed using the non-parametric Kruskal-Wallis test. All pairwise comparisons were performed whenever significant differences ($P < 0.05$) were detected.

The maturation stages of *T. orientalis* ovaries were assessed following the five classifications of Kizhakudan (2014) with slight modifications as described in Table 1. The size at sexual maturity of *T. orientalis* ($n: 179$) in the population was ascertained when 50% of the females were Sexually Mature (SM_y-50) (Atherley et al. 2021). The ratio of sexually mature lobsters in each size class was then fitted to the sigmoid curve using the formulae $P_{CL}: 1/(1+e^{-(a+bxCL)})$ with P being the ratio of mature to immature lobsters, assigned to the size classes in the 5 mm CL interval, and the intercept a and the slope b as parameters estimated using the logistic regression equation in the statistical software RStudio (function ‘glm,’ version 2023.09.1+494). In R, immature females were labeled “0,” and mature females (early developing, late developing, mature, and spent) were labeled “1”. Maturation stages of the ovaries of *T. orientalis* were assessed following the classifications adopted by Kizhakudan (2014) albeit with slight modifications. Comparison of mean body weight and CL between males ($n: 473$) and females ($n: 449$) was calculated using the Mann-Whitney U test.

RESULTS AND DISCUSSION

Results

Sex ratio, sex composition, and size-frequency distribution

Throughout the 10-month sampling period, the overall sex ratio of males to females was 1:0.95 ($\chi^2: 0.54$, $P: 0.46$) (Table 1), which was not significantly different from the expected ratio of 1:1, as confirmed by the chi-square test.

However, notable variations in the monthly sex ratio were observed whereby females outnumbered males in April (1:1.07), June (1:1.09) and especially in October (1:1.59). In contrast, more males were caught in July (1:0.6), November (1:0.74) and December (1:0.47). An equal sex ratio (1:1) was observed in May, August, September, and January. Significant differences in the sex ratio were found between December and January ($P: 0.019$) and between October and December ($P: 0.08$), while no significant differences were detected from April to September and in November.

The total population was composed of 51.30% males and 48.70% non-berried females (Figure 1). In addition, 92 berried females (20.49%) were captured during the sampling periods. The highest number of males was captured between November and December, but this abundance decreased from January to October (Figure 2). The frequency distribution of the size of wild male and female lobsters was divided into 5 mm CL intervals (Figure 3). The population showed a normal distribution, with an average size of 64.14 mm CL. A total of 180 (19.53%) male *T. orientalis* dominated the lower size class up to 64.99 mm CL, while 16.05% of the females dominated the higher size class between 65 and 69.99 mm CL.

Table 1. Monthly and overall sex ratio of male to female slipper lobster *Thenus orientalis* during the 10-months sampling period on the West Coast of Sabah, Malaysia ($n: 922$)

Month	Sex ratio
April 2019	1:1.07
May 2019	1:1
June 2019	1:1.09
July 2019	1:0.6
August 2019	1:1
September 2019	1:1
October 2019	1:1.59
November 2019	1:0.74
December 2019	1:0.47
January 2020	1:1
Overall	1:0.95

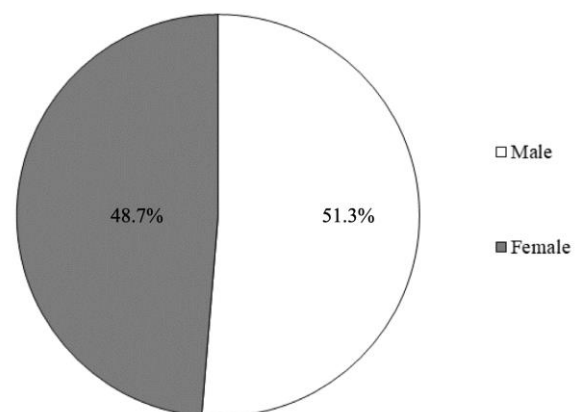


Figure 1. Sex composition of *Thenus orientalis* in Sabah Waters, Malaysia, throughout the 10-month sampling period ($n: 922$)

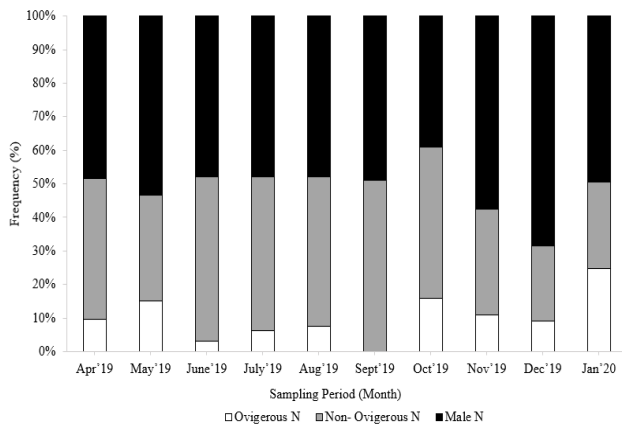


Figure 2. Abundance and sex composition of males and females (ovigerous, non-ovigerous) of *Thenus orientalis* from April 2019 to January 2020 (n: 922)

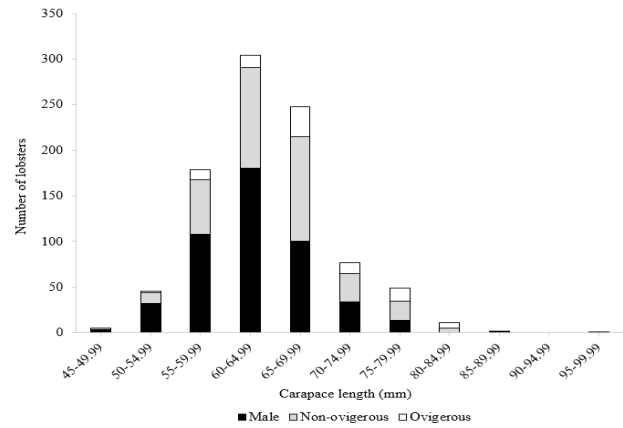


Figure 3. Size-frequency distribution of wild male and female (ovigerous and non-ovigerous) *Thenus orientalis* during the period of April 2019 to January 2020 (n: 922)

