Gonad maturation in *Octopus vulgaris* during ongrowing, under different conditions of sex ratio

Juan Estefanell, Juan Socorro, Francisco J. Roo, Rafael Guirao, Hipólito Fernández-Palacios, and Marisol Izquierdo


*Octopus vulgaris* is a suitable candidate for aquaculture, but there are problems with breeding in captivity, such as aggressive behaviour among males and the frequent death of females after the eggs hatch. To avoid these problems and further understand the sexual maturation of common octopus in captivity, males and females were reared together and separately under similar culture conditions. In all trials, the initial rearing density was 10 kg m⁻³. Females (n = 15, sex ratio 0:1) and males (n = 11, sex ratio 1:0) were kept in circular tanks, and a mixed group (n = 209, sex ratio 4:1) in floating cages. Trials started in November 2008 and octopuses from each treatment were examined macroscopically and histologically in December and January to assess sexual maturation. All the males matured, regardless of the sex ratio during rearing, as did all females in the mixed group. In contrast, a large proportion of the females kept isolated from males was still immature in December and January. Although maturation was successful in floating cages, there was 76% mortality there, in contrast to the zero mortality in tanks. Moreover, most of the dead octopuses from the cages were in post-reproductive condition, with a low digestive gland index, suggesting that this was natural post-reproductive mortality. Therefore, sex segregation is deemed advantageous to avoiding early mortality.

**Keywords:** digestive gland, gonad maturation, mortality, *Octopus vulgaris*.

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**Introduction**

The common octopus (*Octopus vulgaris*) is a merobenthic species that lives from the coast to the outer edge of the continental shelf, up to 200 m deep (Guerra, 1992), all along the western and eastern Atlantic coasts, in the Mediterranean Sea, and in the North West Pacific, around Taiwan and Japan (Söller et al., 2000; Warnke et al., 2004). It has been proposed as a candidate for diversification of European aquaculture, the further development of which is constrained by market saturation because of the small number of species produced commercially (Vaz-Pires et al., 2004). The common octopus commands high market prices and there is increasing demand for it in Europe, South America, and Asia (Vaz-Pires et al., 2004). It grows rapidly (Mangold, 1983) and easily adapts to culture conditions (Iglesias et al., 2000), so would seemingly have an excellent potential for culture (Socorro et al., 2005; Chapela et al., 2006; Rodríguez et al., 2006; García García et al., 2009). Despite unsolved problems in rearing the larvae (Navarro and Villanueva, 2000, 2003; Villanueva et al., 2002, 2004, 2009; Iglesias et al., 2004, 2006, 2007; Villanueva and Bustamante, 2006), a few companies based in Spain (Galicia and the Canary Islands) already ongrow wild subadults in floating cages, using low-price species as feed.

One factor influencing the industrial development of octopus culture is sexual maturation under rearing conditions. Male octopuses die after mating, and females die immediately after the eggs hatch (Guerra, 1992; Hernández-García et al., 2002) and can lose as much as 30–60% of their initial body weight during egg-laying (Iglesias et al., 2000). Although there have been several successful ongrowing experiments with males and females reared together (Socorro et al., 2005; Rodríguez et al., 2006; García García et al., 2009), the effect of sex segregation during broodstock maturation has not been studied well, and the results are contradictory. Whereas Chapela et al. (2006) stated that both males and females grow at similar rates even though 60% of females spawn during summer, Rey Méndez et al. (2003) documented slower growth and greater survival in females than in males.

Sexual maturation of the common octopus in wild populations has been extensively studied (Fernández Nuñez et al., 1996; Quetglas et al., 1998; Hernández García et al., 2002; Silva et al., 2002; Rodríguez de la Ruía et al., 2005; Otero et al., 2007), but little attention has been paid thus far to evaluating sexual maturation under rearing conditions (Mangold and Boletzky, 1973; Cerezo et al., 2007). The aim of the present work was to study gonad maturation in males and females reared together and
separately under similar culture conditions, compared with animals taken from local fisheries. The effect of sexual maturation on the digestive gland index (DGI, an indicator of condition; Cerezo Valverde et al., 2008) and under two dietary treatments is also discussed.

