Histological and morphological aspects of the sexual organs and condition in *Octopus vulgaris* from Moroccan Mediterranean waters

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Abstract. The common octopus, *Octopus vulgaris* (Cuvier, 1797), is the most important cephalopod in fisheries along the Moroccan Mediterranean coast. This work presents different stages of gonads development and allocation of energy during the development of germ cells in different phases of the reproductive tract of *O. vulgaris* in the study area. Monthly samples were collected during a year from 137 specimens, which are caught by commercial catches of trawling fleet. Based on microscopic and macroscopic observations of the reproductive system, the following stages of maturation for both sexes were determined: immature1, immature2, maturing, mature and post spawning. Histological examination of gonads shows that: vitellogenesis begins in December; gonad development in females occurs later than in males and spermiogenesis is active until mating; oviduct gland of most females in previtellogenesis stage stored sperm in spermathecae, showing that females copulate when they are immature. The spawning period begins in spring and extends until summer. The digestive gland constitutes the primary energy source for the spermatogenesis process.

Key Words: gonad development, energy allocation, oogenesis, spermatogenesis, reproductive system.

Introduction. The common octopus, *Octopus vulgaris*, is an exclusively coastal species, a semelparous cephalopod with a short life (Mangold-Wirz 1963), inhabiting mostly in coral reefs or rocks. In other areas it is more abundant over sandy, muddy bottom or sea grass. It is captured by various methods, mainly by trawlers (Borges et al 2000).Females stop feeding many weeks before the spawning period and die after the eggs hatch. Males die after mating (Hernàndez-García et al 2002). *O. vulgaris* is one of the most important harvested cephalopod in the world (Guerra 1997). In the Mediterranean Sea is the most important marketed octopus species (Sauer et al 2019). In the Moroccan Mediterranean *O. vulgaris* is also the main fished cephalopod species with a high economic value. The study area is located in the western basin of the Alboran Sea, representing the western part of the Mediterranean Sea. Atlantic water flows into the Mediterranean Sea through the Strait of Gibraltar, while an undercurrent of Mediterranean origin flows into the Atlantic Ocean (Morán & Estrada 2001). These particular hydrodynamic characteristics makes water richer in biological productivity (VargasYáñez et al 2009).

To understand the reproductive process, which represents a key factor in the management of a marine species (González et al 2011), it is necessary to study the development of gonads. To ensure a precise classification of the stages of development, it's important to combine microscopic to macroscopic analysis (Gonçalves et al 2002). Khallahi (2001), Golçalves et al (2002) and Rodriguez-Rua et al (2005) studied the gametogenesis of *O. vulgaris*. Other studies described different maturity scales of the gonad development of *O. vulgaris* by comparison between histological and morphological changes (Gonçalves et al 2002; Khallahi & Inejih 2002; Cuccu et al 2013b).

Condition is affected by reproduction (Cortez et al 1995) and the degree of this relationship differs between species (Otero et al 2007). In *O. vulgaris* there are two sources of energy devoted to maturation: mobilization of endogenous reserves (Tait 1986) or exogenous sources in recent study (Rosa et al 2004).

The aim of this work was: (1) to understand the oogenesis, spermatogenesis and the source of energy allowed for the reproduction of *O. vulgaris* from the Moroccan Mediterranean Sea, (2) to establish a sexual maturity scale through macroscopic and microscopic descriptions of the reproductive system and (3) to estimate the spawning season through the histological examination of the ovary.

Material and Method

Collection of data. Specimens of *O. vulgaris* were monthly sampled, from June 2013 to May 2014, by trawling fleet operating on the continental shelf, in a depth down to 200 m, and landed in the main ports (M'diq and Jebha) of the western coast of the Moroccan Mediterranean (Figure 1). Among the sample specimens, there were 83 females and 54 males. Body weight (BW) was determined for each octopus and the samples were dissected for the collection of the reproductive system. Gonad weight (GW) and the digestive gland weight (DGW) were measured to the nearest 0.1 g.