Ovarian maturation and Gonadosomatic Index (GSI)

The H-shaped ovaries (Figure 4) of the female *T. orientalis* consisted of a pair of tubular structures connected by a transverse bridge. The third pair of pereopods had two slender oviducts that emerged from the ovary posterior to the transverse ridge and opened via the genital pores on the coxa. In this study, five stages of ovarian development were categorized based on morphological and histological observation of the ovaries. The size of the ovary and GSI (Figure 6) increased simultaneously with changes in the ova throughout the maturation process (Figures 4 and 5, Table 2). The immature ovary (Figure 4.A, Figures 5.A and B) was transparent, thin, elongated and rod-like. The ovary occupied a small part of the cephalothoracic cavity. Histologically, oogonia and pre-vitellogenic oocytes were

observed only at this stage of pre-vitellogenesis; visible nuclei were observed. Immature females (46.24-65.6 mm CL) recorded the lowest mean GSI value (Table 3) of 0.1256, which was significantly lower ($P < 0.05$) than that of the other reproductive stages, with the exception of the early maturing stage. As the early maturing stages of *T. orientalis* progressed, the ovary became creamy white and slightly enlarged (Figures 4.B and 5.C) with a GSI value of 0.2181, which was also significantly lower ($P < 0.05$) than the GSI values of the late maturing and mature females. The ovaries were filled mostly with previtellogenic oocytes. Several larger oocytes started to accumulate yolk droplets around the periphery of the vitellogenic oocytes. At this stage, the cytoplasm was still basophilic and took on a hematoxylin color.

Table 2. Classification and description of the macroscopic and microscopic stages of ovarian development for female *Thenus orientalis*

Stage	Macroscopic appearance	Histological description
I- Immature	Transparent, thin, and elongated rod-like ovaries. The ovary occupies a small part of the cephalothoracic cavity	Only oogonia and pre-vitellogenic oocytes were observed at this pre-vitellogenesis phase. Visible nuclei were observed
II- Early maturing	Creamy white and slightly enlarged ovaries	Ovaries were primarily packed with previtellogenic oocytes. Several larger oocytes began to accumulate yolk droplets around the periphery of vitellogenic oocytes. At this stage, the cytoplasm was still basophilic and assumed a hematoxylin stain
III- Late maturing	Yellowish to light orange ovaries occupied 20-50% of the cavity	More yolk deposition occurred and extended further toward the nucleus in the vitellogenic oocyte. The cytoplasm of the most advanced oocyte in this stage turned slightly into eosinophilic and assumed an eosin stain
IV- Mature	Dark orange ovaries with folds occupied a major part of the cavity	Oocyte reached maximum size and dense yolk granules could be seen. The nuclei of the mature oocyte were shrunken and difficult to observe. Between the mature oocyte and chorion could be identified. The germinal zone of oogonia completely vanished, with only a very few oogonia strands between mature oocytes. Limited numbers of previtellogenic oocytes were also seen
V- Spent	Creamy or yellowish flabby ovaries occupy less than 20% of the cavity	Only immature oocytes could be seen in this stage. Atretic oocytes that were not extruded during spawning could be observed, together with the presence of previtellogenic oocytes and the germinal zone of oogonia

Maturation patterns

The monthly appearance of various maturity stages in commercial catches reflects the breeding season of the animal. In this study, 179 females of *T. orientalis* were examined over 10 months from April 2019 to January 2020. Figure 7 shows the monthly percentage occurrence of different maturity stages during this period. The immature stage was observed throughout the year except in October, with the highest percentage recorded in July (40%). For other months, this stage occurred in lower percentages of between 0% and 20%. The early maturing stage peaked in October at 45%, while the late maturing stage was less prominent, with high percentages recorded in May (40%), June (30%) and September (30%). The mature stage was most abundant between November and January (60-70%), whereas spent females occurred throughout the year, with higher percentages observed in July and August (20%).

Based on the 10-month sampling from April 2019 to January 2020, berried females occurred throughout the year except in September, with the highest number observed in January (50%). Between June and August, the percentage of berried females was very low (6-15%). The smallest berried female recorded in this study had a size of 54.22 mm CL. Berried individuals were rarely observed in October, with a peak in January. The highest number of ovigerous females was observed in January. Notably, no ovigerous females were observed in September 2019. The monthly distribution of maturing stages and berried conditions (Figures 7 and 8) showed that maturation in *T. orientalis* began in June. The maturation process progressed from the immature stage through early and late maturity stages to full maturity, starting in September and peaking between November and January.

Estimation of size at 50% maturity

The size of the first sexual maturity of female *T. orientalis* (Table 4, Figure 9) was determined on the basis of a combination of macroscopic appearance (color) and histological analysis of the examined ovaries. In this study,

the smallest mature female (stage II to V) measured 50.34 mm CL, while the largest mature female was 82.66 mm CL. In contrast, the smallest immature female measured 48.32 mm CL, while the largest immature female was 65.6 mm CL. The highest frequency and the frequency of sexual maturity of the studied females were observed in the CL size range of 63.32-68.31 mm CW with 26.26% and 29.30%, respectively. Logistic curve fitting of the CL data resulted in an SM₅₀ value of P_{CL} 51.9 mm (Figure 9); this indicates that 50% of female *T. orientalis* on the West Coast of Sabah reached sexual maturity at 51.9 mm CL.