Material and methods
Capture and acclimation of the stock
Octopuses were caught in Mogán (Gran Canaria, Canary Islands). Local fishers used cylindrical trawls (1.5 m diameter, 0.4 m high, with a metallic net of 31.6 mm mesh) placed 20–30 m deep. Octopuses were kept on board in open flow-through seawater reservoirs and transported to the laboratory by truck in 500-l square tanks. This operation took 60–80 min and oxygen was provided so that its concentration would not be limiting (＞12 ppm).

Acclimatization was in rectangular 1.5 m³ tanks, and (previously frozen) blue crab (Portunus pelagicus) was provided ad libitum once daily. After 1 week, the octopuses were PIT-tagged subcutaneously on left arm III, using immersion in seawater with 1.5% ethanol (96%) as anaesthetic, following the procedures described by Estefanell et al. (2007). One week later, tagging was verified as successful by reading the PIT digits in all octopuses, prior to the start of the experiment.

Experimental design
For males and females reared separately, octopuses were kept in circular tanks of 1 m³ and the water level was kept up to 500 l to prevent the octopuses from escaping. The initial density in these tanks was 9.9 ± 1.2 kg m⁻³ of water. In all, 11 males were split between two tanks (M₁ and M₂) and 16 females among three tanks (F₁, F₂, and F₃; Table 1). Where males and females were being reared together (M:Fcontrol and M:Ffish), two galvanized stainless-steel floating cages (3 m long, 1.5 m wide, and 3 m high) with 20 mm mesh size and a total internal volume of ~10 m³ were used. More than 100 octopuses, male:female sex ratio 4:1, were placed in each cage, and the initial density was 10.9 ± 0.2 kg m⁻³ of water (Table 1).

Different rearing systems were used for practical reasons. The relative scarcity of females provided by the local fishers (natural sex ratio 4:1), along with the difficulty gathering the octopuses, led us to place the single-sex groups in tanks.

Rearing conditions
Regardless of rearing system, shelters (PVC T-shaped tubes, 160 mm diameter) and shadowing nets were provided (Villanueva, 1995; Hanlon and Messenger, 1996). In tanks, an open flow-through seawater system was adjusted to 500 l h⁻¹. All treatments were fed ad libitum 6 d per week under the natural photoperiod. Tank treatments and one floating cage (M:Fcontrol) were fed a “control” diet based on a 40–60% bogue–crab mix on alternate days (Garcia Garcia and Cerezo Valverde, 2006). The other floating cage (M:Ffish) was fed a monospecific diet of bogue (Boops boops). The daily feeding rate was 6% of initial biomass for bogue, and 10% for crab. The bogue used in the trials were provided by local farms as discard species from offshore sea-bream cages. The blue crab was purchased from a local trader. The trials lasted 11 weeks in the floating cages (1 November 2008–22 January 2009) and 8 weeks in the tanks (26 November 2008–24 January 2009). Mean water temperature and oxygen levels, measured once a day, were 18.4 ± 0.4°C and 7.1 ± 0.2 ppm in tanks and 19.0 ± 1.0°C and 7.0 ± 0.3 ppm in floating cages, respectively. Light intensity, measured at the water surface with a portable luxometer (Model HT170N; Italy), was 30–50 lux in the tanks and 250–300 lux in the floating cages. Octopuses were observed by scuba three times per week; dead animals were removed daily.

Sampling procedure
One tank per treatment (n = 5) was randomly sampled in December, and all the tanks were sampled in January. From each cage, six octopuses were sampled randomly in December (by scuba), and all surviving animals at the end of the experimental period. We also dissected any dead octopuses collected each month from the cages that showed no signs of cannibalism. Animals were sacrificed by immersion in ice-cold seawater. Finally, during the experiment, 11 males and 1 female from the local fishery (wild group) were sacrificed after catching to assess the biological parameters in wild populations.

Biological parameters
Mortality was recorded daily. After sacrifice, all animals were weighed and the sex was determined. The following indices were calculated, and data were expressed in relation to a monthly mean value of each parameter.

