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PRIMARY RESEARCH PAPER

Ovarian development in Argentinean shortfin squid *Illex argentinus*: group-synchrony for corroboration of intermittent spawning strategy

Dongming Lin (ORCID) · **Xinjun Chen** · **Yong Chen** · **Guoping Zhu** · **Jizhou Yan**

Abstract Modern living cephalopod species have evolved a wide phenotypic diversity and flexibility of reproductive strategies, which is closely linked to the pattern of oogenesis and oocytes ovulation of species. Although it has been suggested that Argentinean shortfin squid *Illex argentinus* lay eggs intermittently, there is still little evidence for the mode of oocyte production and development. In this study, the ovarian development of *I. argentinus* was investigated by using histological analysis of ovaries, and six distinct histological stages of ovarian development were found among the ovaries. For each histological stage of the ovary, the frequency distribution of both number and occupied areas by each oogenesis stage was unimodal, and that gradually progressed along with ovarian development. The oocyte size distribution in ovaries before reaching vitellogenic stage was unimodal, bimodal in vitellogenic ovaries, and polymodal in ripe and partially spent ovaries. This evidence indicates that *I. argentinus* undergoes group-synchronous ovarian development, with a single dominant oocyte stock being produced to develop in several batches for a multiple-batch group-synchronous ovulation and confirms the intermittent spawning strategy of this species.

Keywords *Illex argentinus* · Oocyte development · Spawning · Reproduction · Southwest Atlantic

D. Lin · X. Chen (✉) · G. Zhu
College of Marine Sciences, Shanghai Ocean University,
Huchenghuan Rd 999, Shanghai 201306, China
e-mail: xjchen@shou.edu.cn

D. Lin · X. Chen · G. Zhu
The Key Laboratory of Sustainable Exploitation of
Oceanic Fisheries Resources, Ministry of Education,
Shanghai Ocean University, Huchenghuan Rd 999,
Shanghai 201306, China

D. Lin · X. Chen · G. Zhu
Collaborative Innovation Center for National Distant-
water Fisheries, Shanghai Ocean University,
Huchenghuan Rd 999, Shanghai 201306, China

D. Lin · X. Chen · Y. Chen · G. Zhu
National Engineering Research Center for Oceanic
Fisheries, Shanghai Ocean University, Shanghai 201306,
China

Y. Chen
School of Marine Sciences, University of Maine, 218
Libby Hall, Orono, ME 04469, USA

J. Yan
College of Fisheries and Life Science, Shanghai Ocean
University, Huchenghuan Rd 999, Shanghai 201306,
China

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Introduction

Life-history theories suggest that the rate of sexual maturation in the animal kingdom is dependent on the reproductive strategy of a species (Cornwell et al., 2006). The modern living coleoid cephalopods, which are considered to share common themes of fast growth and short lifespan (<1–2 years in most cases) (Boyle & Rodhouse, 2005), have evolved a wide phenotypic diversity and flexibility of reproductive strategies (Boyle et al., 1995; Pecl, 2001; Rocha et al., 2001). Such strategies that only involve a monocyclic lifespan, but with a continuum between one-time and multiple spawning events, are now known to be common in these species (Rocha et al., 2001; Hanlon et al., 2004). Furthermore, these strategies in reproduction are inextricably linked to ovarian development on the progress of maturation and spawning, and the level of gamete maturation and subsequent ovulation pattern greatly impacts the potential spawning events (Wallace & Selman, 1981; Murua & Saborido-Rey, 2003). By investigating the production of oocytes and the pattern of ovarian development, it is possible to determine the spawning strategy in these short-life species.

The Argentinean shortfin squid, *Illex argentinus,* is one of the most abundant shelf-slope squid species and supports an extremely productive cephalopod fishery in terms of landing volume (Jereb & Roper, 2010; FAO, 2014). This species distributes along the continental shelf and slope of the southwest Atlantic Ocean from approximately 22°S–54°S (Jereb & Roper, 2010). Even though several distinct stocks have been identified according to their size structures, spawning seasons, and spawning grounds (Haimovici et al., 1998; Crespi-Abril et al., 2013), there seems to be little evidence of any genetic differences among these stocks (Carvalho et al., 1992; Adcock et al., 1999; Bainy & Haimovici, 2012). Moreover, because of its high abundance and productivity, this species has been considered to be a southern example of a western boundary current species (Anderson & Rodhouse, 2001), and plays a key role in the southwest Atlantic ecosystem (Arkhipkin, 2012). There is, however, only a half to one year lifespan for this species(Lu & Chen, 2012; Schwarz & Perez, 2013), and successful reproduction and subsequent annual recruitment are likely to be essential to sustain the population (Boyle & Rodhouse, 2005).

