Brief Communication

Maturity stages and estimation of spawning season in cephalopods

Geetha Sasikumar[1*], M. Kavitha[2] and K. K. Sajikumar[1]
[1]ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, Kerala
[2]Tuticorin Regional Station of ICAR-Central Marine Fisheries Research Institute, Thoothukudi–628 001, Tamil Nadu

*E-mail: gs.pallath@gmail.com

The reproductive systems of cephalopods in cuttlefish, squid, and octopuses exhibit similarity. These species have separate sexes. In females, maturation primarily involves development of gonads and the enlargement of accessory reproductive organs, namely the oviducal, nidamental, and accessory nidamental glands. Typically, assessing maturity stages relies on a descriptive scale and indices based on the relative development of reproductive tissues. In males, mature spermatozoa are enclosed in spermatophores that are stored in the spermatophoric sac, also known as Needham's Sac. Fertilization occurs through individual mating, during which spermatophores are transferred from males to females using a modified arm (hectocotylus) specialized for this mating process. Females can store sperm, resulting in delayed fertilization. Egg deposition varies among species; some attach egg masses to the seabed (common in octopuses, loliginid squid, and cuttlefish), while others release gelatinous egg masses into the water column (typical of most squid families).

Maturity stages in Cephalopods

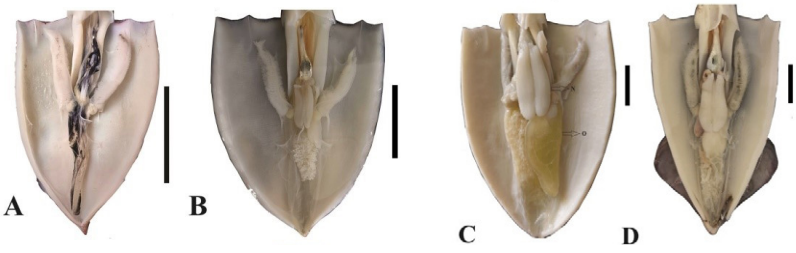
According to the gross morphology of the gonads and the accessory glands four stages of sexual maturity in cephalopods species are defined. In females, oogenesis begins when germ cells differentiate and become primary oogonia. Primary oogonia enlarge and develop into secondary oogonia, which then develop into oocytes. Depending on the degree of follicular cell development associated with oocytes, oogenesis can be defined by four stages. Spermatogenesis in males occurs within the functional units of the testis, known as the seminiferous tubules. Each seminiferous tubule in the mature testis contains spermatogonia, spermatocytes, spermatids and spermatozoa, respectively from the periphery to the center. These different germ cells are derived from spermatogonial differentiation. Spermatogenesis can be divided into four stages according to the degree of development within the seminiferous tubules. In contrast to the testis, the composition of the ovary changes significantly during sexual maturation. As well as the eggs, the female cephalopods develop large glands: the nidamental gland, the accessory nidamental gland (in sepiids, sepiolids and loliginids) and the oviducal gland, which secrete egg coatings at the time of release. A general scale for maturity stages is described using distinctive characteristics for the determination of each stage for squids (Table 1), cuttlefishes (Table 2) and octopus (Table 3).

Maturity stages of Squids

Table 1. A general scale of maturity stages for female and male squids

Maturity stage	Female	Male
Stage I Immature	Nidamental glands appear as transparent pair of strips. The accessory nidamental glands not apparent. Oviduct meander not visible. The ovary is tiny, filiform, and devoid of granulose structure.	Spermatophoric (Needham's) sac small with not visible vas deferens. Spermatophores are absent. Testis are very small and appear as a pale oval structure.
Stage II Maturing/ Developing	Nidamental glands appear as lobe like, small to large in size. The ovary is conspicuously developed; occupies nearly half of posterior body with granulate structure and uniform-sized developing white oocytes. Oviducts thickened, absence of oocyte in the oviduct.	Spermatophoric sac (Needham's Sac) with visible vas deferens. There are small and few spermatophores in the spermatophoric sac. Testis is small and whitish.

Maturity stage	Female	Male
Stage III Mature	Nidamental glands large, firm and whitish cream. Ovary enlarged with plenty of mature oocytes; occupies entire posterior mantle cavity. Oviduct (not paired in neritic squids) filled with fully mature transparent oocytes. The mature oocytes tend to be placed proximally and the immature oocytes are distal.	Needham's sac completely packed with plenty of well-developed spermatophores; spermatophores occur in the penis. Testis large and fully developed.
Stage IV Partially spawned/spent	Decrease in gonad volume/ degenerating oocytes in oviduct/ or oocytes absent. Nidamental glands flaccid or diminished, noticeably smaller in volume and weight in squid. Ovary with few striated loose eggs and few medium to small eggs attached to the connective tissue core of the ovary.	Spermatophores in gonoduct. Needham's sac flaccid with degenerating spermatophores. Testis shrunk and small.