Table 3. Comparison of Gonadosomatic Index (GSI; mean±SD and 95% confidence interval) of female *Thenus orientalis* (n: 179) from April 2019 to January 2020

Stage	n	Size range (mm CL)	Body weight (g)	GSI
I	22	48.32-65.6	64.56-137.54	0.1256±0.03 ^a
II	33	51.94-64.28	82.92-159.79	0.2181±0.07 ^{ab}
III	25	50.34-78.84	74.83-280.45	0.6384±0.22 ^c
IV	68	50.5-82.66	74.89-324.32	2.8942±1.00 ^d
V	31	52.26-75.12	83.42-244.51	0.3612±0.14 ^{bc}

Table 4. Carapace length (mm) frequency, percentage of frequency, maturity frequency, and percentage of maturity for mature female *Thenus orientalis* categorized by ovarian maturation stages

Size range (mm CL)	Frequency (n)	Frequency (%)	Maturity frequency	Maturity (%)
48.32-53.31	24	13.41	9	5.73
53.32-58.31	35	19.55	31	19.75
58.32-63.31	32	17.88	30	19.11
63.32-68.31	47	26.26	46	29.30
68.32-73.31	30	16.76	30	19.11
73.32-78.31	8	4.47	8	5.10
78.32-83.31	3	1.68	3	1.91
Total	179	100	157	100

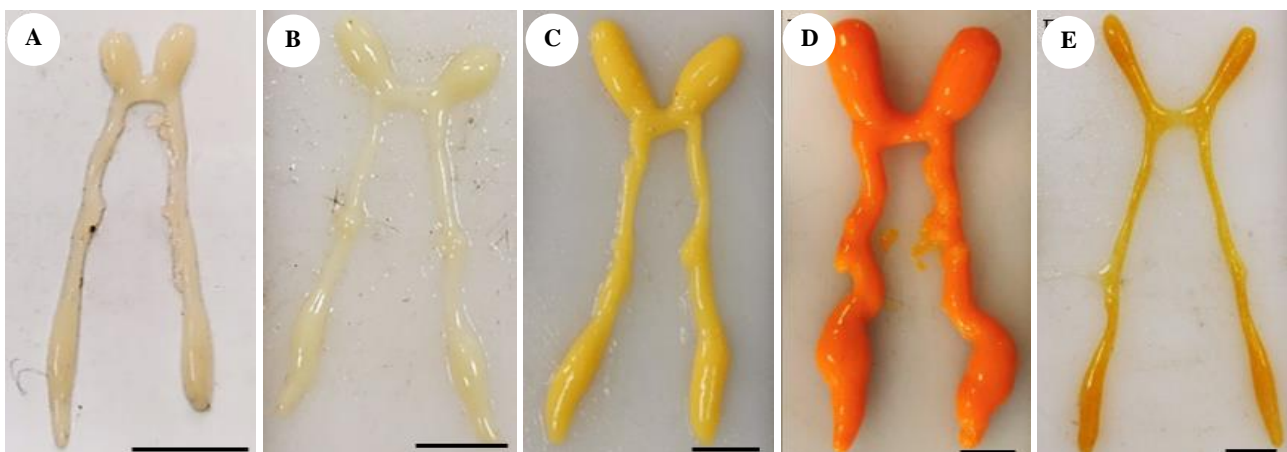


Figure 4. Macroscopic appearance of ovarian development in *Thenus orientalis*. A. Immature; B. Early maturing; C. Late maturing; D. Mature; E. Spent. Scale bar: 10 mm

