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Reproductive strategy in jumbo squid *Dosidicus gigas* (D'Orbigny, 1835): A new perspective

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ABSTRACT

The jumbo squid *Dosidicus gigas* has been considered to be semelparous by several authors. However, to date no studies to support this assumption have been conducted. The present work tests the hypothesis that female jumbo squid do not die after a single reproductive event and provides evidence of the presence of postovulatory follicles (Pof) in females of different sizes. They indicates previous spawning in females in which they are present.

Histological analysis was performed on reproductive structures of 73 female jumbo squid from the northwest of Mexico using hematoxylin-eosin staining. Using image analysis five ovarian stages were identified: (I) previtellogenesis, (II) vitellogenesis, (III) postvitellogenesis, (IV) spawning (in which 3.73% show atresia and the presence of Pof) and (V) Postspawning, in which a higher proportion (4.86%) show atresia and Pof. It is already known that the jumbo squid has asynchronic ovarian development with partial spawning during the reproductive period. However, the consistent presence of postovulatory follicles and the presence of oocytes of different sizes and development support the hypothesis that the jumbo squid is a multiple spawner with more than one reproductive event during its life cycle.

1. Introduction

The jumbo squid *Dosidicus gigas* (D'Orbigny, 1835) is the largest species in the family Ommastrephidae. Its distribution is from California to central Chile, and it has a complex population structure in which different groups at different sizes have been identified based on mature squids (Nigmatullin et al., 2001).

Off the Coast of California, large squid have been reported ranging in size from 500 to 820 mm ML (Field et al., 2007). Litz et al. (2011) describe squids ranging in size from 360 to 790 mm ML and suggest that the species' distribution is related to large scale climate events such as El Niño and the effects of global warming. Off the east coast of the Baja California Peninsula in Mexico reported size groups range between 240 and 820 mm ML (Mejía-Rebollo et al., 2008), 620–770 mm ML (Rosas-Luis, 2007) and 670–810 mm ML (Bazzino, 2008). Elsewhere, in the Gulf of California there are reports of different size groups ranging between 300 and 875 mm ML (Markaida et al., 2004) and 400–680 mm ML (Guerrero-Escobedo et al., 2002), providing evidence for the presence of medium and large size groups of squid.

The jumbo squid *D. gigas* is dioecious with females outgrowing males in size but sexual dimorphism is not pronounced. The species has high fecundity (up to 32 million of eggs per female), the highest fecundity recorded for any cephalopod species (Nigmatullin and Markaida, 2009). In northwest Mexican waters, jumbo squid reproduce all year round and three spawning peaks have been identified, January–February, March–April and May–June (Ehrhardt et al., 1986; Markaida and Sosa-Nishizaki, 2001). The presence of paralarvae suggest the squid spawn near Santa Rosalía and San Pedro Martir Island (Gilly et al., 2006; Camarillo-Coop et al., 2010) and off the eastern coast in Baja California Peninsula (Camarillo-Coop et al.,2007). The fertilized eggs are embedded in a floating gelatinous mass where embryonic development occurs protected from microbial infections (Staaf et al., 2008).

In an early paper on reproductive strategy, Cole (1954) proposed the terms semelparity and iteroparity for species that respectively reproduce only once in their lifecycle and those that reproduce repeatedly. However, is was emphasised that these terms will not always apply exactly to all species due to variability in the timescale of life histories. More recently, Kirkendall and Stenseth (1985) proposed redefinition of the the terms taking into account the spatial and time scales of spawning.

The use of these terms in the case of cephalopods has given rise to discussion of their applicability in a direct way, where some

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Table 1
Stages of gonadal development in jumbo squid *Dosidicus gigas* according to oogenesis. Ovarian stages are included along with oocyte substages.

Ovarian stage	Description and diameter $\mu \pm$ S.D.
Previtellogenesis	Oogonia (PoO): rounded cells, with a spherical nucleus that fills almost all the cytoplasm, without nucleolus or follicle cells, with
I	basophilic cytoplasm (diameter 26.63 ± 14.87).
	Early previtellogenic oocytes (Pol): irregularly shaped cells, nucleus with well-defined nucleoli, with a thin layer of flat follicular cells and basophilic cytoplasm (diameter 121.81 ± 48.30).
	Intermediate previtellogenic oocytes {Po2): rounded and irregular cells, very well defined nucleus and nucleoli, with basophilic cytoplasm and a clear layer of follicular cells from flat to cubic (diameter 210.94 ± 34.32).
	Late previtellogenic oocytes (Po3): rounded and oval cells, with very well defined nucleus and nucleoli and basophilic cytoplasm, with an epithelium covered with cubic follicular cells that start to penetrate (invaginate) inside the oocyte forming invaginations (diameter 269.28 ± 49.44).
Vitellogenesis II	Early vitellogenic oocytes (Vol): rounded or oval cells with cubic epithelium and basophilic cytoplasm that starts turning to acidophilus due to vitellum synthesis, with more prominent invaginations of fol licular cells towards the interior of the oocyte, which start to dispalce the nucleus to the animal pole (diameter 430.45 ± 50.38).
Postvitellogenesis III	Late vitellogenic oocytes (Vo2): oval cells acquiring an acidophilus coloration due to a higher concentration of vitellum, with the nucleus completely displaced to the animal pole, invaginations of follicular cells are very deep and almost reach the animal pole (diameter 642.22 ± 80.56).
	Postvitellogenic oocytes (Pvo): rounded cells, completely filled with vitellum, with acidophilus cytoplasm, the invaginations of follicular cells are reduced to the increase of vitellum, which is to the point of forming a layer that wraps the oocyte (diameter 706.63 ± 49.96).
Spawning IV	Atresia (A): Reabsorbing oocytes characterized by the loss of vitellum granules and follicular cell deterioration.
Postspawning V	Postovulatory follicles (Pof): Layer of follicular cells where pyknotic nuclei are observed; additionally, they do not present vitellum.[a]