(i) Reproductive status in males [where W₅ is the Needham complex + spermatophoric sac weight (in g), W₇ the testis weight (in g), and W the octopus weight (in g)]:
(a) the Hayashi index, H, as modified by Guerra (1975), i.e.
H₅ = W₅/(W₅ + W₇);
(b) the gonadosomatic index, GSI (Otero et al., 2007):
GSI₅ = (W₅/W − W₅) × 100.

(ii) Indices for the reproductive status of females [where WOG is the oviducal gland weight (in g) and WO the ovary weight (in g)]:
(a) the Hayashi index, H, as modified by Guerra (1975), i.e.
H₇ = WOG/(WOG + WO);
(b) the gonadosomatic index, GSI (Otero et al., 2007):
GSI₇ = (WOG/W − WOG) × 100.

<table>
<thead>
<tr>
<th>Rearing system</th>
<th>Treatment</th>
<th>Number of tanks or cages</th>
<th>Number per treatment</th>
<th>Number of males</th>
<th>Number of females</th>
<th>W₅ (g)</th>
<th>W₇ (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tank</td>
<td>Mₙ</td>
<td>2</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>1122 ± 267</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Fₖ</td>
<td>3</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>−</td>
<td>865 ± 226</td>
</tr>
<tr>
<td>Floating cage</td>
<td>M:Fcontrol</td>
<td>1</td>
<td>109</td>
<td>88</td>
<td>21</td>
<td>1041 ± 249</td>
<td>916 ± 322</td>
</tr>
<tr>
<td></td>
<td>M:Ffish</td>
<td>1</td>
<td>105</td>
<td>84</td>
<td>21</td>
<td>1060 ± 326</td>
<td>888 ± 267</td>
</tr>
</tbody>
</table>

Table 1. The rearing system used for the experiment, showing the initial body weight of males (W₅) and females (W₇) ± s.d.
(iii) Digestive gland index: 

\[ \text{DGI} = \left( \frac{W_{\text{DG}}}{W} \right) \times 100 \]

where \( W_{\text{DG}} \) is the digestive gland weight (in g).

In addition, a macroscopic maturation stage (Dia and Goutschine, 1990) was assigned to every octopus collected (I, immature; II, maturing; III, mature; IV, post-reproductive), for both males and females.

**Histological and statistical analyses**

Histological studies were performed on gonad and digestive gland from sacrificed octopuses obtained from the floating cages. The organs were fixed in 10% neutral-buffered formalin, embedded in paraffin, then stained with haematoxylin and eosin (H&E) for optical examination (García del Moral, 1993). Micrographs of gonads were taken from the paraffin sections using a Nikon Microphot-FXA microscope and an Olympus DP50 camera.

In terms of the statistics, means and standard deviations were calculated for initial body weight, initial rearing density, and temperature. Data from each treatment were submitted to the Levene test, and when that showed no difference in variance among groups \((p \geq 0.05)\), individual means were compared using the one-way ANOVA along with the Tukey test for multiple comparisons (Sokal and Rolf, 1995).

**Results**

No differences in initial body weight density (Table 1) and rearing temperature were found among treatments. The mean values for each index calculated for males and females are listed in Table 2. Daily observations revealed mating from the start of the experiment. In females, \( H_f \) decreased with maturity, but then increased sharply after spawning. In males, \( H_M \) increased with maturity. In females, both GSI and DGI increased with maturity, but collapsed after egg-laying. In males, no immature or maturing stages were found regardless of treatment. The DGI decreased after mating and the GSI remained static. No mortality was recorded in any of the male-only or female-only treatments, but in cages, mortality reached 76 and 77% for M:F\text{control} and M:F\text{fish}, respectively.

The numbers of individuals dissected per treatment, either sacrificed or collected dead, final weight, \( H \) index, GSI values, and DGI values are listed in Table 3 for males and in Table 4 for females. Macroscopic maturity stages in males and females from all treatments are shown in Figures 1 and 2, respectively. Males were consistently mature throughout the experiment, and octopuses collected dead, regardless of sex and diet, were mainly in the post-reproductive stage (IV). In contrast, a smaller proportion of the males sacrificed was at stage IV. In terms of females, data were absent from random sampling in floating cages in December, and wild specimens (those dissected immediately after capture) were scarce throughout the experimental period. The females sampled from the cages in January were mainly at stage IV, in contrast to females kept separately from males in tanks. In fact, in the tank treatment, immature females represented 40 and 80% of those sampled in December and January, respectively.