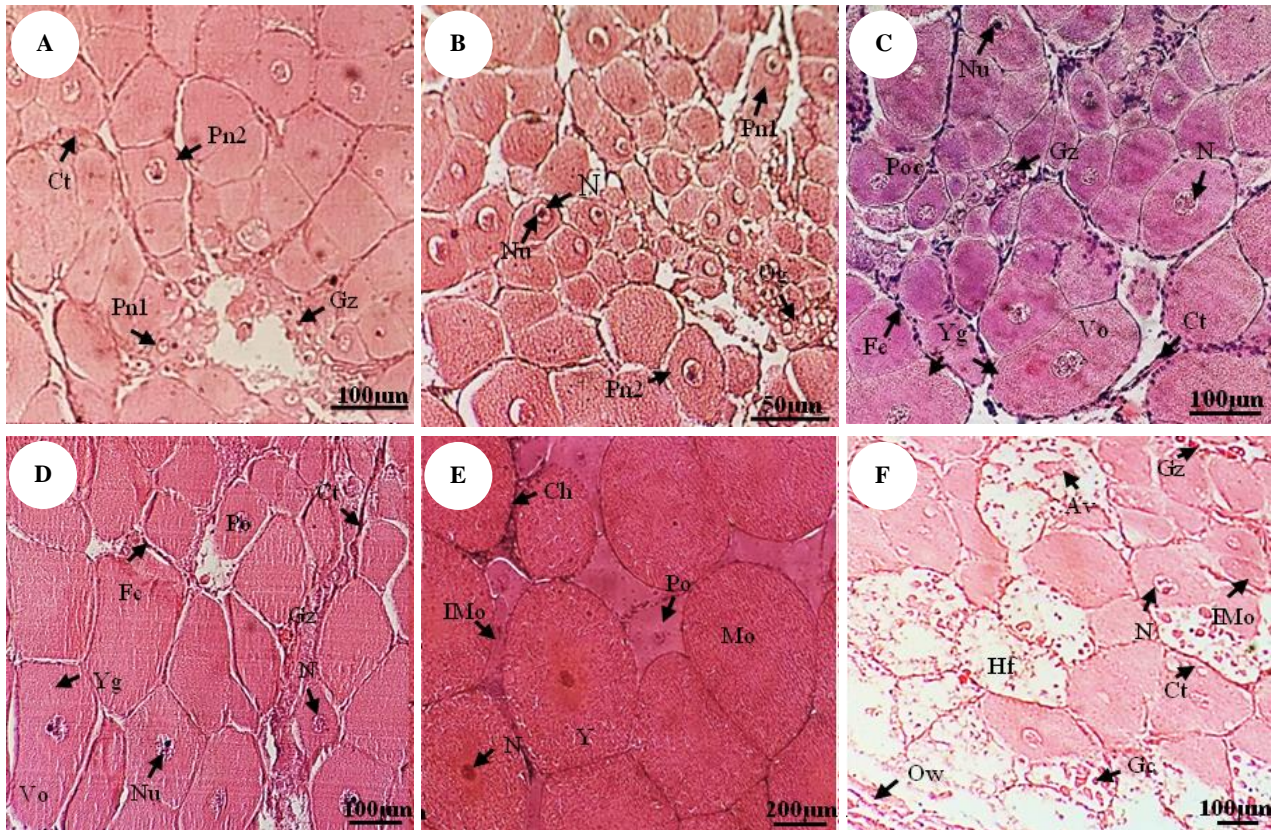


Figure 5. Histological oogenesis development from a cross-section of wild *Thenus orientalis*. A,B. Stage I (pre-vitellogenesis) ovary with a germinal zone of oogonia, early perinucleolus oocytes, and late perinucleolus oocytes; C. Stage II (primary vitellogenesis) ovary with the onset of the deposition of yolk globules, vitellogenic oocytes, and previtellogenic oocytes; D. Stage III (Secondary vitellogenesis) ovary with more yolk deposition observed in vitellogenic oocytes; E. Stage IV (mature) oocyte fully packed with yolk, the masked nucleus was hardly distinguishable, and chorion could be seen; F. Stage V (spent) ovary consisted of atretic oocytes or empty hyaline follicles. Ao: Atretic ova; Ch: Chorion; Ct: Connective tissues; Fc: Follicle cell; Gc: Granulosa cells; Gz: Germinal zone of oogonia; Hf: Hyaline follicle; IMo: Immature oocyte; Mo: Maturing oocyte; N: Nucleus; Nu: Nucleolus; Og: Oogonia; Pn1: Early perinucleolus oocytes; Pn2: Late perinucleolus oocytes; Po: Previtellogenic oocyte; Vo: Vitellogenic oocyte; Ow: Ovarian wall; Y: Yolk; Yg: Yolk droplets

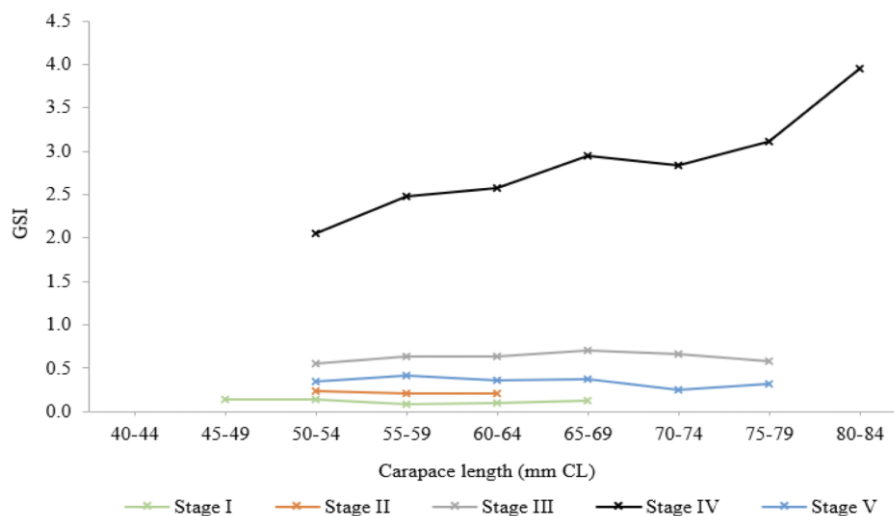


Figure 6. The Gonadosomatic Index (GSI) of each maturity stage in female *Thenus orientalis* is classified based on the carapace length (mm) (*n*: 179)

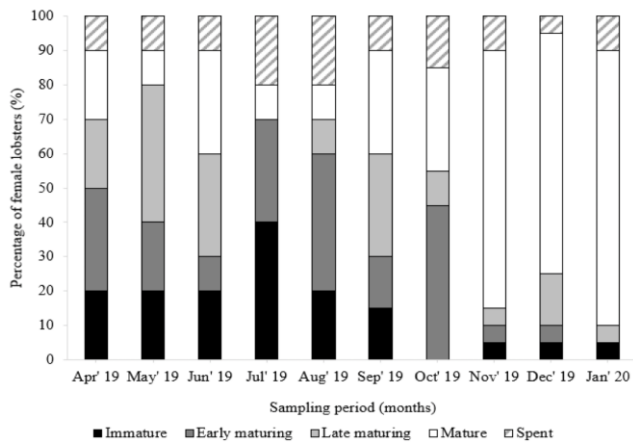


Figure 7. Monthly characterization of ovarian maturation stages (immature, early maturing, late maturing, mature, and spent) in female *Thenus orientalis* (n : 179) sampled from April 2019 to January 2020

