Reproductive biology of the female shovel-nosed lobster *Thenus unimaculatus* (Burton and Davie, 2007) from north-west coast of India

Joe K. Kizhakudan

Madras Research Centre of Central Marine Fisheries Research Institute
75, Santhome High Road, R.A. Puram, Chennai-600 028, Tamil Nadu, India
[E-mail: jkkizhakudan@gmail.com]

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Reproductive biology of the female shovel-nosed lobster *Thenus unimaculatus* from the north-west coast of India was studied from samples obtained from the fishery along Gujarat coast. Development of secondary sexual characters, gonadal maturation, size at sexual maturity, fecundity and Gonadosomatic Index (GSI) were assessed. Development of ovigerous setae on abdominal pleopods and size of gonopore were identified as secondary sexual characters indicative of the maturation state of the lobster. Onset of maturity in females is marked by the development of ovigerous setae. There are no mating windows (ventral sternite region) in female *T. unimaculatus*. Female reproductive tract in *T. unimaculatus* comprises of ovaries, oviducts and genital opening. Ovarian development in *T. unimaculatus* could be classified into six stages - Immature, Early Maturing, Late Maturing/Mature, Ripe, Spawning and Spent/Recovery. The process of ovarian maturation in *T. unimaculatus* could be traced through three distinct phases depending on the extent of yolk deposition - Pre-vitellogenesis, Primary vitellogenesis and Secondary vitellogenesis. Morphological, physiological and functional maturity is almost simultaneous in *T. unimaculatus*, with the size at first maturity in females attained at 61-65 mm CL. Size at onset of sexual maturity, judged from the 25% success rate in development of different sexual characters is 51-55 mm CL. Average fecundity for reproductively active females in the size range of 60-102 mm CL is 39300 eggs. Ovarian development corresponds well with the changes in GSI, with maximum GSI values coinciding with the occurrence of mature individuals.

[Keywords: *Thenus unimaculatus*, Maturation, Fecundity, Gonadosomatic Index, Size at first Maturity]

**Introduction**

A lot of information on the reproductive biology and behaviour of lobsters, particularly palinurid and homarid lobsters, has been documented over the last five decades. Several studies on the histological structure and development of the gonads have been carried out, particularly in palinurid lobsters 1-6. In comparison with spiny lobsters, there is very little information on the biology and reproductive behaviour of scyllarid lobsters. Fecundity and spawning seasons have been studied in the Mediterranean locust lobster, *Scyllarides latus*7 and the slipper lobster, *S. nodifer*, from the northeastern Gulf of Mexico8,9. Size at first maturity and reproductive biology of *Ibacus peronii* and *Ibacus* sp. from Australian waters10 and fecundity and egg size in *I. peronii*11 have been described. Fecundity, egg size12 and indicators of sexual maturity in *S. squammosus*13 have been studied. While there is some documentation on the reproductive characteristics of *T. orientalis* from Australia14,15, Red Sea16 and Bay of Bengal off Bangladesh17,18, studies from Indian waters are limited. Breeding peaks, fecundity and size at first maturity of *T. orientalis* were studied from the Mumbai coast19 and aspects of egg development and moulting have been described20,21.

The species of shovel-nosed lobsters available in Indian waters has now been confirmed as *Thenus unimaculatus*22 (Burton and Davie, 2007) and earlier records of *T. orientalis* now correspond to *T. unimaculatus*. In the present study, a comprehensive assessment of the reproductive biology of female *T. unimaculatus* has been made by examining the development of secondary sexual characters, size at first maturity, morphological and histological changes...
in gonadal structure, changes in the Gonadosomatic Index (GSI) and fecundity in lobsters collected from the fishery along the Gujarat coast in north-west India.

Materials and Methods

All animals for the study were collected from the trawl landings at Veraval and Mangrol fish landing centres in Gujarat in the north-west coast of India. Lobsters in the size range of 35-100 mm CL were chosen, so as to get sufficient representation of different maturity stages.

Descriptions of secondary sexual characteristics were made from observations on 221 females of *T. unimaculatus* (35-100 mm CL) collected from the wild. Morphological descriptions were made using standard lobster terminology. Structural variations in different body parts were studied through direct observation under a Carl Zeiss Stemi-2000 stereozoom trinocular dissection microscope.