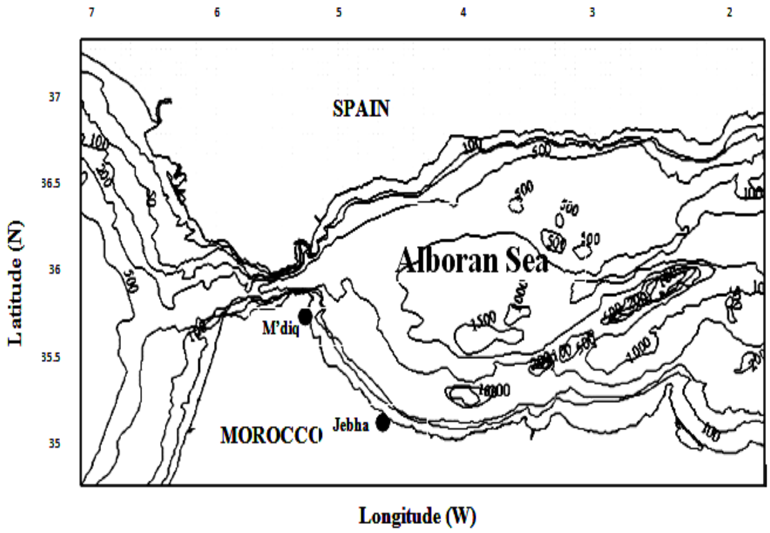


Figure 1. Map of the study area of *Octopus vulgaris* between M'diq (35.68° N / -5.32° W) and Jebha (35.21° N/-4.66° W).

Condition study. The condition of individuals was evaluated using the digestive gland index (DGI) (Castro et al 1992): DGI=DGW/(BW-DGW).

Macroscopic study. Macroscopic maturity stages were assigned to every octopus collected, using the scales proposed by Dia (1988) and Sanchez & Obarti (1993).

Microscopic analysis. For both sexes, the middle sections of the gonad at each macroscopic stage were collected for the histological analysis. Pieces of testis, ovary and oviducal gland (gland placed halfway along the oviducts in females) were fixed in the alcoholic Bouin solution and transferred for dehydration in increasing concentrations of ethanol, from 70 to 100%. Then each sample was embedded in paraffin. Sections were cut at 5 μm thickness using a biological rotary paraffin microtome and stained with hematoxylin and eosin before being examined through a light microscope. Different development levels of gonads were described using histological studies conducted by Khallahi (2001), Gonçalves et al (2002), Khallahi & Inejih (2002), Idrissi et al (2006) and Cuccu et al (2013b).

Statistical analysis. The correlation between GW and DGW was performed by the MATLAB software. DGI comparisons between the maturity stages were made by a Wilcoxon Mann Whitney test (α=0.05).

Results

Macroscopic and microscopic study. The macroscopic and histological study of the reproductive organs in both sexes of *O. vulgaris* allowed us to identify five stages which are described in Tables 1 and 2 and shown in Figures 2 and 3.

Table 1

Female *Octopus vulgaris* gonads: macroscopic (Ma) and microscopic (Mi) aspects of the ovary and oviducal gland (OG) at different maturity stages

Stage	*Description*
Previtellogenesis 1	Ma: The ovary and oviduct glands are small, round and translucent. Sometimes, a small white edged is present at the insertion of the oviduct in the glands. The oocytes are not visible in the ovary (Figure 1A). Mi: The oocytes have a spherical shape with a central nucleus and it is surrounded by a single layer of follicular cells. (Figure 1F) OG: Two compartments are present, the central and the peripheral glands, separated by a basal lamina, oviduct in the centre of the gland, while the spermathecae is absent. (Figure 2A).
Previtellogenesis 2	Ma: The ovary and the glands are larger but remain rounded. They gradually become creamy white, with even translucent parts. In the glands of the oviduct, a brown disc that may become dark surrounds the white border (Figure 1B). Mi: The oocyte has an ovoid shape and is surrounded by a double layer of follicle cells, with the nucleus displaced to the polar zone of the cell. (Figure 1G) OG: The first small spermathecae are formed without sperm inside. (Figure 2B, C).
Vitellogenesis	Ma: The ovary and glands, always spherical, become creamy white. The brown disc becomes dark. Oocytes become apparent but they are not yet individualized (Figure 1C) Mi: The ooplasm presents many morphological alterations. Follicular cells fit together in the form of cords which penetrate inside. The first production of yolk globules takes place. (Figure 1H) OG: The spermathecae, already storing sperm inside, increase their size proportionately with the size of the oviducal glands. (Figure 2D,E).
Mature	Ma: The ovary is larger and becomes yellow lemon. The glands are round at their first development stage, but later they become flatten. The brown disk grows, but without striation (it has not yet the form of a crown). The oocytes are already exposed and begin to be individually discernible (Figure 1D). Mi: During the vitelligenesis, the elaborated yolk forces the follicular syncytium to the periphery. The cytoplasme is completely filled with yolk granules. The oocyte is surrounded by the chorion, leaving a mature oocyte (Figure 1I).
Post spawning	OG: the majority of sparmathecae complete their development, having a large size and storing conspicuous sperm inside (Figure 2D, E). Cell residues are present (Figure 1J). Eggs with a peduncle are freed from their follicular sheath and descend the proximal oviduct one by one, passing through the oviducal glands to be fertilized by the sperm (Figure 2D, E).

