

Bivalve maturity scales and estimation of spawning season

R. Vidya^{1*}, P. Gomathi², M.K. Anil², Geetha Sasikumar¹ and P.S. Alloydious¹

¹ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, Kerala

²Vizhinjam Regional Centre of ICAR-Central Marine Fisheries Research Institute, Vizhinjam P.O., Thiruvananthapuram – 695 521, Kerala

Molluscan reproduction is primarily regulated by the interaction of external and endogenous variables. The principal method of assessing the reproductive cycle in bivalves is based on histology. A simplified method for assessing the maturity stages of the gonad is by visual examination of the colour of the mantle tissue, followed by inspection of the fresh gonad smears under a microscope (10X magnification). Many schemes have been used to describe the seasonal reproductive cycle in bivalves. The gametes in bivalves are produced by the germinal epithelium within a web of follicles enveloped by connective tissues. Generally, these schemes recognise: indeterminate stage (immature/inactive stage); then the active stage begins with the multiplication of germ cells; growth of germinal tubules and germinal maturation including vitellogenesis in females, which is followed by sexual maturity including spawning. Usually 35-50 specimens are randomly collected at fortnightly or monthly intervals to assess maturity stages. They are measured and weighed, and a tissue smear from

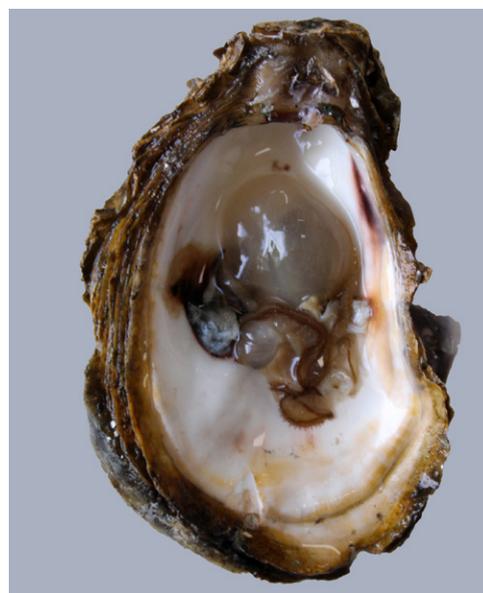
the middle part of the gonad is examined in the laboratory for maturity assessment. The mantle serves not only as the site of gametogenesis but also as the primary storage site for nutrient reserves, particularly glycogen. These reserves are accumulated before spawning and are utilized in gamete formation. Gametes proliferate within the mantle and during the spawning season, they are transported through ciliated channels to paired gonoducts that release them into the mantle cavity.

Stage I Indeterminate

No distinguishable differences exist in the gonads or follicles between females and males. Initially, the gonads appear translucent and gelatinous. This stage encompasses the period during which glycogen and other nutrients gradually accumulate, which are subsequently utilised during the active reproductive phase.



Indeterminate gonad stage in Brown mussel



Indeterminate gonad stage in Edible oyster

Stage II Early maturing

In this stage, the gonad of the female is somewhat thick, and pale yellowish with underlying ductules more prominent. Follicle walls contain primary oocytes in various developmental stages. Follicles become larger and denser with lumina filling with growing oocytes, with connective tissue between the follicles. During the maturing stage in mussels, the gonads of females develop into thick, yellowish to light orange tissues, while the mantle cavity becomes engorged. Male gonads are creamy in colour; however, it has to be confirmed by histological examinations.

Stage III Late maturing

During the late maturation phase, secondary oocytes are attached to the follicular wall by slender stalks. These oocytes exhibit a pear-shaped morphology rather than being perfectly spherical, and they remain attached to the inner walls of follicles via connections. Some free oocytes are present in the lumen, displaying distinct nuclei. There is a noticeable rapid expansion in the size of the follicle, occupying a larger area within the connective tissue. Concurrently, there is a reduction in connective tissue volume. In males, the gonad becomes thicker, firm and white with follicles occupying the entire area of the gonad. Spermatogonia, spermatocytes and spermatids are present in rounded to expanded tubules. At the centre, a few spermatozoa proliferate. In the late maturing phase, follicle increase in size and the periphery contains numerous spermatogonia and a few spermatids radiate towards the lumen of the follicle. As the maturing phase advances, the secondary spermatocytes occur in large numbers along with the primary spermatocytes. The primary and secondary spermatocytes can be differentiated by size and staining intensity.