In terms of histology, Figure 5 shows a mature and a post-reproductive testis and digestive gland. The lumen was full of spermatocytes in the mature testis (Figure 5a), whereas few spermatozoa and abundant empty spaces in the lumen were indicative of the spermatocytes in the post-reproductive testis (Figure 5a), whereas few spermatozoa and abundant empty spaces in the lumen were indicative of the spermatocytes having been expelled in the post-mating stage.

**Table 2.** Mean value (± s.d.) for each index calculated for female and male O. vulgaris, the Hayashi index modified by Guerra (\( H \)), the GSI, and the DGI.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Maturity stage</th>
<th>Description</th>
<th>( H )</th>
<th>GSI (%)</th>
<th>DGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>I</td>
<td>Immature</td>
<td>0.21 ± 0.06</td>
<td>0.20 ± 0.09</td>
<td>4.07 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Maturing</td>
<td>0.14 ± 0.02</td>
<td>0.10 ± 0.15</td>
<td>4.21 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Mature</td>
<td>0.06 ± 0.02</td>
<td>3.45 ± 2.05</td>
<td>4.52 ± 1.35</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Post-reproductive</td>
<td>0.47 ± 0.06</td>
<td>0.46 ± 0.14</td>
<td>0.98 ± 0.32</td>
</tr>
<tr>
<td>Male</td>
<td>III</td>
<td>Mature</td>
<td>0.54 ± 0.08</td>
<td>0.39 ± 0.10</td>
<td>2.71 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Post-reproductive</td>
<td>0.78 ± 0.05</td>
<td>0.58 ± 0.17</td>
<td>1.14 ± 0.58</td>
</tr>
</tbody>
</table>

**Table 3.** The number of male O. vulgaris dissected (sacrificed or collected dead, \( n \)), weight, Hayashi index modified by Guerra (\( H \)), GSI, and DGI in December and January (mean values ± s.d.).

<table>
<thead>
<tr>
<th>Month</th>
<th>How collected</th>
<th>Treatment</th>
<th>( n )</th>
<th>Weight (g)</th>
<th>( H )</th>
<th>GSI (%)</th>
<th>DGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December</td>
<td>Sacrificed</td>
<td>( M_1 )</td>
<td>5</td>
<td>1 648 ± 529</td>
<td>0.52 ± 0.12</td>
<td>0.43 ± 0.08</td>
<td>2.93 ± 1.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( M_{\text{fish}} \text{control} )</td>
<td>6</td>
<td>2 943 ± 1 369</td>
<td>0.66 ± 0.16</td>
<td>0.37 ± 0.10</td>
<td>1.89 ± 0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( M_{\text{fish}} )</td>
<td>6</td>
<td>2 117 ± 596</td>
<td>0.65 ± 0.16</td>
<td>0.44 ± 0.06</td>
<td>1.74 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>4</td>
<td>1 187 ± 78</td>
<td>0.43 ± 0.09</td>
<td>0.38 ± 0.17</td>
<td>4.39 ± 1.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collected dead</td>
<td>( M_{\text{fish}} \text{control} )</td>
<td>23</td>
<td>1 400 ± 453</td>
<td>0.76 ± 0.07</td>
<td>0.61 ± 0.13</td>
<td>1.52 ± 0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( M_{\text{fish}} )</td>
<td>18</td>
<td>1 624 ± 609</td>
<td>0.77 ± 0.10</td>
<td>0.65 ± 0.26</td>
<td>1.20 ± 0.40</td>
</tr>
<tr>
<td>January</td>
<td>Sacrificed</td>
<td>( M_2 )</td>
<td>6</td>
<td>2 168 ± 777</td>
<td>0.50 ± 0.09</td>
<td>0.45 ± 0.16</td>
<td>3.77 ± 1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( M_{\text{fish}} \text{control} )</td>
<td>14</td>
<td>3 021 ± 1 373</td>
<td>0.65 ± 0.12</td>
<td>0.36 ± 0.07</td>
<td>1.70 ± 0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( M_{\text{fish}} )</td>
<td>12</td>
<td>2 957 ± 930</td>
<td>0.59 ± 0.11</td>
<td>0.41 ± 0.10</td>
<td>2.52 ± 1.62</td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>7</td>
<td>1 883 ± 344</td>
<td>0.41 ± 0.04</td>
<td>0.33 ± 0.09</td>
<td>3.46 ± 0.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collected dead</td>
<td>( M_{\text{fish}} \text{control} )</td>
<td>14</td>
<td>2 358 ± 1 250</td>
<td>0.73 ± 0.10</td>
<td>0.52 ± 0.14</td>
<td>1.28 ± 0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( M_{\text{fish}} )</td>
<td>12</td>
<td>1 697 ± 514</td>
<td>0.80 ± 0.06</td>
<td>0.57 ± 0.09</td>
<td>1.06 ± 0.37</td>
</tr>
</tbody>
</table>
Table 4. The number of female *O. vulgaris* dissected (sacrificed or collected dead, \(n\)), weight, Hayashi index modified by Guerra (\(H\)), GSI, and DGI in December and January (mean values ± s.d.).