In females, the identified stages were: previtellogenesis 1, previtellogenesis 2, vitellogenesis, mature, post spawning (Table 1). In males, there were determined also 5 stages: immature1, immature2, maturing, mature, post spawning.

Histological analysis in the female demonstrate that the ovary contains: oogonia scattered between the follicular cells, richly vascularized connective tissue, immature and mature oocytes. We differentiate previtellogenesis 2 from previtellogenesis 1, in the immature stages, through the formation of a second layer of follicular cells surrounding the oocyte. The formation of yolk by the invagination of follicular cells within the oocyte characterized the maturing stage (vitellogenesis); it’s the mostly present stage during December, which means that vitellogenesis begins in this month. The end of vitellogenesis and the regress of follicular envelope characterize the mature oocyte. Post spawning stage marks the presence of residual cells. Mature and post spawning stages are more present in spring and summer; so these two seasons represent the spawning period of the *O. vulgaris* in the study region. The cells observed at each sexual development stage showed an asynchronous oogenesis progression. The microscopic analysis of the oviducal gland showed the presence of two glandular regions and of spermatozoa attached to the wall of spermathecae since the immature stages.

In males, the beginning of genital maturation is marked by the development of seminiferous tubules, where the process of spermatogenesis is following a centripetal and regular sequence. From the periphery to the center of the seminiferous tubule, there can be observed the spermatogonia, spermatocytes, spermatids and the differentiation of spermatids into spermatozoa, which are located in the central lumen of the cyst. The presence of mature males throughout the year reveals a fast spermatogenesis process.

Table 2

Octopus vulgaris: macroscopic (Ma) and microscopic (Mi) aspect of the male gonad at different maturity stages

Stage	*Description*
Immature1	Ma: Small compact and translucid gonad without spermatophores (Figure 1K). Mi: The beginning of the spermatogenesis is marked by the development of seminiferous tubules. The spermatogonia are less differentiated close to the tubule walls (Figure 1P).
Immature2	Ma: Some spermatophores are present, without a spot at the base of spermatophoric complex (Figure 1L). Mi: Spermatogonia are divided and become spermatocytes, which have a smaller nucleus (Figure 1Q).
Maturing	Ma: The testicle becomes spherical and a neat spot becomes visible at the base of the spermatophoric complex (Figure 1M). Mi: Spermatocytes are followed by spermatids; they are initially spherical and lengthen thereafter (Figure 1R).
Mature	Ma: A full translucent gonad, with a neat spot, can be observed (Figure 1N). Mi: The mature specimens are marked, due to the differentiation of spermatids into spermatozoa (which are in the central lumen of the cyst) (Figure 1S). At this stage there are all types of cells, with abundant spermatozoa in the central lumen.
Post spawning	Ma: A flaccid greyish testicle can be observed, with packed spermatophores inside (Figure 1O) Mi: After mating, the testicular cyst shrinks and the central lumen becomes empty of sperm. A few spermatocytes, spermatidia and spermatozoa are observed (Figure 1T).