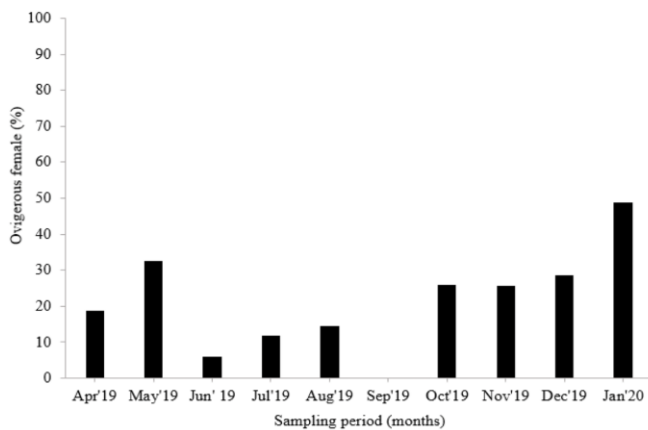


Figure 8. Percentage of ovigerous females *Thenus orientalis* (n : 92) observed on the West Coast of Sabah, Malaysia, from April 2019 to January 2020

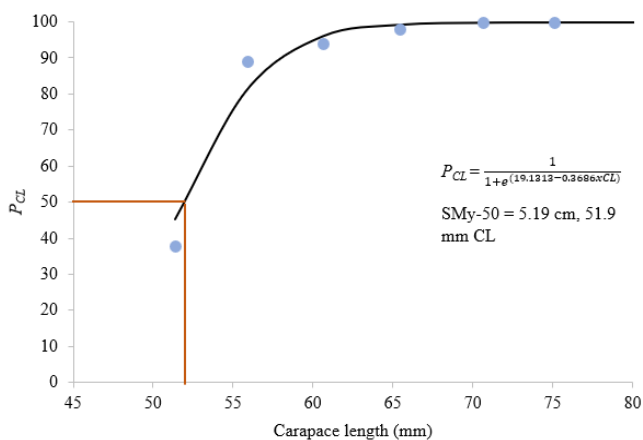


Figure 9. The sigmoid curve for the estimation of size at sexual maturity in female *Thenus orientalis* derived from physiological maturity indices through microscopic examination of ovaries. The analogous perpendicular lines are 50% of the sexually matured lobsters where SMY-50: 51.9 mm CL

Discussion

Sex ratio, sex composition, and size-frequency distribution

This current study is the first to report on the biological aspect of *T. orientalis* in Malaysia. The population dynamics of *T. orientalis* demonstrated a non-uniform pattern of monthly sex distribution, in agreement with Kagwade and Kabli (1996). The overall sex ratio (1:0.95) did not deviate significantly from the expected ratio of 1:1, suggesting a balanced sex ratio in the population distribution of *T. orientalis* on the West Coast of Sabah. A similar observation was reported in *Scyllarides nodifer* (Stimpson, 1866) on the Northeastern Gulf of Mexico (Hardwick and Cline 1990). However, notable differences were identified between December and January and between October and December. Previous studies suggested these differences to be due to factors such as migration patterns and spawning seasons (Hardwick and Cline 1990; Kailola et al. 1993; Kizhakudan et al. 2019). In this study, the variation in sex ratio was associated with the spawning season, suggesting a complex interaction between environmental factors and reproductive biology. Figures 7 and 8 show the monthly distribution of different maturity stages of ovaries and ovigerous females, indicating a specific spawning season, with a high percentage of mature specimens recorded between September and January and berried females between October and January. Kagwade and Kabli (1996) reported two peaks of spawning activity in *T. orientalis* between October and March; the increased abundance of males from November to December and May in our study supports this assessment, providing a reassuring continuity with previous research.

The high number of spent individuals forming a peak in February and fertilized individuals in January suggests the occurrence of a large peak in January and a minor peak in May. In comparison, the spawning period of *T. orientalis* may definitely be between October and May, with the major peak occurring in January and the minor in May. The females of *Thenus* spp. usually spawn twice, with the possibility of additional occurrences from August to September and January to February (Kailola et al. 1993). The peak spawning period determined in this study was almost identical to the results of Branford (1980) and Kagwade and Kabli (1996). In the Bombay waters, *T. orientalis* spawned between September and April, with two prominent spawning peaks in November and March (Kagwade and Kabli 1996). However, in the Tokar Delta, Sudan, *T. orientalis* was found to spawn between February and March (Branford 1980). These findings suggest *T. orientalis* as exhibiting comparable spawning patterns across different locations, although differences in peak periods have been reported.

The monthly variations in the sex ratio could also be related to local migrations. Although *T. orientalis* is found in sandy, coarse substrate with mud at depths between 8 and 100 m, it is usually caught between 10 and 50 m water depth (Kizhakudan 2017). In addition, Kizhakudan et al. (2004) observed mass movements of juvenile and subadult *T. orientalis* to shallow grounds with an abundance of *Babylonia* spp. This indicates the *Thenus* spp. preference to

migrate to deeper waters as they mature and grow in size (Kizhakudan et al. 2004). Scyllarid lobsters pass through a series of developmental stages during their life cycle and inhabit different ecological habitats (Radhakrishnan et al. 2019). Berried, mature, and spent females have been found to constitute the majority of coastal migratory populations, with very few active males (Kizhakudan et al. 2019). Differences in spawning time may vary due to seasonal variations and water temperature. Hardwick and Cline (1990) hypothesized adult slipper lobsters migrate to warmer and shallower waters during the breeding season. In Northern Australia, Jones (2007) reported *T. orientalis* as having its peak spawning season in September, i.e. in spring, and a lower spawning season in midsummer, i.e. in January. Kailola et al. (1993) also observed a peak in spawning activity during the spring season.

Ovarian development stages

There is little literature on the reproductive biology of *Thenus* species. The five stages of ovarian development in *T. orientalis* on the West Coast of Sabah were comparable to the results of earlier studies by Kagwade and Kabli (1996). The ovary undergoes a series of color and size changes after the maturation cycle, consistent with the findings of Kagwade and Kabli (1996), Kizhakudan (2014), and Tirtadanu et al. (2016). Branford (1980) documented two stages, namely immature and mature stages. This change results from the accumulation of yolk in the oocytes (Ikhwanuddin et al. 2014). In addition, histological changes in the ovaries during development through several stages of vitellogenesis are consistent with the description of *T. unimaculatus* (Kizhakudan 2014). There was a pre-vitellogenesis, a primary vitellogenesis, and a secondary vitellogenesis until complete maturation of the oocyte (Ikhwanuddin et al. 2014; Kizhakudan 2014; Rodríguez-García et al. 2015).

