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Workshop on Cephalopods Maturity Stages

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Workshop on Cephalopods Maturity Stages

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1. Introduction

In Portugal, there are five species of cephalopods with commercial value: an octopus species *Octopus vulgaris*, two longfin squids *Loligo vulgaris* and *Loligo forbesi*, two shortfin squids *Illex coindetii* and *Todaropsis eblanae*, and *Sepia officinalis*, the common cuttlefish. Each species is targeted by the artisanal fleet although the common octopus artisanal fleet is by far the most important. With the exception of cuttlefish, these species are also important as a by-catch of the multi-species bottom trawl fisheries.

The Portuguese Fisheries Institute, IPIMAR, began the biological data collection for squid species in the early 1990's within the EUROSQUID I project (FAR 1.146) and later for octopus under the CEPHVAR Project (FAIR-CT96-1520). Nowadays, under the Data Collection For The Fisheries Sector and the PRESPO project (INTERREG IV B, co-financed by European Union, ERDF funds) the invertebrate fisheries group collects biological information of two sources: from the bottom trawl surveys targeting demersal fish species conducted regularly in the autumn and ranging the entire Portuguese coast; and monthly samples collected from landings in a national port of the commercial artisanal fisheries, targeting each one of the cephalopod commercial species. The regular biological sampling from commercial fisheries is conducted in the lab and adapted to the specific characteristics of each species.

2. Data collection and Sampling Programme

2.1 *Octopus vulgaris*

The collection of biological data regarding the common octopus *Octopus vulgaris* began in 1996 and gathers information from both the bottom trawl surveys and the commercial fleet landings (mostly using pots). Since January 2007, we have an additional monthly sample of the artisanal fleet of the Portuguese south coast (Figure 1). Considering this fairly long time series and samples in two oceanographically different regions, we are aiming to describe the differences between the spawning timing and reproductive investment of the octopus population from those two regions. In the biological sampling conducted on the lab for each individual we collect the weight, dorsal mantle length, sex and maturity stage, digestive gland weight and stomach fullness index. For males, the testis and Needham's complex are weighted separately, and for females ovary and oviducal gland are also weighed separately. In bottom trawl surveys, all of the individuals captured are weighed, measured, sexed and their maturity staged. The maturity scale adopted for the *Octopus vulgaris* is described by Gonçalves (1993) (Table 1). This is a four stage maturity scale for males (I: immature, II: maturing, III: spawning, IV: post-spawning) and five stages for females (I: immature, II: maturing, III: pre-spawning, IV: spawning, V: post-spawning).

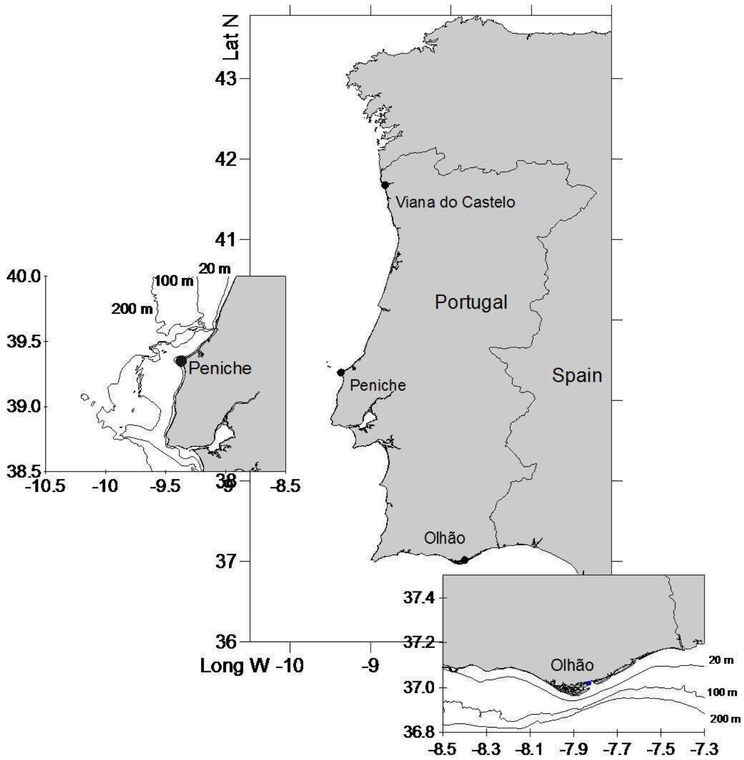

Figure 1. Sampling ports and coastal characteristics.