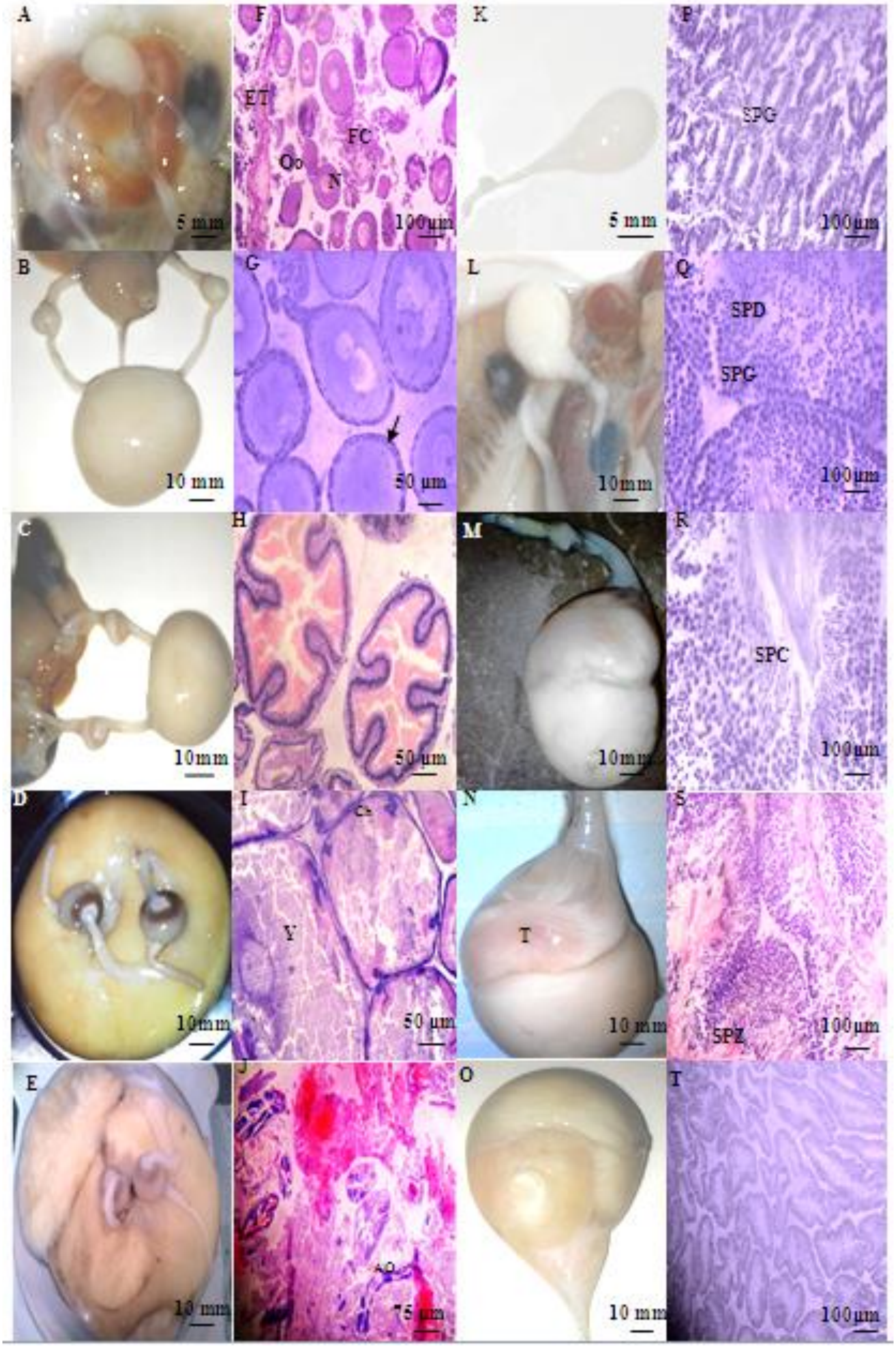


Figure 2. *Octopus vulgaris*: macroscopic and microscopic photos at five maturity stages: stage 1 (females: A, F; males: K, P), stage 2 (females: B, G; males: L, Q), stage 3 (females: C, H; males: M, R), stage 4 (females: D, I; males: N, S), stage 5 (females: E, J; males: O, T). ET-epithelium tissue; Oo-oogonia; FC-follicle cells; N-nucleus; G-double layer of follicle cells; Ch-chorion; Y-yolk; AO-atretic oocyte; SPG-spermatogonia; SPC-spermatocytes; SPD-spermatids; SPZ-spermatozoa; T-spot.