During its annual life cycle, *I. argentinus* leaves about 10–20% of its lifespan for sexual maturation and spawning condition to the terminal (Schwarz & Perez, 2013), while maturation of subadults to reproductive condition occurs relatively quickly, generally as the squids undergo spawning migration (Jereb & Roper, 2010). Immediately after the beginning of spawning, the species lays eggs in an intermittent manner, while number of eggs at each release decreases as a result of the decreasing production of eggs over time (Laptikhovsky & Nigmatullin, 1992, 1993). Additionally, because of ceasing growth after the commencement of spawning (Arkhipkin, 1993), Rocha et al. (2001) assigned *I. argentinus* to the category of "intermittent terminal spawning" by assuming that this species might spawn oocytes in a pattern of group-synchrony. However, knowledge about the oogenesis and ovarian development in this species is still insufficient. To more deeply understand how this species develops oocytes to release them in an intermittent manner, a description and quantification of aspects of ovarian development are necessary through a histological observation due to the importance of reflecting the crucial changes during sexual maturation (ICES, 2010).

Thus, the aims of this study were to determine the pattern of ovarian development of *I. argentinus* by using the histological analysis of ovaries, an analysis which has been used previously to confirm the asynchronous ovarian development in many species [e.g., *Loligo reynaudii* (Melo & Sauer, 1999), *Octopus vulgaris* (Sieiro et al., 2014), *Dosidicus gigas* (Hernández-Muñoz et al., 2016)], and also to confirm the synchronous oogenesis in *Lolliguncula panamensis* (Arizmendi-Rodríguez et al., 2012). Furthermore, this study also aimed to clarify the mode of oocyte development and subsequent ovulation to provide a comprehensive understanding of the intermittent spawning strategy exhibited by this species as suggested above.

Materials and methods

Sampling

The Argentinean shortfin squid, *Illex argentinus,* was sampled from commercial jigging fishery fleets in the

high seas of the southwest Atlantic (41°56′S–47°11′S and 58°06′W–61°15′W) (Fig. 1), throughout January to March 2013 and April to June 2014, during which it would comprise the greatest period of sexual maturation for the major stocks of this species (Brunetti et al., 1998a, b). Similar to the approaches adopted by Jackson & Mladenov (1994) and Laptikhovsky et al. (2007), the whole specimens were placed on iron plates neatly and frozen immediately (−20°C) after being fished aboard for later analysis in the laboratory.

In the laboratory, dorsal mantle length (DML) was measured to the nearest 1 mm. Then the ventral mantle was cut open for sex identification when the specimens were at semi-thawed state, and the ovary was removed immediately and preserved in 10% buffered formalin solution (Laptikhovsky et al., 2007). In order to avoid any bias from the macro-maturity

assignment, no female specimens were assigned a macro-maturity stage, and a total of 123 ovaries were collected from females of 170–320 mm DML, which included the complete size range of the specimens.

Histological procedure and oogenesis classification

Histological methodologies were modified from the ICES's technique (ICES, 2010): all ovaries were fixed in the formalin solution for at least two months; small subsamples (3–5 mm thickness) from the middle of each ovary were dehydrated in a graded series of alcohol from 70 to 100%, and tissue transparency was improved by using absolute xylol three times; then embedded in paraffin for cutting into thin histological sections (5–6 μm) and stained with Harris's