Based on the data collected between January 2007 and June 2010, proportion of mature individuals, monthly mean Gonad-somatic index (GSI) and maturity ogives were determined for the northwest coast and south coast populations of *Octopus vulgaris*. Concerning the results on proportion of mature individuals and monthly GSI, is possible to find mature individuals across all year. Despite that, the two populations preferentially spawn in different seasons. In the northwest coast, the months with higher frequency of mature individuals occur between February and August with a bimodal distribution for females, and in the south coast frequently occurs a spawning peak in September (45% of the females sampled mature in this month). Regarding maturity ogive, significant differences were found between sexes, with females maturing at larger sizes than males. Comparing the maturity ogives between the two regions, northwest coast and south coast, no differences were found.

Table 1 . *Octopus vulgaris* maturity scale adopted (Gonçalves, 1993).

Females	Males
I. Immature Ovary and oviducal glands white small and homogeneous. Oviduct thin and transparent	**I. Immature** Needham's complex: spermatophores absent; Testis and vas deferens transparent
II. Maturing Ovary medium size, white and homogeneous. Oviducal glands small with a proximal band of small denticules. Oviducts white and thick.	**II. Maturing** Needham's complex: Presence of few and empty spermatophores. Testis: white; Vas deferens: thick and whitish.
III. Pre-spawning Ovary 1/3 of the mantle cavity, yellow and heterogeneous. Oviducal glands developed, the denticulate band is bigger, occupying almost half of the gland. Oviducts white and thick	**III. Spawning** Needham's complex: Several spermatophores with stored spermatozoa; Testis homogeneous white-yellowish. Vans deferens yellowish
IV. Spawning Ovary: biggest organ inside the mantle cavity, yellow with visible eggs. Oviducal glands: 3 different parts, with proximal part darker, longitudinal septa Oviducts: white and thick.	**IV. Post-spawning** Needham's complex: Few spermatophores; Atrophy of the Testis, darker and heterogeneous; Vas deferens, also darker
V. Post-spawning Ovary smaller, darker and heterogeneous, without eggs; Oviducal glands smaller, darker and heterogeneous; Oviducts: yellowish and thick.	

2.2 Loligo vulgaris

The biological sampling and data collection on longfin squids *Loligo vulgaris* and *Loligo forbesi* started in 1990 with the EUROSQUID I project. The occurrence of *Loligo forbesi* on the Portuguese coast seems to be cyclical and the regular sampling of this species depends of its availability. Our sampling gathers information both from the bottom trawl surveys and the commercial fleet landings (mainly from trawl fisheries). In the biological sampling conducted in the lab, we collect for each individual, the weight, dorsal mantle length, sex and maturity stage, mantle weight and stomach fullness index. The gonads and spermatophoric complex of males are weighed separately, as well as the weight of the ovary, nidamental plus accessory glands and the oviducal gland plus distal oviduct and the proximal oviduct of females. During bottom trawl surveys, all of the individuals captured are weighed, measured and sexed and their maturity staged. The maturity scale adopted for the long-fin squids is the five stage maturity scale described by Boyle and Ngoile (1993) for *Loligo forbesi* (Table 2) which in fact is akin to the Lipinsky (1979) scale for the *Illex illecebrosus.*

The maturation ogives as well as the size of maturation and monthly evolution of the gonadal index for *Loligo vulgaris* and *Loligo forbesi* off the Portuguese coast are described in Moreno *et al* (1994) and

Moreno *et al* (2002). Recently, the reproduction of *Loligo vulgaris* has been revisited with the aim of comparing potential fecundities of squid under different environmental regimes, based on histological methods. Results of this research indicate that maturation development is strongly dependent on environmental conditions, not only in the timing of events, but also in determining the quality and quantity of the reproductive output. Results also support the presence of batches of oocytes in different microscopic maturity stages within what is normally considered stage four of the macroscopic scale (Boavida- Portugal et al., 2010).

Table 2. Longfin squids maturity scale adopted (Boyle and Ngoile, 1993).