Gonadosomatic Index (GSI) and size at sexual maturity

The Gonadosomatic Index (GSI) provides useful information about ovarian cycles and offers comprehensive details from morphological, anatomical, and histological studies (Kizhakudan 2014). Kizhakudan (2014) showed a strong correlation between changes in GSI and ovarian development, especially in the lower-size classes. In addition, GSI was found to closely reflect the histological changes that occurred in response to gonadal maturation in *P. argus* (Atherley et al. 2021). As observed in *Panulirus* species, GSI values were also useful in distinguishing ovarian maturation (Kumar et al. 2018). Compared to other stages, the mature females in this current study had the highest GSI, consistent with findings of studies on other *Thenus* and *Panulirus* species (Kizhakudan 2014; Yusnaini et al. 2020; Atherley et al. 2021). The size range of immature (Stage 1), early developing (Stage 2), and 50% of females with mature (Stage V) ovaries in this study was the same as *T. unimaculatus* (Kizhakudan 2014). However, the smallest mature female with a CL of 50.5 mm was smaller than the mature *T. unimaculatus* reported from India (Kizhakudan 2014).

Apart from possible geographical variations, the difference in maturity size between the current study and the existing literature could be influenced by environmental parameters such as water temperature (Johnston and Yeoh 2021). Johnston and Yeoh (2021) demonstrated that the size at maturity of *Portunus armatus* (A.Milne-Edwards, 1861) varied spatially and temporally along the West Coast of Australia, with water temperature and population density playing crucial roles. Similarly, variations in the size of sexual maturity observed in *T. orientalis* could be associated with environmental changes, but more research is needed to validate these impacts in Sabah Waters. Although continuous overfishing of larger adult *Homarus americanus* (H.Milne Edwards, 1837) has been associated with reaching sexual maturity at a smaller size (Le Bris et al. 2017; Haarr et al. 2018), the specific explanation for sexual maturity at a smaller size in this current study cannot be proven due to the small sample size, especially at Stages III, IV and V. Furthermore, no literature on Sabah Waters provides comparative perspectives on potential environmental or anthropogenic influences on *T. orientalis*. Oocyte diameter was found to be closely correlated with gonad maturity. These results are consistent with studies on other crustacean species such as *Scylla* sp. (Ikhwanuddin et al. 2014; Kahar et al. 2022), *Panulirus* sp., and *Homarus* sp. (Erkan and Ayun 2014; Comeau and Benhalima 2018). The mean oocyte size increased steadily with the progression of the maturity stage until the onset of spawning (Comeau and Benhalima 2018).

Size at first maturity, which represents the minimum size at which 50% of the population has reached a state of advanced maturity, is an important stock assessment tool (Kizhakudan 2014; Soares et al. 2020). The size of sexual maturity of female *T. orientalis* from the current study (SM_y-50: 51.9 mm CL) differed from the result reported by Kizhakudan (2014), whereby the size at first sexual maturity was reached at 61-65 mm CL in females of *T. unimaculatus*. In addition to the differences between species, dissimilar geographical locations and periods may also be responsible for the changes in size at sexual maturity (Kizhakudan 2014). The population distribution of *T. orientalis* observed during the sampling periods in this study showed a prevalence of males in the smaller size class (45-64.99 mm CL) while females were predominant in the larger size class (65-84.99 mm CL). Males of *T. orientalis* usually reach sexual maturity earlier than females, resulting in smaller males mating with larger females, possibly from the same brood (Kizhakudan et al. 2019).

Conclusively, the sex ratio (1:0.95) of the population distribution of *T. orientalis* on the West Coast of Sabah was dominated by male lobsters (51.30%) in the smaller size class, while females (48.7%) dominated in the larger size class. However, significant differences were observed between December and January and October and December. The spawning season could be the reason for the fluctuating monthly sex ratio in the present study. According to our findings, *T. orientalis* does not spawn throughout the year. Instead, there is a distinct spawning period from October to May, with a notable peak in

January and a minor peak in May. The maturation of an immature ovary in June leads to spawning activity in October. This means that individual females are able to spawn twice a year. The study revealed that the critical first spawning phase of female *T. orientalis* occurs in a size range of 50-69.99 mm CL, which is derived from the maximum proportion of egg-laying females in a size range of 65-69.99 mm CL and size at Sexual Maturity (SM_y-50) of 51.90 mm CL in the population. Macroscopic and histological observation revealed five developmental stages of the ovaries of wild female *T. orientalis*. The ovaries differed macroscopically in size and color at each gonadal maturity, and histologically, three distinct phases (pre-vitellogenesis, primary vitellogenesis, and secondary vitellogenesis) were found to correspond to the ovarian stages. Both morphological and histological analysis indicated that the ovarian maturation stages of *T. orientalis* correlated with increasing GSI levels during maturation development.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Higher Education Malaysia (MOHE) through its Fundamental Research Grant Scheme (FRGS/1/2022/WAB05/UMS/03/2). The authors declare no known competing interests or personal relationships that may have influenced the work in this article.