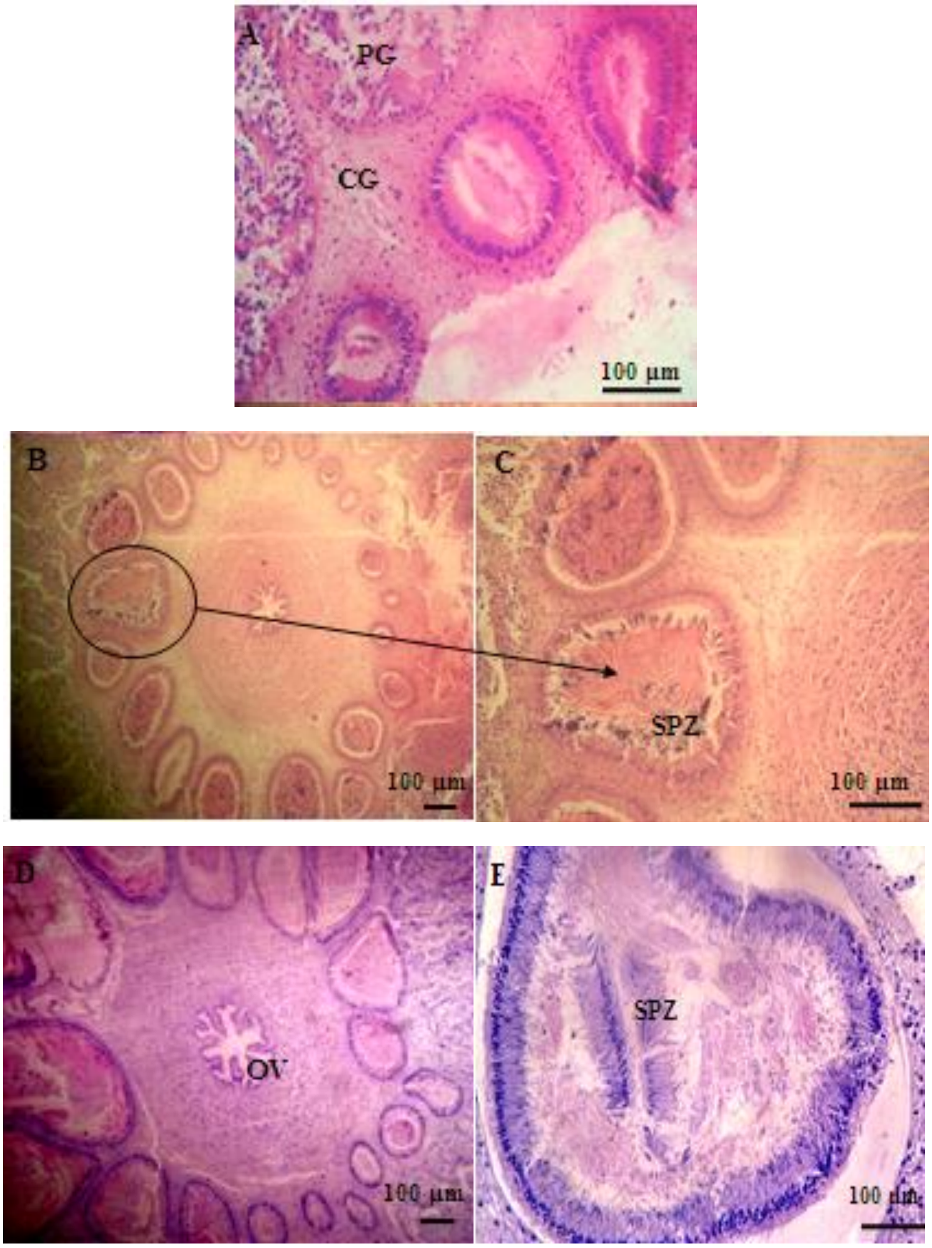


Figure 3. Transversal cut of the oviducal gland of *Octopus vulgaris* at different maturity stages. (A): OG of immature females without sperm in spermathecae; (B), (C): stage II showing spermathecae with spermatozoa inside; (D), (E): stages III, IV and V, showing large size spermathecae. PG-peripheral glands; CG-central glands; OV-oviduct; SPZ-spermatozoa.