	Females	Males
Stage I	The nidamental glands are transparent and appear as two strands anterior to the stomach and bellow the digestive gland. The acessory nidamental glands are transparent and cannot be distinguished from the nidamental glands. The oviduct and oviducal gland are transparent and appear as droplets posterior to the base of the left ctenidium. The ovary is transparent and membranous.	The testis is transparent and membranous and is recognised by tracing the genital aorta from the posterior tip of the stomach. It appears as an elargement of the genital aorta. The spermatophoric organ appears as a clear droplet just below (posterior to) the base of the left ctenidium. The opening of the penis is located dorsally to the base of the left ctenidium.
Stage II	The accessory nidamental glands appear as scarlet specks dorsally to the tips of the nidamental glands. The nidamental gland is opaque. The oviduct and oviducal gland are translucent and cannot be distinguished one from another. The ovary is translucent and appears as fluffy cotton wool emanating from genital aorta.	All the components of the reproductive system can be seen with the naked eye. The testis is small, translucent and soft. The spermatophoric organ is translucent and easily distinguishable into its components-espermatophoric sac, spermatophoric complex and penis. The opening of the penis is anterior to the base of the left ctenidium. No spermatophores in the spermatophore sac. The vas deferens is transparent and not easily visible. Hectocotylization of fourth left arm has begun.
Stage III	The accessory nidamental glands are mottled scarlet and reddish-brown and partly covered by the anterior tips of the nidamental glands. The nidamental glands have increased in size and are opaque. The oviduct is large and transparent, and its opening is halfway along the length of the ink sac. The oviducal gland is opaque. Eggs are visible at naked eye.	The testis is large and extends from the posterior tip of the stomach to the posterior apex of the mantle. It is opaque and ridged. The vas deferens is full of sperm and appears creamy white. Spermatophoric complex with a white streak. Spermatophoric sac without functional spermatophores. The penis extends half way the length of the ink sac. Hectocotylization of fourth left arm easily recognisable.
Stage IV	The accessory nidamental glands are mottled scarlet, reddish-brown and orange and are very large. The nidamental glands are large and exude a viscous fluid when cut and squeezed, the anterior tips overgrow the accessory nidamental glands. Eggs present in the proximal oviduct. The oviducal glands are creamy white. Ovary with eggs of different sizes. Free eggs found in the body cavity. Spermatheca may contain sperms in which case it appears as white spot on the ventral oral area	The testis extends anteriorly past the posterior tip of the stomach. It is large, opaque and ridged. Spermatophoric sac packed with functional spermatophores. The tip of the penis at level of the anus and ink sac opening. Vas deferens full of sperms. Hectocotylization complete.
Stage V	Same as IV. Distal oviduct with eggs. Spermatheca with sperms.	Same as IV. Penis with spermatophores.

2.3 *Illex coindetti* and *Todaropsis eblanae*

Although biological information on these species is being collected since the early 1990's based on bottom trawl surveys, the regular biological sampling of the shortfin squids *Illex coindetii* and *Todaropsis eblanae* from commercial landings (mainly trammel and gill nets) started in 2002. As both species partially share the same habitat, samples are characterized by a mixture of both, although *Todaropsis eblanae* is far more abundant in the commercial samples and *Illex coindetii* more abundant in the demersal scientific surveys, a reflection of differences in targeted depths. In the biological sampling conducted in the lab, for each individual we collect the weight, dorsal mantle length, sex and maturity stage, mantle weight and stomach fullness index. For the males the testis and spermatophoric complex are weighed separately, for females we take separate weights of the ovary, nidamental plus accessory glands, the oviducal gland plus distal oviduct and proximal oviduct. During bottom trawl surveys, all of the individuals captured are weighed, measured, sexed and their maturity staged. The maturity scale adopted for the long-fin squids is a five stages maturity scale adapted from Lipinski (1979) for *Illex illecebrosus* (Table 3).

Table 3. Shortfin squids maturity scale adopted.

