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**Maturity staging for squid**

*China*

## A common macroscopic maturity scale for Teuthida

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### 1. Macroscopic maturity scale using in Chinese Laboratory

The scale is after the same scale used for *Uroteuthis chinensis* (Duanwu Ou, 1983), including five stages for females and males.

#### 1.1 For females

**Stage I, Immature.** Ovary is small and elongated, with granular structure and tiny oocytes visible to the naked eye. Oviduct is small but no any visible eggs. Nidamental glands (NG) and oviducal glands (OG) small and translucent.

**Stage II, Developing.** Ovary enlarged, reaching the posterior about one fourth of the mantle cavity, with different size of oocytes. Small and white oocytes occupied half of the ovary. Milky oviduct, not visible ripe eggs. Developing NG and OG.

**Stage III, mature.** Ovary enlarged further, occupying one third of the mantle cavity and containing different size of oocytes. Small white oocytes occupied one third of the ovary. Oviducts visible ripe eggs. NG and OG fully developed.

**Stage IV, Spawning.** Ovary enlarged to the largest size, occupying about half of the mantle cavity, with few small opaque oocytes. Oviducts contained ripe eggs. Enlarged and turgid nidamental glands, smooth surface, nearly covering all internal organs. Spermatophores visible inside the mantle cavity particularly close to the gill base.

**Stage V, post spawning.** Flaccid ovary appeared in loose status, with few small oocytes. Loose oviduct contained few or none eggs. Flaccid NG and OV.

#### 1.2 For males

**Stage I, Immature.** Small testis is white color. Spermatophoric Complex (SC) is semitransparent, and vas deferens is not visible.

**Stage II, Developing.** Testis enlarged. Spermatophoric complex developed in a larger size. Vas deferens developed, enlarging the end to spermatophoric sac. The Needham's Sac may contain few underdeveloped spermatophores.

**Stage III, mature.** Testis enlarged to the largest size, with large and white vas deferens. SC fully developed, visible developing spermatophore inside. Turgid Needham's Sac visible packed spermatophores.

**Stage IV, Spawning.** Testis flaccid and reduced. Needham's Sac fully packed spermatophores, with some spermatophores outside the terminal organ.

**Stage V, post spawning.** Flaccid testis and SC, with light grey color. Needham's Sac contained few spermatophores, some of which were broken, with sperms leaked.

## 2. Macroscopic maturity scale proposed by ICES (2010)

[Report of the Workshop on Sexual Maturity Staging of Cephalopods, 8-11 November 2010, Livorno, Italy. ICES CM 2010/ACOM:49. 97 pp.]

### 2.1 For females

**Stage 0, Undetermined.** Sex not distinguished with the naked eye.

**Stage 1, Immature.** Ovary is semi-transparent, stringy and lacking granular structure. Small and translucent Nidamental Glands (NG) and Oviducal Glands (OG). Oviduct meander not visible. Oocytes not visible to the naked eye.

**Stage 2a, Developing.** Whitish ovary with visible granular structure, not reaching the posterior half of the mantle cavity. Developing NG/OG. NG enlarged, covering some internal organs. Oviduct meander clearly visible. Very small oocytes visible to the naked eye.

**Stage 2b, Maturing.** Ovary occupies the whole posterior half of the mantle cavity, containing tightly packed oocytes. Large NG covering the viscera below. Oviducts fully developed but empty. Very small isodiametric oocytes visible to the naked eye.

**Stage 3a, Mature / Spawning.** Ovary containing a higher percentage of large oocytes. Enlarged and turgid NG/OG. Plenty of oocytes in the oviducts. Amber-coloured oocytes in the oviducts.

**Stage 3b, Spent.** Flaccid ovary with strikingly loose disorderly aspect. Few oocytes, which may be attached to the central tissue. Flaccid NG/OG. Few oocytes which may be attached to the central tissue.

### 2.2 For males

**Stage 0, Undetermined.** Sex not distinguished with the naked eye.

**Stage 1, Immature.** Small and translucent testis. Thin and semitransparent Spermatophoric Complex (SC) with not visible vas deferens. No spermatophores.

**Stage 2a, Developing.** Whitish-grey testis, filling almost  $\frac{1}{2}$  of the posterior part of the mantle cavity. SC whitish with visible vas deferens. Penis appears as a small prominence of SC. Absence of spermatophores.

**Stage 2b, Maturing.** Whitish-grey testis, filling the posterior part of mantle cavity, with visible structure. The vas deferens is white, meandering and enlarged. The Needham's Sac (SS) may contain few partially developed spermatophores (visible as whitish particles) and/or few fully developed spermatophores. Few spermatophores partially or fully developed.

**Stage 3a, Mature / Spawning.** Yellowish well-developed testis with large and white vas deferens. Spermatophores packed in the Needham's Sac; spermatophores may occur in the penis. Plenty of well-developed spermatophores.

**Stage 3b, Spent.** Testis flaccid. SS empty or with few spermatophores. None or few spermatophores.

### 3. Macroscopic maturity scale proposed by Perea et al. (2018)

[Ángel Perea, Javier Sánchez, Betsy Buitrón. 2018. Gonadal maturity scale of the jumbo flying squid *Dosidicus gigas* (d'Orbigny, 1835) (Cephalopoda: Ommastrephidae). Bol Inst Mar Perú, 33 (2):137-152]

#### 3.1 For females

**Stage I, Immature.** The ovary is characterized by being an organ in the form of a piriform sac, elongated, smooth to granular, translucent or white, being translucent in its initial phase and somewhat opalescent (opaque) in its final phase. No oocytes are observed in oviducts and oviductal glands.

**Stage II, Maturing.** The ovary is granular, opaque in appearance and varies in color from white to cream. In the oviducts and oviductal glands, no oocytes are yet observed.

**Stage III, Mature.** The ovary is larger, swollen and piriform in appearance; its wall is thin and transparent, so mature oocytes can be seen with the naked eye. Its color varies from yellowish cream to amber. Highly developed nidamental glands.

**Stage IV, Spawning.** The ovary is less turgid, grainy, with a large number of yellow and amber cream oocytes, its wall is very thin. The amber oocytes are predominant and give the ovary an orange hue.

### 3.2 For males

**Stage I, Immature.** The testicle is thin and translucent. The spermatophoric complex is in formation and without visible spermatophores in the spermatophoric sac.

**Stage II, Maturing.** The testicle is characterized by a larger volume than in the previous phase, whitish, opaque.

**Stage III, Mature.** The testicle is characterized by its turgid consistency, white color and milky appearance. The spermatophores are found both in the spermatophoric sac and in the visceral cavity.

By comparing with the scales proposed by ICES (2010) and Perea et al. (2018), we recommend to scale the macroscopic maturation for *Dosidicus gigas* at five different stages. Although the scale proposed by ICES (2010) covers the unidentified stage, it is rarely to detect individuals with such stage from the catches. Meanwhile, both stages 1 and 2a in the scale of ICES (2010) would be better to identify as immature stage (stage I), because sometimes it will be too subjective to identify whether the oviducts meander or not for females and the vas deferens visible or not for males. In terms of the scale proposed by Perea et al. (2018), the significance should be the lack of post-spawning (or spent) stage for females and spawning and spent stages for males. Regarding the scale currently used in our laboratory, the five stages appear better to describe the reproductive growth at a macroscopic level. Particularly, it is important to separate mature and spawning stages due to the inherent characteristics of storage of gametes before spawning and synchronous ovulation once spawning commencing.