Condition and allocation of energy. The condition, expressed as DGI, varied as a function of the sexual maturity stage. In males, there was a decrease in the DGI from immature to mature individuals. In females, the condition increased with the maturation stage (Figure 4). There was no statistically significant difference between sexes ($p>0.05$).

In males, the DGW increased significantly with the GW ($R^2=0.678$, $p<0.05$), indicating that growth of testis was made at the expense of the digestive gland reserves. In females, a poor correlation between GW and DGW was observed ($R^2=0.414$, $p>0.05$), suggesting that the digestive gland is not the source of energy devoted to the ovary development (Figure 5).

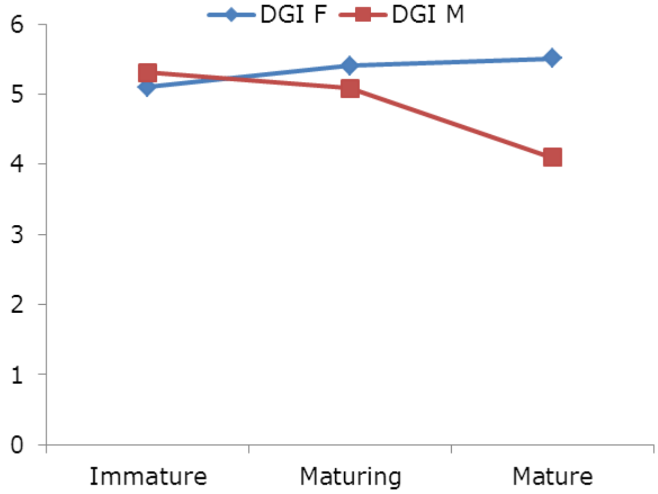
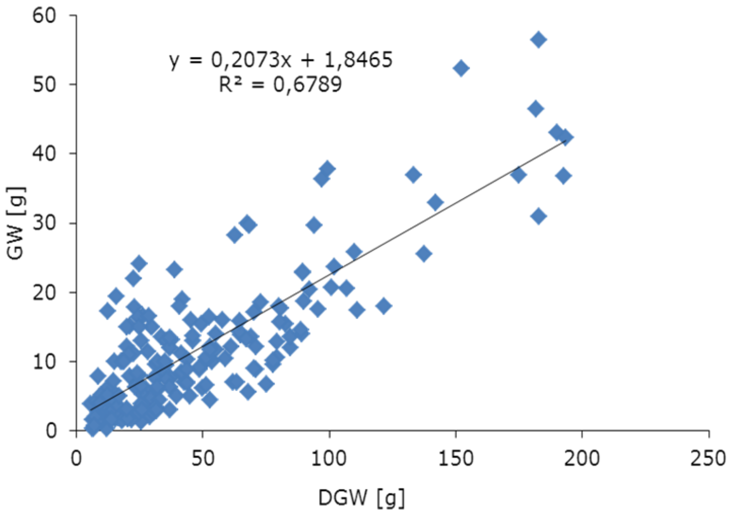


Figure 4. Changes in the digestive gland index (DGI) appear as a function of the maturity stage in the *Octopus vulgaris* females and males. Immature = Immature1 + Immature2; Mature = Mature + post spawning.

A



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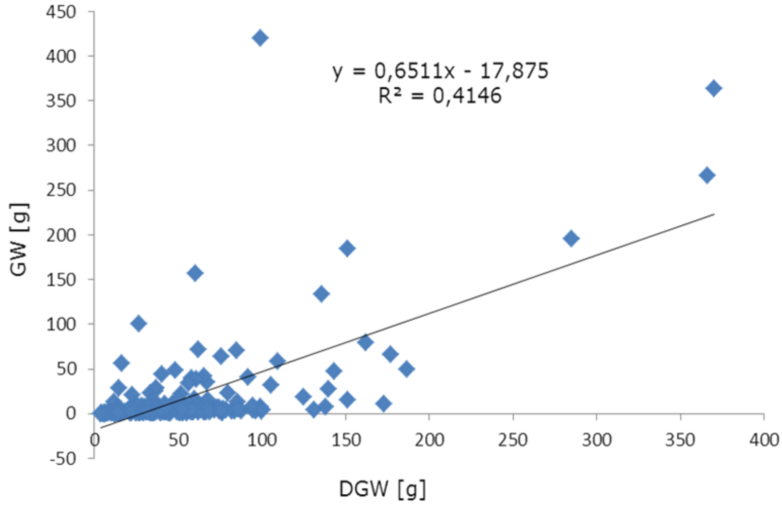

Figure 5. Correlation between digestive gland weight (DGW) and gonad weight (GW) in in males (A) and females (B).