	Females	Males
I. Juvenile	The reproductive structures are only visible at microscope. The oviducts and nidamental glands appear as very fine transparent strips. The ovary is translucent, membranous.	Spermatophoric complex is transparent or appears as a translucent spot. The testis is transparent or translucent spot. The testis is transparent and membranous.
II. Immature	The reproductive structures appear as translucent or whitish. The oviducts and nidamental glands form clearly visible translucent or whitish strips. The oviduct meander visible. Nidamental glands are small. The ovary clearly visible, in most cases without visible structures.	The reproductive structures appear as translucent or whitish. The separate parts of the spermatophoric complex are clearly and visible. The testis small and its structure invisible.
III. Preparatory	Meander of the oviduct is extended. The nidamental glands are enlarged, covering some internal organs. The structures inside the ovary are clearly visible	The vas deferens whitish or white, spermatophoric organ with white strip. The testis in most cases is white or pink, its structure is invisible.
IV. Maturing	The nidamental glands are large, also cover kidneys and distal part of the liver; the external glandular oviducts are fleshy and swollen. Plenty of eggs in the oviducts; the meanders hardly noticeable. The eggs not transparent/ roughly 95%/ and are pressed together at least in the proximal part of the oviduct. Is possible to find different stages of eggs in the distal part of the oviduct.	The vas deferens white, meandering, enlarged. Needham's sac long, with structureless whitish particles inside, but without formed spermatophores. The testis tight, crispy, its surface covered with structure.
V. Mature	As above, but the eggs are translucent/more then 60%/ at least in the proximal part of the oviduct. Cut open, the nidamental glands secrete a viscous substance.	As above, except that spermatophores are present in the Needham sac.

2.4 *Sepia officinalis*

The regular collection of biological information regarding the cuttlefish *Sepia officinalis* began in 2002 with samples collected from the commercial fleet landings (mostly trammel nets). In the biological sampling we collect individual weights, dorsal mantle lengths, sex and maturity, the mantle weight and the stomach fullness index. For males the testis and spermatophoric complex are weighed separately, and for females we weight the ovary plus proximal oviduct, nidamental plus accessory glands, the oviducal gland plus distal oviduct. The maturity stage scale adopted for cuttlefish is a simplified three stage maturity scale in which stage I, is immature, stage II, maturing and stage III, mature (Table 4).

Table 4. Three stages scale adopted to assess maturity in cuttlefish.

	Females	Males
I. Immature	Reproductive structure of reduced size, without eggs.	Reproductive structures of reduced size, without spermatophores
II. Maturing	oviducal glands developed, without eggs	Testis and spermatophoric complex developed without spermatophores
III. Mature	eggs fully formed in the ovary	spermatophores formed in the complex

3. Main Results and Problems

We believe that the extensive data collection on commercial cephalopod species conducted by IPIMAR over the years allows us to have an important perspective of the spawning cycles and minimum maturity sizes of the common octopus, longfin squids, shortfin squids and cuttlefish of commercial importance in Portugal. Regarding the interpretation of the macroscopic maturity scales, we identify some inaccuracies in the attribution of the maturity stages of *Octopus vulgaris*, which reproductive aspects we are specially addressing this year. By the sole evaluation of the macroscopic characteristics of the different reproductive structures, frequently we observe that in the same animal, different structures give different maturity stage indications. This problem leads to a subjective interpretation of the maturity scale often with different results if calibration exercises are attempted between technicians:

a) It is especially difficult to establish a frontier between males II and males III. In this case, the testis has often characteristics of a stage III but the spermatophoric complex and spermatophoric sac are still half full (stage II);

b) Another interpretation problem regards stage IV females. Normally due to the size and appearance of the oviducal gland, and size and appearance of the ovary (biggest organ in the mantle cavity, heterogeneous with yellow visible eggs) we tend to attribute a maturity stage IV, although, the gravimetric analysis done in some samples of ovaries of females in that condition shows that the oocytes are not fully developed at that stage.

Presently we need to validate some aspects of the common octopus maturity staging, focusing on independent functional aspects of gonads and accessory structures such as the Needham's complex and oviducal gland. A histological approach seems to be the most appropriate, although the histological processes described for fish in general seem to be inadequate for these invertebrates due to the form and the structure of the reproductive system of Cephalopods.

For the Workshop on Cephalopods Maturity Stages, we aim to present the maturity scale adopted for the five species sampled regularly in our lab, as well as the recent results of our study regarding the different reproductive strategies adopted by the *Octopus vulgaris* populations of different coastal areas of Portugal. Under the reproductive strategies of common octopus work, we are interested in discuss which histological methodologies are more useful to validate the macroscopic maturity scale adopted and also which methodologies (histological and/or gravimetric) should be adopted in order to determine total and potential fecundity of this and other cephalopod species.

We intend to bring to the workshop, our data on macroscopic maturity scales documented with a photographic collection of the relevant reproductive structures (Table 5).

Table 5. Summary of the information and collections presented to the Workshop on Cephalopods Maturity Stages.

	Maturity Scales	Macroscopic photos	Microscopic photos	Gonadal indices	Oral presentation
Octopus vulgaris					
Sepia officinalis					
Loligo vulgaris					
Illex coindetii					
Todaropsis eblanae					

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