Stage IV Mature

At this stage, the female gonad is full and plump. Large free oocytes predominate within the follicles, which are brimming with mature oocytes. The fully yolked ova adopt a perfectly spherical shape with a distinctive round nucleus and nucleolus. Follicles are densely packed without any interspace and there is no interfollicular tissue observed. As the male gonad reaches ripe condition, spermatids undergo differentiation into spermatozoa, forming a core within the follicle's lumen. A mature gonad exhibits clusters of spermatozoa, generally arranged in a radial pattern with their tails directed towards the centre of the follicular lumen. In fully mature specimens, the lumen becomes filled with spermatozoa. In mussels, gender can be distinguished through visual inspection at this stage. Ripe female mussels exhibit an orange-red hue, with their entire mantle cavity filled with gonad tissue. In contrast, males have cream-coloured gonads that release milt.

Stage V Partially spent

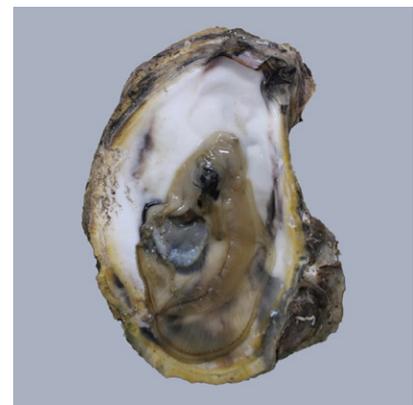
At this stage, the female gonad is flaccid and has a loose consistency. Some follicles contain a small number of free oocytes, while others are empty due to the release of gametic material, causing the follicle walls to rupture. In female clams, the follicles display varying degrees of emptiness, with vascular tissue, connective tissue cells, and free oocytes scattered in the lumen. Ripe ova may be present in some follicles, while others remain empty. Partially spawned and spent female mussels show thin, flaccid tissue with a faded orange coloration and a decreased presence of gametes in the tissue. In male gonads, partially empty follicles contain residual sperm and spermatids. As follicle cells rupture, they become empty, with a few residual gametes remaining. The vesicular connective tissue increases in volume.



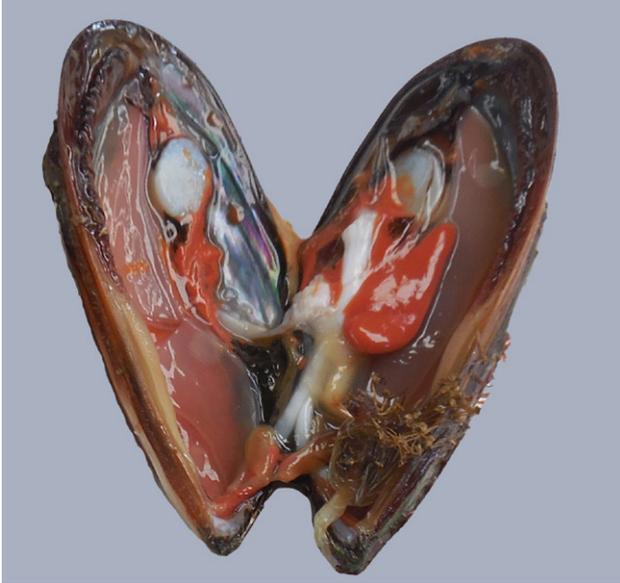
Ripe female (left) and male (right) Edible oyster



Partially spent female Edible oyster



Fully spent female Edible oyster



Ripe stage female Brown mussel



Ripe stage male Brown mussel



Partially spent female Brown mussel



Partially spent male Brown mussel

Stage VI Fully spent

At this stage, the follicles appear fragmented, significantly reduced in size, dispersed, and relatively empty. Vesicular and connective tissue increase and fill the spaces between the follicles. In females, the gonads are loose and translucent. Residual oocytes are scarcely visible within the follicles, which appear collapsed. Connective tissue is present. In males, connective tissue is evident. Some tubules are empty but typically contain residual spermatozoa with numerous gaps.

Spawning season

Females in stages III (Late maturing), IV (Mature) and V (Partially spent) were considered to assess the proportion of mature animals in the pooled monthly samples. The spawning period is determined as the month(s) or season with the highest spawning proportion.

$$\text{Monthly Spawning proportion (\%)} = \frac{\text{Number of females in stages III (Late Maturing)+IV (Mature)+V (Partially spent)}}{\text{Total number of females (N) (stages II+III+IV+V+VI) sampled for the month}} \times 100$$