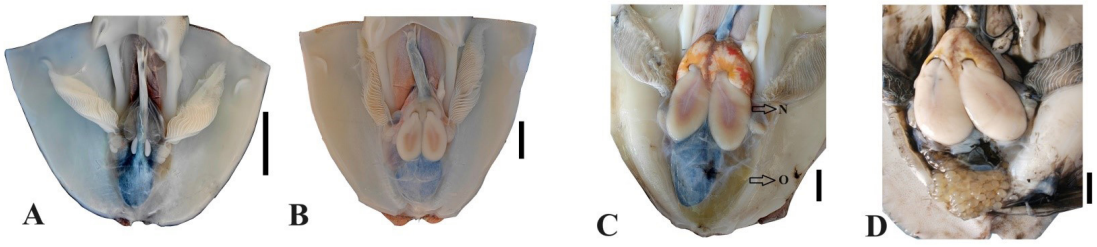


General overview of the development of the reproductive organs of *Uroteuthis duvaucelii*. (A=immature, B=maturing, C= mature (N=nidamental gland, O=ovary) and D= spawned, Scale bar=2 cm)

Maturity stages of Cuttlefish

Table 2. A general scale of maturity stages for female and male cuttlefish

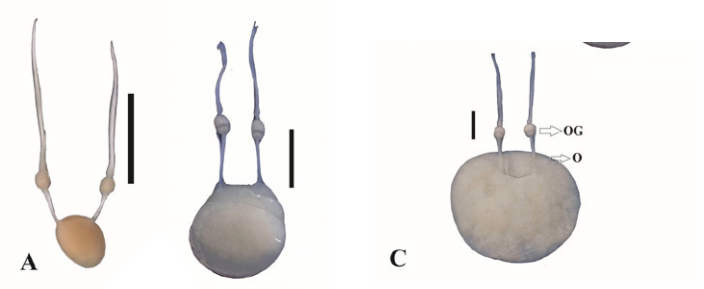
Maturity stage	Female	Male
Stage I Immature	Nidamental glands appear as very thin and transparent, small in size, accessory nidamental glands are not apparent. Ovary is very small, translucent, membranous, occupying the posterior mantle as a whitish patch. No oocyte apparent. Oviduct meander is not visible.	Spermatophoric (Needham's) sac small and transparent with no visible vas deferens. Spermatophores are absent. Testis small and thin.
Stage II Maturing/ Developing	Nidamental glands thicker and transparent to translucent, pearł-shaped; accessory nidamental gland small and creamy white to beige. Oviduct thickened and developing. Ovary with clearly visible small – medium sized oocyte and very few of them reticulated.	Spermatophoric (Needham's) sac with visible vas deferens and few spermatophores, most of them poorly developed. Testis has enlarged considerably.
Stage III Mature	Nidamental glands large, thick and white, with distant anterior pore; Accessory nidamental gland show an intense orange-red coloration in mature female. Ovary occupies entire posterior mantle cavity with small, medium, large reticulated and large smooth transparent oocyte. The proximal oviduct in cuttlefish with smooth transparent mature oocytes, the distal part of ovary with striated eggs and small eggs.	Needham's sac completely packed with plenty of well-developed spermatophores; spermatophores occur in the penis. Testis large and fully developed.
Stage IV Partially spawned/spent	Nidamental glands large but soft flabby. Accessory nidamental gland mottled, reddish brown. Ovary with few striated loose eggs and few medium to small eggs attached to the connective tissue core of the ovary.	Spermatophores in gonoduct. Needham's Sac flaccid with degenerating spermatophores and appear as white mass. Testis small.



General overview of the development of the reproductive organs of *Sepia pharaonis*. (A=immature, B=maturing, C= mature (N=nidamental gland, O=ovary) and D= spawned, Scale bar=1 cm for A,B and 2 cm for C,D)

Table 3. A general scale of maturity stages for female and male octopus

Maturity stage	Female	Male
Stage I Immature	Ovary very small, semi-transparent with no signs of ovarian granulation. Oocytes not visible to naked eyes. Oviducal glands small and translucent, diameter is slightly larger than the oviduct diameter.	Testis small, thin and translucent. Needham's sac small and white. Spermatophoric complex with vas deferens not visible. Absence of spermatophores.
Stage II Maturing/ Developing	Ovary with granular structure clearly visible through the membrane, not reaching the posterior half of mantle cavity. Oviducal gland developing and larger.	Developing and whitish testis larger than in stage I. Spermatophoric complex transparent with visible vas deferens. A white streak may appear spermatophores not fully formed.
Stage III Mature	Large ovary containing clearly visible oocytes, a high percentage of large reticulated oocytes. Well-developed oviducal glands.	Testis whitish with large and white vas deferens. Large Needham's Sac full of packed spermatophores.
Stage IV Spent	Shrunken flaccid ovary, with only immature oocytes attached to the central tissue and a few loose large oocytes in the coelom (generally spent females are not collected in routine sampling)	Testis flaccid. Needham's sac soft empty or with a few well-formed spermatophores.



General overview of the development of the reproductive organs of *Amphioctopus neglectus*. (A=immature, B=maturing and C= mature (N= Oviducal gland, O=ovary), Scale bar=1 cm)

Maturity stages of Octopus

The reproductive system is simplest in the octopus. The female gonad is oval with two tubular oviducts. The oviducal glands are set on the oviducts like a ring and are attached to the sexual coelom. The single testis is rounded in octopus.

Spawning season

Cephalopods have relatively short lifespans and rapid growth rates, which can influence their reproductive timing. Females may spawn all their eggs in a short period (terminal spawning) and die shortly after reproduction. Alternatively, they may have distinct spawning bouts, referred to as batch or repeated spawning. To determine the seasonality of spawning in cephalopods, samples are collected fortnightly, to assess the development of the gonads. This assessment involves determining the stages of gonadal development, which may include visual observation of external characteristics or histological analysis. The peak spawning period is determined from the monthly percentage of occurrence of the mature/ spawning females. The spawning fraction is estimated by considering the distribution of mature females in stages III and IV in random samples. It will be quite challenging to calculate how the sample relates to the whole natural population projected over the entire life cycle in these short-lived cephalopods with seasonal spawning migration, spawning congregation (*Sepia pharaonis*, *Uroteuthis duvaucelii*) and parental care (*Octopus* spp.)

$$\text{Monthly Spawning proportion (\%)} = \frac{\text{Number of females in Stage III (Mature)+Stage IV (Partially spawned/spent)}}{\text{Total number of females sampled (Stages I to IV)}} \times 100$$