REFERENCES

- Alborés I, García-Soler C, Fernández L. 2019. Reproductive biology of the slipper lobster *Scyllarus arctus* in Galicia (NW Spain): Implications for fisheries management. *Fish Res* 212: 1-11. DOI: 10.1016/j.fishres.2018.12.001.
- Anna Z, Hindayani P, Suryana AAH, Ihsan YN, Salsabila A. 2020. Sustainability study of scalloped hammerhead shark (*Sphyrna lewini*) in Indramayu Waters. *Sustainability* 12 (4): 10459. DOI: 10.3390/su122410459.
- Arieta S, Gunawan B, Sumadinata RWS, Ihsan YN. 2022. Calling for institutional arrangements in Napoleon Wrasse conservation and management in Indonesia: The Anambas Islands case. *Mar Policy* 145: 105258. DOI: 10.1016/j.marpol.2022.105258.
- Atherley NAM, Dennis MM, Behringer DC, Freeman MA. 2021. Size at sexual maturity and seasonal reproductive activity of the Caribbean spiny lobster *Panulirus argus*. *Mar Ecol Prog Ser* 671: 129-145. DOI: 10.3354/meps13762.
- Berlinsky DL, Kenter LW, Reading BJ, Goetz FW. 2020. Regulating reproductive cycles for captive spawning. *Fish Physiol* 38: 1-52. DOI: 10.1016/bs.fp.2020.09.001.
- Bertini G, Baeza JA. 2014. Fecundity and fertility in a freshwater population of the neotropical amphidromous shrimp *Macrobrachium acanthurus* from the Southeastern Atlantic. *Invertebr Reprod Dev* 58 (3): 207-217. DOI: 10.1080/07924259.2014.894948.
- Branford JR. 1980. Notes on the Scyllarid Lobster *Thenus orientalis* (Lund, 1793) off the Tokar Delta (Red Sea). *Crustaceana* 38 (2): 221-224. DOI: 10.1163/156854080X00670.
- Burton TE, Davie PJF. 2007. A revision of the shovel-nosed lobsters of the genus *Thenus* (Crustacea: Decapoda: Scyllaridae), with descriptions of three new species. *Zootaxa* 1429 (1): 1-38. DOI: 10.11646/zootaxa.1429.1.1.
- Comeau M, Benhalima K. 2018. Functional anatomy of the female reproductive system of the American lobster (*Homarus americanus*). *J Morphol* 279: 1603-1614. DOI: 10.1002/jmor.20889.
- Department of Fisheries Malaysia. 2013. Annual Fisheries Statistics. <https://www.DepartmentofFisheriesMalaysia.gov.my/en/resources/fisheries-statistics-i/>.
- Duarte LFA, Severino-Rodrigues E, Gasalla MA. 2010. Slipper lobster (Crustacea, Decapoda, Scyllaridae) fisheries off the Southeastern Coast of Brazil: Exploitation patterns between 23°00' and 29°65'S. *Fish Res* 102: 141-151. DOI: 10.1016/j.fishres.2009.11.004.
- Erkan M, Ayun YT. 2014. Morphological and histochemical examination of male and female gonads in *Homarus gammarus* (L. 1758). *Central Eur J Biol* 9 (1): 37-48. DOI: 10.2478/s11535-013-0148-7.
- Gordula RP, Lamoso FB, Favor CC. 2022. Socio-economic assessment of the slipper lobster (*Thenus orientalis*, Lund, 1793) fishery in Ragay Gulf. *J Ecosyst Sci Eco-Gov* 4: 33-37. DOI: 10.54610/jeseg/4.Special_Issue.2022.004.
- Haarr ML, Sainte-Marie B, Comeau M, Tremblay MJ, Rochette R. 2018. Female American lobster (*Homarus americanus*) size-at-maturity declined in Canada during the 20th and early 21st Century. *Can J Fish Aquat Sci* 60: 0434. DOI: 10.1139/cjfas-2016-0434.
- Hardwick Jr CW, Cline GB. 1990. Reproductive status, sex ratios and morphometrics of the slipper lobster *Scyllarides nodifer* (Stimpson) (Decapoda: Scyllaridae) in the Northeastern Gulf of Mexico. *Northeast Gulf Sci* 11 (2): 131-136. DOI: 10.18785/negs.1102.05.
- Ikhwanuddin M, Fatimah SN, Nurul JR, Zakaria MZ, Abol-Munafi AB. 2014. Biological features of mud spiny lobster, *Panulirus polyphagus* (Herbst, 1793) from Johor Coastal Water of Malaysia. *World Appl Sci J* 31 (12): 2079-2086. DOI: 10.5829/idosi.wasj.2014.31.12.69.
- Johnston DJ, Yeoh DE. 2021. Temperature drives spatial and temporal variation in the reproductive biology of the blue swimmer crab *Portunus armatus* A. Milne-Edwards, 1861 (Decapoda: Brachyura: Portunidae). *J Crustacean Biol* 41: ruab032. DOI: 10.1093/jcabi/ruab032.
- Jones CM. 2007. Biology and fishery of the bay lobster, *Thenus orientalis*. In: Lavalli KL, Spanier E. *The Biology and Fisheries of the Slipper Lobster*. CRC Press, Boca Raton. DOI: 10.1201/9781420005165.ch16.
- Kagwade PV, Kabli LM. 1996. Reproductive biology of the sand lobster *Thenus orientalis* (Lund) from Bombay waters. *Indian J Fish* 43 (1): 13-25.
- Kahar NAS, Yong ASK, Sharif MNA, Hussein SMA, Ransangan J. 2022. Reproductive cycle and size at maturity of wild mud crab, *Scylla tranquebarica* (Fabricius, 1798) in Marudu Bay, Sabah. *Borneo J Mar Sci Aquacult* 6 (1): 20-29. DOI: 10.51200/bjomsa.v6i1.1543.
- Kailola PJ, Williams MJ, Stewart PC, Reichelt RE, McNee A, Grieve C. 1993. Australian Fisheries Resources. Bureau of Resource Sciences, Department of Primary Industries and Energy, and Fisheries Research and Development Corporation, Canberra, Australia.
- Kellee RS, Jocelyn SS. 2021. Histological analysis of gonads in zebrafish. In: Dosch R (eds). *Germline Development in the Zebrafish: Methods and Protocols*. Humana Press, New York.
- Kizhakudan JK, Radhakrishnan EV, Lakshmi PS. 2019. Reproductive biology of spiny and slipper lobster. In: Radhakrishnan EV, Phillips BF, Achamveetil G (eds). *Lobsters: Biology, Fisheries and Aquaculture*. Springer, Singapore. DOI: 10.1007/978-981-32-9094-5.
- Kizhakudan JK, Thirumulu P, Manibal C. 2004. Marine Fisheries Information Service. Central Marine Fisheries Research Institute, India.
- Kizhakudan JK. 2014. Reproductive biology of the female shovel-nosed lobster *Thenus unimaculatus* from North-West Coast of India. *Indian J Geo-Mar Sci* 43 (6): 927-935.
- Kizhakudan JK. 2017. Prioritized Species for Mariculture in India. ICAR - Central Marine Fisheries Research Institute, Kochi.
- Kumar PG, Biswas TK, Ghoshal M, Kailasam L, Christina KK, Vijayan. 2018. Current knowledge on the biology, captive breeding and aquaculture of the brackishwater catfish, *Mystus gulio* (Hamilton, 1822): A review. *Aquaculture* 499: 243-250. DOI: 10.1016/j.aquaculture.2018.09.045.
- Lavalli KL, Spanier E, Goldstein JG. 2019. Scyllarid lobster biology and ecology. In: Diarte-Plata G, Escamilla-Montes R (eds). *Crustacea*. Intech Open, London.
- Le Bris A, Pershing AJ, Gaudette J, Pugh TL, Reardon KM. 2017. Multi-scale quantification of the effects of temperature on size at maturity in the American lobster (*Homarus americanus*). *Fish Res* 186: 397-406. DOI: 10.1016/j.fishres.2016.09.008.
- Liu M, Feng Q, Francis DS, Turchini GM, Zeng C, Wu X. 2019. Tamoxifen affects the histology and hepatopancreatic lipid