Gonadal maturation was assessed primarily from the anatomy and appearance of the gonads in 221 female lobsters. Classification of ovarian developmental stages was done through descriptive macroscopic staging. Gonadal smears and eggs were observed under a Carl-Zeiss Axiostar trinocular compound microscope. Histological observations on gonadal maturity were made from 100 immature, maturing and mature gonads of female *T. unimaculatus*. The colour and condition of the fresh gonads were noted and the wet weights were measured to the nearest 0.1 g after drying the ovaries with blotting paper. The gonads were then preserved in 10% formalin and kept for standard histological processing. Dissections were done in crustacean saline between 16:00 - 18:00 hours (to avoid interference of circadian rhythms in the maturation process). The tissues fixed in Aqueous Bouin’s Fixative (ABF) for 24 hours, were treated through tap water, graded series of tertiary butyl alcohol and chloroform, before impregnation and embedding in paraffin wax. Transverse sections of 6-8 μm thickness cut from the blocks were spread on albumen-coated slides, stained in Harris’s Haematoxylin and spirit soluble Eosin, cleared in xylene and mounted in DPX. Photomicrographs were taken with a Ricoh camera attached to a Carl-Zeiss Axiostar trinocular dissection microscope and were used to study the histological variations with maturation.

The size at sexual maturity was assessed from observations on 221 females (35-100 mm CL) collected from the wild by determining the size at which the animals were morphologically, physiologically and functionally mature. The size at physiological maturity was assessed from the maturity condition of the gonad, based on its colour and structure. Histological observations were also done simultaneously to ratify the developmental phase judged from the anatomy of the gonads. All animals in an advanced state of gonadal maturation, those that had mature or ripe gonads and those that were in a state of rematuration were collectively grouped as “mature”, while immature animals and those in which gonadal maturation had just begun were grouped as “immature”. The frequency distribution of “immature” and “mature” females in the sampled population was analysed, and the size at first (physiological) maturity was read as the size at which 50% of the lobsters were “mature”.

The size at morphological maturity was assessed by following the development of external indices of maturity like ovigerous setae and changes in the growth of the last three pairs of pereiopods (walking legs), the ventral sternite length, and the maximum width of the carapace relative to carapace length in males and females of both species. Linear plots of the somatic lengths against the carapace lengths in size groups <50 mm CL and > 50 mm CL were used to assess changes in growth patterns from juvenile phase to sub-adult and adult phases. The sizes at which deflections (if any) in regression lines between juveniles and sub-adults were studied for indications of the onset of sexual maturity. Analysis of covariance was used to test the statistical significance of difference between the slopes of the regression lines obtained for juveniles and sub-adults.

The size at functional or physical maturity was assessed from the frequency distribution of females in ovigerous condition which establishes beyond doubt that the animal has entered a phase of active breeding.

Fecundity was estimated from 53 egg batches in the size range of 60-102 mm CL. The ovigerous animals were collected from the trawl landings at Veraval and Mangrol fish landing centres in the months of November and December, when there is an increased abundance of ovigerous females in the fishery.

The egg masses were oven dried and cleaned of adhering material like setae, pleopods etc. The total dry weight (to the nearest 0.01 g) was measured using an electronic balance. The average number of eggs in three 0.1 g sub-samples was raised to the total dry
weight of the clutch to obtain the total number of eggs. The relationship between fecundity and carapace length was estimated by linear regression.

The Gonadosomatic Index (GSI) was calculated using the standard formula:

\[ I = \frac{W \times 10^5}{L^3} \]

where,

\[ I = GSI \]

\[ W = \text{weight of gonad (g)} \]

\[ L^3 = \text{carapace length (mm)} \]

The GSI values for individual lobsters were used to arrive at the average GSI for different size groups (CL, mm) in different stages of maturity.

Results and Discussion

Secondary sexual characters

(a) Ovigerous setae on abdominal pleopods: (Pl I a-f) In *T. unimaculatus*, the endopods of the abdominal pleopods in mature females bear ovigerous setae which are used for attaching spawned eggs until they are hatched, whilst in juvenile females, the pleopods are devoid of setae. As the female enters into the sub-adult phase, the pleopods enlarge and the leaf-like endopods bifurcate and develop long ovigerous setae. Development of the ovigerous setae marks the onset of sexual maturity in females. Mature females possess a small club-shaped process midway along the inner margin of the endopod of the first pair of pleopods. The endopods of the other three pairs of pleopods are ovoid at the proximal end and narrow and tubular at the distal end.

(b) Gonopore: (Pl.I g-h) The female gonopore, situated in the form of a simple aperture at the base of the third walking leg, is seen to increase in size as the animal grows but is much smaller than the male gonopore.

Morphology of the female reproductive tract

The female reproductive tract in *T. unimaculatus* comprises of ovaries, oviducts and genital opening. The ovaries (Pl. II h-i) are a pair of tubular structures connected by a transverse bridge, giving an H-shaped appearance. They are located dorsal to the alimentary tract. A pair of thin oviducts arising from the ovary, posterior to the transverse ridge, open through the genital pores on the coxa of the third pair of pereiopods.