Discussion

Microscopic stages of gonads. Histological examination of the ovary revealed that vitellogenesis begins in December by a proliferation of the follicular cells that form follicular cords, invading the ooplasm oocyte, which will trigger the synthesis and deposition of yolk until the spawning period, when the oocytes become mature. Different stages of development of the oocytes are present in mature ovary, revealing that oogenesis is not synchronous in all oocytes, as confirmed by Gonçalves et al (2002), Olivares et al (2001) and Idrissi et al (2006).

Storing spermatozoa after mating inside the sparmathecae of oviducal glands of the immature females is a common pattern in cephalopods (Fernández-Núñez et al 1996). This could explain the earlier maturation of males. The white denticulate apical region appearing in the oviducal glands (Immature stage), is a morphological indicator of presence of spermatozoa inside the spermathecae (Cuccu et al 2013b). When mature oocytes reach the central cavity of the oviducal glands, sperms are mobilized from spermathecae (Frösh & Marthy 1975). Histochemical differences between the two glandular regions reflect the role of the oviducal glands in storing sperm and secreting substances (Di Cosmo et al 2001).

Through this study we have identified five stages of development of the ovarian follicle: previtellogenesis 1, previtellogenesis 2, vitellogenesis, mature and post-spawning. Other studies also investigated the sexual development in the female *O. vulgaris*: Khallahi (2001) identified three stages: previtellogenesis, vitellogenesis and post-spawning, Olivares et al (2001) described nine stages of oogenesis, Idrissi et al (2006) identified eight stages (in the Moroccan Atlantic *O. vulgaris*) and Cuccu et al (2013b) identified sex stages of ovarian development (in the Mediterranean *O. vulgaris*).

The beginning of genital maturation in males is marked by the development of seminiferous tubules, where the process of spermatogenesis is initiated by a transformation of spermatogonia in spermatocytes, after the first meiotic division. The second meiotic division transforms the spermatocytes into spermatidia, which differentiate into spermatozoa with a flagellum in the central lumen of the cyst. This process was similarly described by Rodríguez-Rúa et al (2005) and Idrissi et al (2006).

Spermatogenesis in the area of investigation is active throughout the annual cycle, with five stages of gonad development. Olivares et al (2001), Khallahi (2001), Idrissi et al (2006) and Cuccu et al (2013b) identified respectively: three, four, five and six stages in their study areas.

The particularity of the present study is that we have analyzed a high number of gonads in each macroscopic stage: 54 males (8 immature1, 10 immature2, 10 maturing, 24 mature, 2 post spawning) and 83 females (21 previtellogenesis 1, 19 previtellogenesis 2, 24 vitellogenesis, 14 mature, 5 post spawning), which allowed us to identify the most frequently observed microscopic stages. The presence of mature and post spawning stages indicated a spawning period over the spring and summer (in the study region), the same results being reported in other studies conducted in the Mediterranean basin (González et al 2011; Cuccu et al 2013a).

Energy allowed to gonads development. During their maturation process, gonads (like other cells) need energy and the digestive gland was investigated as the candidate source, in the current study which demonstrated that females condition increased with the maturation stage and that the relationship between DGW and OW shows a poor correlation. In males, we detected a DGI decrease with maturation, while the DGW and GW development shows an isometric correlation. These results are in agreement with those obtained by Rosa et al (2004) and Otero et al (2007), suggesting that maturation in females is reached directly from exogenous sources (food) rather than from endogenous sources (digestive gland or muscles), while in males the digestive gland seems to be the source of energy for the spermatophores production.

Conclusions. The information about the biology of *O. vulgaris* from the Moroccan Mediterranean waters is very limited. The presently reported study provides the first histological and morphological examination of the gonads and condition of this species, resulting in data which can be used in management strategies of *O. vulgaris* in this area.

Conflict of interest. The authors declare no conflict of interest.

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