Fig. 1 Map of the studied area showing the distribution of the sampled specimens of *Illex argentinus*
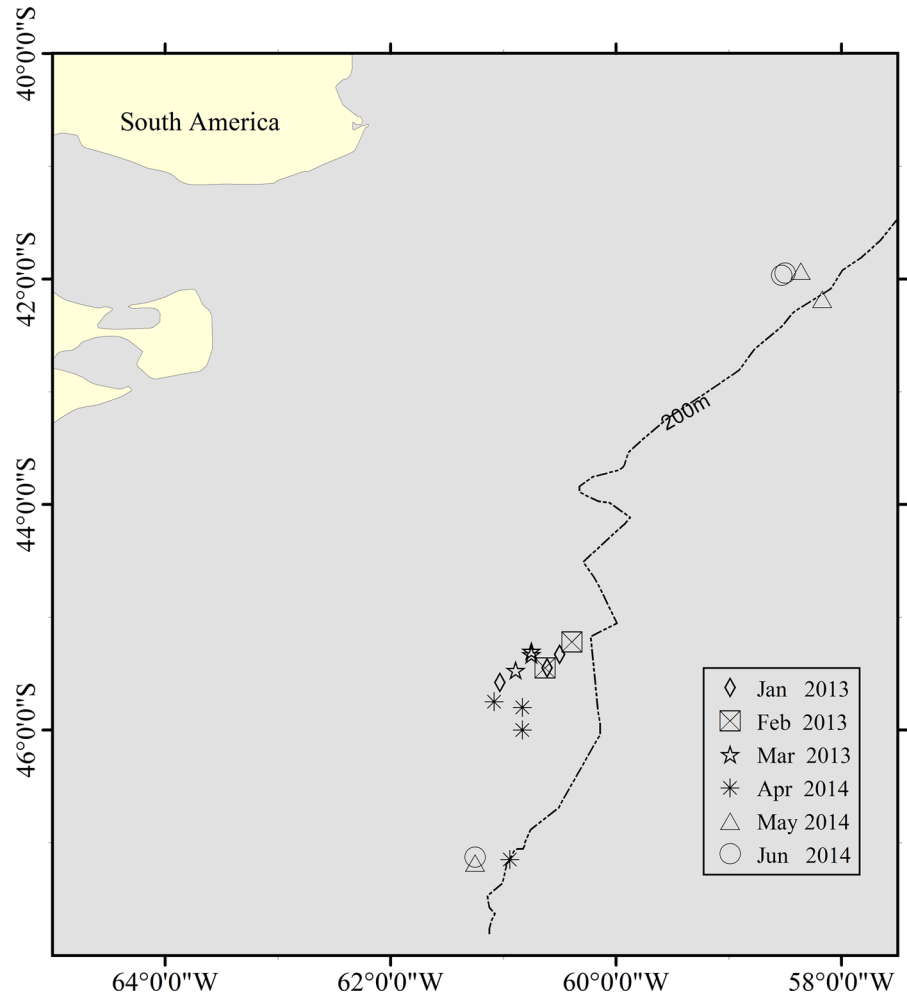
hematoxylin–eosin. Oocytes in each histological section were photographed using an Olympus BX-51 stereo microscope coupled with an Olympus DP-71 digital video camera for oogenesis analysis.

The oogenesis in *I. argentinus* was observed based on the structure of the follicle (Selman & Arnold, 1977; Sauer & Lipiński, 1990), and the stages assigned were similar to those used for *Doryteuthis gahi* by Laptikhovsky & Arkhipkin (2001), for *Sepiella maindroni* by Jiang et al. (2007) and for *Octopus vulgaris* by ICES (2010). However, some modification was made in our observation of *I. argentinus*: we combined the two stages of early yolkless and late yolkless together, as described in *D. gahi*, into the previtellogenic stage; we divided the follicular penetration stage in *S. maindroni* into three stages, the early vitellogenic stage, late vitellogenic stage, and ripe stage; and we also divided the previtellogenic stage in *O. vulgaris* into the multiple follicular stage and previtellogenic stage. Therefore, the oogenesis of *I. argentinus* was classified into seven stages plus the post-ovulatory follicle (*POF*) which are briefly described here (Table 1), and the nomenclature of "Stage with Arabic numbers (i.e., Stage 1)" suggested by Laptikhovsky & Arkhipkin (2001) was used: Stage 1 (*S1*)—oogonium, Stage 2 (*S2*)—primary oocyte, Stage 3 (*S3*)—multiple follicular oocyte, Stage 4 (*S4*)—previtellogenic oocyte, Stage 5 (*S5*)—early vitellogenic oocyte, Stage 6 (*S6*)—late vitellogenic oocyte, and Stage 7 (*S7*)—ripe oocyte. The atretic oocytes were also recorded for all histological sections, using the descriptions given by Melo & Sauer (1998): primary atretic oocyte (*PAO*), previtellogenic atretic oocyte (*PVAO*), and vitellogenic atretic oocyte (*VAO*).