Maturation

The ovary of *T. unimaculatus* undergoes a series of colour and size variations in tandem with the maturity cycle. The immature ovary is translucent (Pl II a) and becomes white as the animal enters into the early stages of maturation (Pl. II b-c). At this stage the posterior lobes of the ovary do not extend into the abdominal region (Pl.II h). As maturation progresses the ovary becomes creamish to dark yellow (Pl.II d-e) and finally dark orange, when it is ready for spawning (Pl. II f). The oviducts, however, remain translucent. In the mature stage, the posterior lobes of the ovary extend to the abdominal region (Pl. II i).

The onset of maturity in females is marked by the development of ovigerous setae. The setae, once developed, lose their density after the first spate of egg...
bearing, but regain it during the next phase of breeding. This continues as cyclic phenomenon, coinciding with the breeding activity of the female. There are no mating windows (ventral sternite region) in female T. unimaculatus.

Five stages of ovarian development have been recognized in T. orientalis\textsuperscript{19}. From the observations made in the present study, ovarian development in T. unimaculatus could be classified into six stages - Immature, Early Maturing, Late Maturing/Mature, Ripe, Spawning and Spent/Recovery. While the earlier study\textsuperscript{19} does not recognize a “Spawning” stage, in the present study a distinct difference in the appearance of the ovary in spawning females was noticed, with the ovary being lighter in colour, with white patches indicating extrusion of ova. Since spawning has been observed to occur in these lobsters over a span of 2 to 3 days (personal observation made during experiments on captive breeding), spawning female lobsters were often encountered in the sampled population.

**Histology of ovary and oviduct**

The ovarian wall consists of an outer layer of connective tissue supplied with blood vessels and an inner germinal epithelium (Pl. III a-b). The thickness of the connective tissue changes with the ovarian maturation cycle, being thin initially and thickest in spent/recovering ovaries. The germinal epithelium (Pl. II d) tends to fold inwards and give rise to the ova. Immature ova are surrounded by follicular cells. The

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>Immature</td>
<td>The ovaries are initially seen as a pair of translucent, white straight, thin structures. There is no evidence of any individual oocytes (Pl. II a).</td>
</tr>
<tr>
<td>Early Maturing</td>
<td>Ovary becomes slightly enlarged and white. Although individual oocytes are not visible initially, as the ovary matures, small transparent immature ova can be seen along with a few larger ones in which yolk deposition has begun (Pl. II b-c).</td>
</tr>
<tr>
<td>Late Maturing/Mature</td>
<td>As development progresses, the colour of the ovary becomes yellow-orange and the ovary is slightly enlarged with some transparent immature ova and opaque, orange maturing ova, which can be seen through the ovarian wall. Towards the end of this phase, the ovary is packed with mature ova (Pl. II d-e).</td>
</tr>
<tr>
<td>Ripe</td>
<td>Dark orange fully ripe ovary, with dark orange opaque ova. The ovary occupies a major portion of the cephalothoracic region and is easily visible through the dorso-thoracic musculature (Pl. II f).</td>
</tr>
<tr>
<td>Spawning</td>
<td>Orange ovary with white patches, retaining part of the ova. Ova slightly smaller, orange and opaque (Pl. II g).</td>
</tr>
<tr>
<td>Spent/Recovery</td>
<td>Flabby ovary, white or light orange with residual opaque ova and a number of immature ova.</td>
</tr>
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The ovaries of immature and maturing individuals lack a lumen; the ovaries of mature individuals were found to have a central lumen. The process of ovarian maturation in *T. unimaculatus* could be traced through three distinct phases depending on the extent of yolk deposition –

**Pre-vitellogenesis phase:** The ovary is in an immature state, with a thin outer wall and immature oocytes. The oocytes have large and well-defined nuclei and are mostly surrounded by follicle cells. There is no yolk deposition. The cytoplasm is basophilic and takes haematoxylin stain.

Primary vitellogenesis phase: The ovary begins developing. Development of the oocytes progresses through two distinct stages -

Stage 1: A number of developing oocytes are seen to have a few peripheral vacuoles scattered in the cytoplasm. The cytoplasm takes haematoxylin stain.

Stage 2: (Pl. II c) As development progresses, there is an increase in the number of peripheral vacuoles which grow larger and tend to be distributed uniformly around the nucleus. Yolk deposition begins in the cytoplasm of the developing oocytes. The cytoplasm turns slightly eosinophilic.