- metabolism of swimming crab *Portunus trituberculatus*. *Aquat Toxicol* 213: 105220. DOI: 10.1016/j.aquatox.2019.06.003.
- Mirzaei MR, Ajdari A, Woo SP. 2023. Spatial and temporal distribution pattern and biomass trend of flathead slipper lobster, *Thenus orientalis* (Lund, 1793) from Gulf of Oman. *Intl J Environ Res Educ* 3 (1): 27-36. DOI: 10.52547/injoere.3.1.27.
- Rabaoui L, Yacoubi L, Lin YJ, Joydas TV, Qurban MA, Premlal P, Gopalan J, Nazeer Z, Vijayakumaran M, Khan SA, Roa-Ureta RH. 2022. Ecology, life history, and fisheries potential of the flathead lobster (*Thenus orientalis*) in the Arabian Gulf. *Fish Bull* 120: 125-137. DOI: 10.7755/FB.120.2.3.
- Radhakrishnan EV, Phillips BF, Achamveetil G. 2019. *Lobsters: Biology, Fisheries and Aquaculture*. Springer, Singapore. DOI: 10.1007/978-981-32-9094-5.
- Rodríguez-García OU, de Lara VCF, Rodríguez-Jaramillo C, Serviere-Zaragoza E. 2015. Nutritional condition and gonad development of juvenile and subadult California spiny lobster *Panulirus interruptus* in two habitats. *Revista de Biología Marina y Oceanografía* 50 (2): 261-270. DOI: 10.4067/S0718-19572015000300005.
- Saher NU, Naz F, Noor SH, Kamal M. 2018. A new record of shovel-nosed lobster *Thenus unimaculatus* Burton and Davie 2007 (Crustacea: Decapoda: Scyllaridae) from the coastal waters of Pakistan. *Thalassas* 35 (1): 223-228. DOI: 10.1007/s41208-018-0113-y.
- Soares BE, Barros TF, Hashiguti DT, Pereira DC, Ferreira KCF, Caramaschi ÉP. 2020. Traditional approaches to estimate length at first maturity (L50) retrieve better results than alternative ones in a Neotropical heptapterid. *J Fish Biol* 97 (5): 1393-1400. DOI: 10.1111/jfb.14505.
- Taridala SAA, Muhammad ALO, Yusnaini, Asriya. 2019. Income and cost efficiency of lobster farming in Soropia, Southeast Sulawesi, Indonesia. *IOP Conf Ser Earth Environ Sci* 382 (1): 012037. DOI: 10.1088/1755-1315/382/1/012037.
- Teoh CF, Tuzan AD, Yong ASK, Liew KS, Lim LS, Liew HJ. 2023. Evaluation of crystalline amino acids as potent stimulatory chemoattractants for the slipper lobster *Thenus orientalis*. *PeerJ* 11: e15607. DOI: 10.7717/peerj.15607.
- Tirtadanu, Kembaren DD, Suprpto. 2016. Stock density and biological aspect of slippery lobster (*Thenus orientalis*) in the Java Sea. *Bawal* 8 (3): 131-136. DOI: 10.15578/bawal.8.3.2016.131-136.
- Wahle RA, Linnane AJ, Harrington AM. 2020. *Lobster fisheries*. In: Lovrich G, Thiel M (eds). *Fisheries and Aquaculture*. Oxford University Press, United Kingdom. DOI: 10.1093/oso/9780190865627.003.0003.
- Yusnaini Y, Nur I, Idris M, Yasidi F. 2020. Morphology of the female gonads of the long-legged Spiny Lobster. *J Fish Aquat Sci* 15 (1): 1-6. DOI: 10.3923/jfas.2020.1.6.