Ovarian development

Ovarian development was investigated by (i) analyzing number of oocytes at each oogenesis stage in a given histological section, (ii) analyzing the occupied areas of oocytes at each oogenesis stage in a given histological section (i.e., the total areas of oocytes at Stage 1 in a given histological section in μm^2), and (iii) analyzing oocyte size–frequency distribution with ovary progress. Preliminary examination indicated that some histological sections showed distortions in some oocytes, which might be the result of materials being frozen before preservation. Thus, an average of

100–300 oocytes with normal appearance per ovary, along two random perpendicular 3–5 mm lines running across the middle of each histological section (Ortiz, 2013), were used to investigate the oocyte composition, occupied areas, and size–frequency distribution. The occupied area of oocytes was estimated as the absolute area of each oocyte being assigned a similar oogenesis stage in the histological section, and measured to the nearest 0.1 μm^2 (Jiang et al., 2007). Oocyte size was measured as the major axis length and was used to plot size frequency for oocyte development analysis (Laptikhovsky et al., 2008; Hoving & Lipiński, 2009; Nigmatullin & Markaida, 2009). Both of these were determined using Image-Pro Plus 6.0 software (1993–2006 Media Cybernetics, Inc).

Every ovary was classified under a comprehensive analysis for both of the most advanced oogenesis stages present (Melo & Sauer, 1999) and the most occupied areas at each of the seven oogenesis stages (Jiang et al., 2007). In addition, the number of oocytes at each oogenesis stage in its ovary would also be used as a reference. The nomenclature "Stage with Latin number (i.e., Stage I)" was used to describe ovary development after Melo & Sauer (1999). All statistical analyses were performed using SPSS 20.0 and Microsoft Excel packages.

Results

Stages of ovary development

Based on the histological analysis and comparison, six stages of ovary development were found in the *Illex argentinus* specimens, in which 16 ovaries were determined at Stage II (primary growth ovary), 15 ovaries at Stage III (multiple follicular ovary), 18 ovaries at Stage IV (previtellogenic ovary), 13 ovaries at Stage V (vitellogenic ovary), 47 ovaries at Stage VI (ripe ovary), and 14 ovaries at Stage VII (partially spent ovary). Unfortunately, the oogonial ovary (Stage I) and fully spent ovary (Stage VIII) were not present.

Stage II, primary growth ovaries These ovaries contained three types of oocyte: oogonia, primary oocytes, and multiple follicular oocytes (Fig. 2A). These oocytes had an average major axis length of 0.031 ± 0.011 mm, 0.110 ± 0.025 mm, and 0.165 ± 0.049 mm, separately. For ovaries at this

Table 1 Oocyte stages in *Illex argentinus*

Oogenesis	Terminology	Diagnosis
Stage 1	Oogonia	Oval or pyriform in shape, a large nucleus without visible cytoplasm or surrounded by a narrow sheet of cytoplasm. Size <0.06 mm
Stage 2	Primary oocytes	Follicle cells beginning to attach the oocyte and proliferate as a layer of follicular epithelium on the surface, with a centrally located nucleus. Size at 0.05–0.20 mm
Stage 3	Multiple follicular oocytes	Two layers of follicular epithelium surrounding the oocyte's surface. The inner follicle cells turning their long axis direction to the oocyte, and an irregular corona occasionally occurring between the inner follicle cells and oocyte. Size at 0.15–0.30 mm
Stage 4	Previtellogenic oocytes	Follicle cells undergoing rapid division and beginning to form the follicular folds to penetrate into the oocyte until forming a follicular syncytium. Central nucleus displacing to the polar zone. Size at 0.20–0.45 mm
Stage 5	Early vitellogenic oocytes	Follicular syncytium engaging in vitellogenesis and the formation of a chorion, beginning to displace toward the periphery of the oocyte owing to yolk materials assembly. Size at 0.40–0.80 mm
Stage 6	Late vitellogenic oocytes	Follicular syncytium noticeably being displaced by the continuing vitellogenesis, with few scattering around less than half of the oocyte periphery. Well-developed chorion surrounding the oocyte. Size at 0.60–1.10 mm
Stage 7	Ripe oocytes	Follicular syncytium completely being displaced, a distinct chorion surrounding around the spherical oocyte. Size at 0.95–1.15 mm
Post-ovulatory follicle		Follicular syncytium collapsing, with materials like cytoplasm distributing around. Follicle cells appearing in an irregular contour. Size at 0.20–0.60 mm