Secondary vitellogenesis phase: In this phase yolk granules accumulate abundantly in the developed oocytes and the ovary becomes mature. Two distinct stages can be recognized in this phase -

Stage 1: Dense yolk deposition takes place and nuclei begin to be masked. The large vitellogenic oocytes are separated from the follicle cells by the egg membrane. The cytoplasm takes eosin stain.

Stage 2: (Pl. II a,b,d) The oocytes are large, globular and non-nucleated or with shrunken nuclei. Yolk deposition is at a maximum and dense yolk granules are visible. The ovarian wall is strong and stretched.

Observations in the present study on histological changes in the gonads during the maturation cycle and the progress of oogenesis through different stages of vitellogenesis conform to descriptions given for other crustaceans and lobsters.

**Size at sexual maturity**

Ovigerous setae

Development of ovigerous setae on the abdominal pleopods begins at 46-50 mm CL. A small proportion...
(18%) of the females in the size range of 51-55 mm CL had developed ovigerous setae. 50% of the females in the CL range of 61-65 mm, and all females above 71-75 mm CL, had well developed ovigerous setae (Fig. 1).

(i) Carapace length (CL) – Maximum width of carapace (MWC): The lines of regression between CL and MWC at the anterior end of the carapace (AMWC) for female lobster <50 mm CL and >50 mm CL differed in their slopes and the intersection of the two lines was estimated to be at 47.6 mm CL (Fig. 2 a). The lines of regression between CL and MWC at the posterior end of the carapace (PMWC) for female lobster <50 mm CL and >50 mm CL differed in their slopes and the intersection of the two lines was estimated to be at 43.9 mm CL, a size preceding the minimum size at which development of the ovary was observed in some females (Fig. 2 b).

(ii) Carapace length (CL) – Ventral sternite length (VSL): The regression lines of VSL versus CL in female T. unimaculatus had different elevations and there was a clear difference in the relationship between the two parameters before 50 mm CL and after 50 mm CL. In Fig 2 c, the area between 46 mm and 55 mm CL marks the area of deflection from one line of

Maturing/mature ovary

Females in the size range of 41-45 mm CL had immature ovaries. Signs of ovarian development were observed in some females in the size range of 46-50 mm CL. 50% of the females in the size range of 61-65 mm CL had ripe ovaries (Fig. 1). All females above 76-80 mm CL were fully mature.

Occurrence of ovigerous females

The minimum size class in which ovigerous females were noticed was 61-65 mm CL. The smallest ovigerous female recorded measured 64.1 mm CL and the largest measured 100 mm CL. However, the percentage frequency of ovigerous females could not be used as an index to estimate the size at first maturity as the percentage did not exceed 26% in any size class from 61-65 mm to 96-100 mm CL (Fig. 1). The percentage of ovigerous females increased from 11% in lobsters of 61-65 mm CL to 26% in lobsters of 76-80 mm CL, followed by a decrease in the larger size classes. The peak occurrence of ovigerous females in the size range of 76-80 mm CL follows the completion of ovarian maturation and the development of ovigerous setae. The frequency distribution of ovigerous females thus serves to corroborate the conclusions drawn from these indices.

Relationship between carapace length, maximum width of carapace, ventral sternite length and leg lengths as indices of sexual maturity

Fig. 1 Size at sexual maturity in female T. unimaculatus derived from physiological and functional maturity indices

Fig. 2 Relationship between carapace length and somatic lengths as indices of sexual maturity in female T. unimaculatus
growth to the next, signifying changes due to sexual maturation.

(iii) Carapace length (CL) – Leg lengths (III WL, IV WL and V WL): The regression lines between CL and lengths of both, the III and IV pereiopods (Figs. 2 d & e) differed in their elevations in the two size groups. The deflection area lay in the range of 49 – 53 mm CL. The intersection of the regression lines for the fifth pereiopod was at 47.5 mm CL (Fig. 2 f).

From the results obtained, it is evident that morphological, physiological and functional maturity in *T. unimaculatus* occurs over a narrow size range, with the size at first maturity in females attained at 61-65 mm CL. The size at onset of sexual maturity, judged from the 25% presence of the development of different sexual characters (Fig. 1), was in the 51-55 mm CL size class for females.