[a] The difference between IV and V refers to the differential proportion of those structures.

authors consider them in general as semelparous (Boyle, 1983; Mangold, 1983). There is, however, evidence of iteroparous species such as *Nautilus* (Saunders, 1984), *Octopus chierciae* (Rodaniche, 1984), *Stenoteuthis ovalanensis* (Harman et al., 1989; Nigmatullin and Laptikhovsky, 1994). In general, *D. gigas* has been considered semelparous (Nesis, 1983). This hypothesis has been maintained until today by some authors (Markaida, 2001; Nigmatullin et al., 2001).

Rocha et al. (2001) proposed changing the concepts of semelparity and iteroparity in squid for "spawning once" and "spawning more than once" with *D. gigas* in the latter category of a multiple spawner with an overall monocyclic spawning pattern. In *D. gigas*, the development of oocytes in the ovary is clearly asynchronous (Nigmatullin and Markaida, 2009). However, Harman et al. (1989) has concluded that ovulation from the ovary to the oviducts occurs in synchronic-groups.

The purpose of this study was to characterize the different ovarian phases based on oogenesis and to describe the spawning and postspawning stages. We propose that *D. gigas* is a partial spawner with more than one reproductive event based on evidence for the presence of indicators of previous spawning events: postovulatory follicles and atresia, in a large proportion of mature females.

2. Material and Methods

2.1. Collection of organisms

All squids studied came from directed sampling conducted in the Gulf of California in September of 2003 and August of 2012 and off of the western coast of Baja California Peninsula in October of 2000 and February of 2012. They were captured manually with five-crown jigs, and the records taken for each specimen were mantle length (ML) ($\pm$0.5 cm) and total weight (TW) ($\pm$0.1 gr) along with sex and macroscopic maturity stage (Lipinski and Underhill, 1995). Ovaries of all females were fixed with Davidson solution over 48 h and were preserved with 70% ethanol solution for later histological analysis.

2.2. Sample processing for histological analysis

A section of each ovary was processed following the procedure for embedding in paraffin, cutting three 4-μm slices (Rodríguez-Jaramillo et al., 2008) that were stained with Harris' hematoxylin and contrasted with eosin-floxin (Sheehan and Hrapchak, 1980).

2.3. Microscopic analysis

From the samples collected, slices were obtained from 73 ovaries and observed under different magnifications (4×, 10×, 20× and 40×) due to cell size, and 10 images were captured with each magnification. In order to make replicate observations of the sample in the present study, three slices were analyzed, from which three to five images were taken for each ovary to ensure a correct estimation of the proportion of oocyte substages (including atresia and postovulatory follicles).

Oocytes were classified as previtellogenesis, vitellogenesis and postvitellogenesis using criteria given by Arizmendi-Rodríguez et al. (2012) following earlier descriptions of the process of oogenesis (Sauer and Lipinski, 1990; Melo and Sauer, 1999; Laptikhovsky and Arkhipkin, 2001). Oocytes were later analyzed with the Image-Pro Plus 6.0. software to count and calculate the area of each oocyte and the theoretical diameter according to Briarty (1975) and Saout et al. (1999): where: D: diameter, A: area, π: 3.1416.

The mean and standard deviation of the theoretical diameter of each oocyte maturity type were calculated. One-factor ANOVA was performed to test for differences between oocyte type. Finally, for the validation of ovarian stages, three to five images from each squid were compared (Rodríguez-Jaramillo *et al.*,2008) using 4×, 20× and 40× magnifications.

3. Results

During histological analysis, seven oocyte substages were identified (oogonium Po0, early previtellogenic oocyte Po1, intermediate previtellogenic oocyte Po2, late previtellogenic oocyte Po3, early vitellogenic oocyte Vo1, late vitellogenic oocyte Vo2, and postvitellogenic oocyte Pvo), along with atresia (A) and Postovulatory follicles (Pof). From these, five ovarian stages were characterized: (I) Previtellogenesis, composed only of immature cells without yolk (Po0, Po1, Po2, Po3); (II) Vitellogenesis, where immature oocytes are observed together with oocytes in the maturation process where yolk synthesis has begun (Vo1, Vo2); (III) Postvitellogenesis, with completely mature oocytes (Pvo) along with the presence of the aforementioned substages; (IV) spawning, where all oocyte substages, atresia (A), and postovulatory follicles (Pof) are found; and (V) postspawning, where atresia and postovulatory follicles are present in greater proportions (Table 1; Fig. 1). Regarding the ANOVA performed for the oocyte substages, significant differences were found between each of the substages (Tukey