stage, the primary oocytes (*S2*) were growing with follicle cells attached to the surface, and these oocytes were the most abundant in both numbers and occupied areas (Fig. 3A).

Stage III, multiple follicular ovaries These ovaries contained a further advanced oocyte stage, previtellogenic oocytes (*S4*) (Fig. 2B). The previtellogenic oocytes underwent a rapid division of the follicle cells, and had an average major axis length of 0.291 ± 0.056 mm. Multiple follicular oocytes (*S3*) grew further increasing to an average major axis length of 0.243 ± 0.045 mm. Additionally, these multiple follicular oocytes predominated in both numbers and occupied areas in ovaries at this stage (Fig. 3B).

Stage IV, previtellogenic ovaries In these ovaries, the most advanced oocytes were at the early vitellogenic stage (*S5*), and had an average major axis length of 0.463 ± 0.082 mm (Fig. 2C). The previtellogenic oocytes (*S4*) had grown to 0.359 ± 0.069 mm, and flourished in both numbers and occupied areas (Fig. 3C). In contrast to multiple follicular ovaries, the previtellogenic oocytes at this stage exhibited a greater proliferation of follicle cells,

which penetrated deeply into the oocyte until forming a follicular syncytium (Fig. 2C).

Stage V, vitellogenic ovaries In these ovaries, the early vitellogenic oocytes (*S5*) had a greater growth in size, with an average major axis length of 0.633 ± 0.078 mm, and exhibited yolk bodies assembling rapidly within the oocyte (Fig. 2D). And these early vitellogenic oocytes predominated in both numbers and occupied areas (Fig. 3D). In addition, there was the presence of some larger oocytes in these ovaries, which had an average major axis length of 0.801 ± 0.153 mm and a noticeable reduction of follicular syncytium (Fig. 2D). This might indicate that some early vitellogenic oocytes had progressed to the late vitellogenic oocyte stage (Stage 6).