<table>
<thead>
<tr>
<th>Month</th>
<th>How collected</th>
<th>Treatment</th>
<th>(n)</th>
<th>Weight (g)</th>
<th>(H)</th>
<th>GSI (%)</th>
<th>DGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December</td>
<td>Sacrificed</td>
<td>F_1</td>
<td>5</td>
<td>1348 ± 349</td>
<td>0.12 ± 0.11</td>
<td>2.99 ± 2.89</td>
<td>4.73 ± 0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M:F control</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M:F fish</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wild</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Collected dead</td>
<td>M:F control</td>
<td>4</td>
<td>831 ± 163</td>
<td>0.24 ± 0.18</td>
<td>1.53 ± 1.57</td>
<td>1.23 ± 0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M:F fish</td>
<td>4</td>
<td>861 ± 285</td>
<td>0.37 ± 0.21</td>
<td>1.42 ± 2.07</td>
<td>0.97 ± 0.40</td>
</tr>
<tr>
<td>January</td>
<td>Sacrificed</td>
<td>F_2 – 3</td>
<td>10</td>
<td>2009 ± 188</td>
<td>0.10 ± 0.03</td>
<td>0.87 ± 0.73</td>
<td>4.92 ± 0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M:F control</td>
<td>1</td>
<td>800</td>
<td>0.42</td>
<td>0.75</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M:F fish</td>
<td>1</td>
<td>120</td>
<td>0.45</td>
<td>0.49</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wild</td>
<td>1</td>
<td>192</td>
<td>0.08</td>
<td>0.65</td>
<td>4.71</td>
</tr>
<tr>
<td></td>
<td>Collected dead</td>
<td>M:F control</td>
<td>5</td>
<td>923 ± 123</td>
<td>0.44 ± 0.04</td>
<td>0.35 ± 0.07</td>
<td>0.99 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M:F fish</td>
<td>3</td>
<td>745 ± 176</td>
<td>0.55 ± 0.03</td>
<td>0.56 ± 0.10</td>
<td>1.24 ± 0.56</td>
</tr>
</tbody>
</table>

(Figure 5b). Histological examination revealed evidence of spermatogonia, spermatocytes, spermatids, and spermatozoa in mature testes. In the digestive gland, octopuses in the post-reproductive state showed degenerative vacuolization in the parenchyma (Figure 5d) compared with that of mature animals (Figure 5c).