The functional criteria of egg-bearing\textsuperscript{32,33}, the presence of fresh or spent spermatophores and resorbing ova, the physiological criteria of ovary colour and size, oocyte size and development of cement glands on the pleopods, and morphological criteria of abdomen and pleopod development\textsuperscript{28} are the critical indicators studied to assess size at first maturity in female lobsters. Staging based on histological examination of gonads provides more detailed information than any other indicator. The percentage of ovigerous (berried) females in the sampled population has been described to give a reliable estimate of the size at maturity in the spiny lobster *J. lalandii*\textsuperscript{34} since it was found that after a certain size there was sharp increase in the percentage of lobsters in ovigerous condition, which continued to increase in larger lobsters. One of the most important external indicators of sexual maturity in female lobsters is the presence of fully developed ovigerous setae on the abdominal pleopods, to which spawned fertilized eggs remain attached until the larvae are hatched. The presence of these setae has been recognized as a useful criterion for the identification of mature females\textsuperscript{13,15} and in some species like *J. lalandii* there is a regular cycle of appearance and disappearance of these setae\textsuperscript{30}. No significant relationship between ovigerous setae and maturity in female *T. orientalis* was reported in studies from Mumbai\textsuperscript{19}. In the present study it was observed that the appearance and development of the ovigerous setae for the first time coincides with the onset of sexual maturity in *T. unimaculatus*. While the number and size of the setae were found to reach a maximum at the time of egg bearing, it was also observed that after one batch of eggs is completely hatched/removed from the pleopods, there is a reduction in the density of the ovigerous setae, which later increases during the successive breeding cycle. Female maturity has been associated with allometric changes in the length of pleopods, the length of the pleopodal setae, telson length or the width of the abdomen relative to carapace length, which all change in relation to preparation for first spawning\textsuperscript{10,13,15,17,33,37,38,39,40}. Easily measured appendage length to body size relations can be routinely applied to provide estimates of female SOM (Size at Onset of Maturity) in lobsters, but only after this approach has been validated by undertaking histological studies of gonadal maturation\textsuperscript{28}. In the present study, the size at maturity in female *T. unimaculatus* was estimated from different indices of maturity and corroborated by histological observations on the progress of gonadal development.

**Fecundity**

The fecundity of *T. unimaculatus* ranged from 19600 eggs (60 mm CL) to 59500 eggs (102 mm CL), with an average fecundity of 39300 eggs for reproductively active females in the size range of 60 – 102 mm CL. The relationship between fecundity and carapace length (Fig. 3) was derived as

\[ \text{Fecundity (000)} = 0.7285 \times \text{CL} - 19.153 \quad (r^2 = 0.9424) \]

**Gonado-somatic Index**

Average GSI values ranged from 0.6 to 0.88 in immature females of 51-65 mm CL and from 1.04 to 2.68 in early maturing females of 56-70 mm CL. In late maturing and mature females of 61-90 mm CL, the average GSI values ranged between 4.16 and 4.9. The highest GSI values were observed in mature

![Fig.3 Relationship between fecundity and carapace length in *T. unimaculatus*](image-url)
females of 85-90 mm CL. In females of 75-100 mm CL, in the spent/recovery stage, the GSI fluctuated between 0.96 and 1.67. The GSI was seen to increase with size among immature and early maturing females (Fig. 4). In the group of lobsters identified as being in the late maturing/mature stage, the GSI increased from 4.16 in the 61-65 mm CL class and thereafter did not show much variation until the spent stage is reached, in the size range of 66-100 mm CL. In the spent/recovery staged females, the GSI remained close to 1.00.

Gonadosomatic Index (GSI) has been used to examine ovarian development and to obtain information on the spawning season and reproductive cycle in a range of crustaceans. Although GSI by itself does not provide useful information on the process of ovarian development, it does provide useful information on ovarian cycles which can supplement detailed information obtained from morphological, anatomical and histological studies. Studies on the reproductive cycle in *P. japonicus*, revealed that ovarian development, particularly, in smaller size classes, corresponds well with the changes in GSI. This was found to be true in the present study also. In size classes between 46 and 60 mm CL, in maturing individuals, the GSI was found to increase. Maximum GSI values correspond with dominance of mature individuals. Thereafter, in sizes above 70 mm CL, the GSI was found to fluctuate, probably due to the influence of repetitive breeding cycles before the next phase of breeding.

**Conclusion**

With the sizes at physiological and morphological maturity of female *T. unimaculatus* derived to be in the 61-65 mm CL range, and maximum proportion of ovigerous females in the sampled population being in the 76-80 mm CL range, it can be concluded that the critical first spawning phase of female *T. unimaculatus* occurs over the size range of 61-80 mm CL. The low fecundity estimates observed in the present study signal the need to regulate fishing of lobsters of 61-80 mm CL so as to ensure at least one successful breeding cycle during the life history of each individual, which in turn will effect recruitment to the population.

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![Fig. 4 Gonadosomatic Index in female T. unimaculatus Im: Immature; Em: early maturing; Lm: late maturing; M: mature; Sp: Spent/recovering](image-url)