**Discussion**

Under rearing conditions, *O. vulgaris* breed frequently regardless of the time of year (Mangold and Boletzky, 1973; Iglesias et al., 2000; Estefanell, 2006). In the Canary Islands, *O. vulgaris* in the wild breed all year-round, with two periods of main activity, one from January to July, with a peak in April, and a second in autumn (October–November; Hernández-García et al., 2002). In the current experiment, males were constantly mature throughout the period of sampling, irrespective of treatment (Figure 1), a finding that supports data obtained under rearing conditions (Mangold and Boletzky, 1973; Cerezo et al., 2007) and several previous reports from wild populations in the Atlantic Ocean (Guerra, 1979; Caveriviere et al., 2002; Silva et al., 2002; Carvalho and Sousa Reis, 2003; Oosthuizen and Smale, 2003; Rodríguez de la Rúa et al., 2005; Otero et al., 2007) and
Mediterranean (Quetglas et al., 1998). In contrast, a large proportion of the females kept isolated from males remained immature or maturing (Figure 2), suggesting that the presence of males may promote female sexual maturation. Indeed, sex steroid hormones have been reported in *O. vulgaris* of both sexes (D’Aniello et al., 1996; Di Cosmo et al., 2001), and they could act as pheromones if released to the water, coordinating maturation in both sexes. Therefore, in sex-segregated females, the absence of hormones from the opposite sex could postpone maturation. On the other hand, females matured in cages where the light intensity at the surface was nine times higher than in tanks inside the experimental facilities, contradicting the statement of Mangold (1987) that high light intensity can retard sexual maturation and that low light intensity can stimulate gonad development in aquarium-held octopuses.

The few females caught in random sampling in cages during December was probably the result of breeding activity then, because females remain in their dens cleaning, ventilating, and caring for the egg strings (Guerra, 1992). It may also explain the few females caught in the wild before and during the experiment. Progressively lower values of $H_F$ were recorded from the start of winter to spring in females living in the wild around Gran Canaria (Hernández García et al., 2002), and deviations from a 1:1 sex ratio were attributed to reproductive behaviour or the sampling strategy (Silva et al., 2002).

In the cages, large proportions of octopuses in post-reproductive state were found (Figures 1 and 2) contrary to the findings of Cerezo et al. (2007). Daily observations showed mating behaviour throughout the experiment, which agrees with the findings of Iglesias et al. (2000). Occasionally, two males were seen introducing a hectocotylus into the same female at the same time. Most octopuses collected dead from either treatment were at the post-reproductive stage, which demonstrates a clear relationship between mating processes under rearing conditions and mortality, recorded here for the first time in floating cages, supporting earlier observations in tanks (Hernández García et al., 2002).

Histological examination confirmed the maturity stages obtained from macroscopic observation and the Hayashi index. Therefore, the presence of spermatogonia/spermatocytes in the tubular wall and spermatids/spermatozoa in the central lumen are evidence of maturing and mature stages in male *O. vulgaris*, and empty spaces in the lumen indicate that spermatozoa have been expelled after mating (Rodríguez de la Rúa et al., 2005). On the other hand, the presence of cells at all stages of maturation suggests different phases of gonad development at the various maturity stages of the testes, as found in the testis of maturing or mature *Octopus mimus* (Avila-Poveda et al., 2009).

In the present experiment, female GSI and DGI increased with sexual maturation, but this was not the case for males (Table 2; Silva et al., 2002; Rodríguez de la Rúa et al., 2005; Otero et al., 2007). The significantly higher DGI in mature than in post-mating *O. vulgaris* (Figure 3) supports other authors’ findings (Otero et al., 2007). A decrease in DGI in post-mating *O. mimus* (Cortez et al., 1995) and *Sepia officinalis* (Castro et al., 1992) has been documented.

In contrast to the findings of Otero et al. (2007), the correlation between $H$ and GSI was very poor, probably related to the fact that few females in an immature or maturing state were analysed here and that few post-reproductive animals were available to Otero et al. On the other hand and also according to Otero et al. (2007), DGI and $H$ are inversely correlated in both sexes (Figure 4).

For egg production, *O. vulgaris* uses energy directly from food, rather than from stored resources (Rosa et al., 2004; Otero et al., 2007).
2007). In our work, sexual maturation of both males and females did not seem to be influenced by diet, because most, irrespective of diet, were in a mature or post-reproductive stage.

To conclude, under the experimental conditions described, there was a clear increase in mortality attributable to the reproductive process. Deterioration of the gonads was confirmed by histological examination (Figure 5) and by a massive decrease in digestive gland weight in post-reproductive animals. Consequently, further work needs to evaluate the benefits of segregating by sex under rearing conditions if mortality reduction and an increase in profitability of octopus culture are desired.

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