Stage VI, ripe ovaries Most of the early vitellogenic oocytes (*S5*) had progressed to late vitellogenic oocytes (*S6*), and had increased to 0.969 ± 0.181 mm (Fig. 2E) and predominated in both numbers and occupied areas (Fig. 3E). A few late vitellogenic oocytes had grown to a further advanced stage of ripe oocytes (*S7*), and had an average major axis length of 1.041 ± 0.112 mm which were mostly found near to the edge of the histological ovary tissue
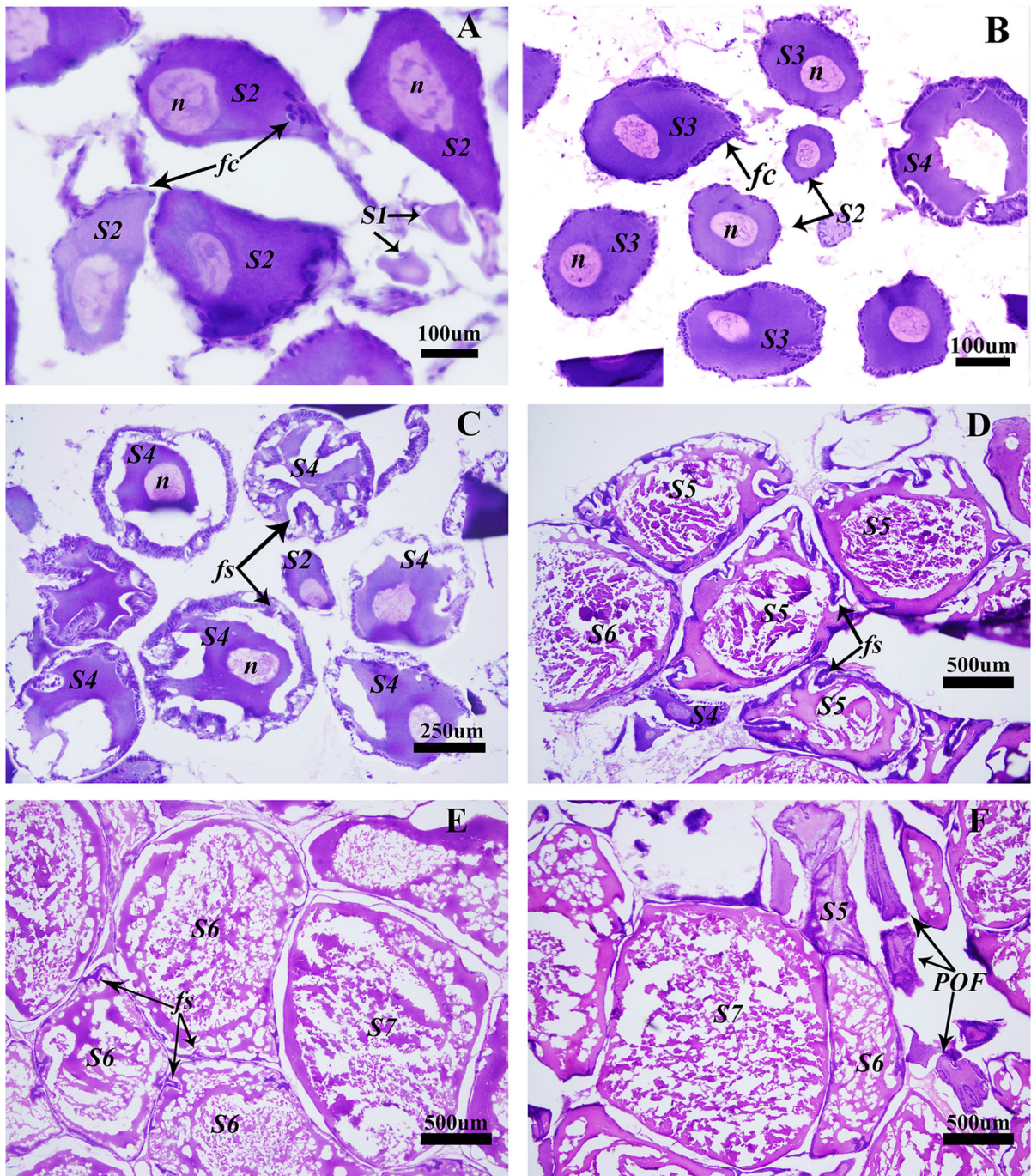
Fig. 2 Ovary development of *Illex argentinus*. **A** Section of stage II ovary showing oogonia (Stage 1, *S1*) and primary oocytes (Stage 2, *S2*). **B** Section of stage III ovary showing primary oocytes (*S2*), multiple follicular oocytes (Stage 3, *S3*), and follicular cells (*fc*). **C** Section of stage IV ovary showing previtellogenic oocytes (Stage 4, *S4*) and follicular syncytium (*fs*). **D** Section of stage V showing previtellogenic oocytes (*S4*), early vitellogenic oocytes (Stage 5, *S5*), late vitellogenic oocytes (Stage 6, *S6*), and follicular syncytium (*fs*). **E** Section of stage VI showing late vitellogenic oocytes (*S6*) and ripe oocyte (Stage 7, *S7*). **F** Section of stage VII showing early (*S5*) and late vitellogenic oocytes (*S6*), ripe oocyte (*S7*), and post-ovulatory follicle (*POF*). *n* nucleus

Fig. 3 Percentages of number and occupied area by each stage oocyte in *Illex argentinus* showing the process of oocyte development at maturation. *S1* Stage 1: oogonia, *S2* Stage 2: primary oocyte, *S3* Stage 3: multiple follicular oocyte, *S4* Stage 4: previtellogenic oocyte, *S5* Stage 5: early vitellogenic oocyte, *S6* Stage 6: late vitellogenic oocyte, *S7* Stage 7: ripe oocyte, *PAO* primary atretic oocyte, *PVAO* previtellogenic atretic oocyte, *VAO* vitellogenic atretic oocyte, *POF* post-ovulatory follicle
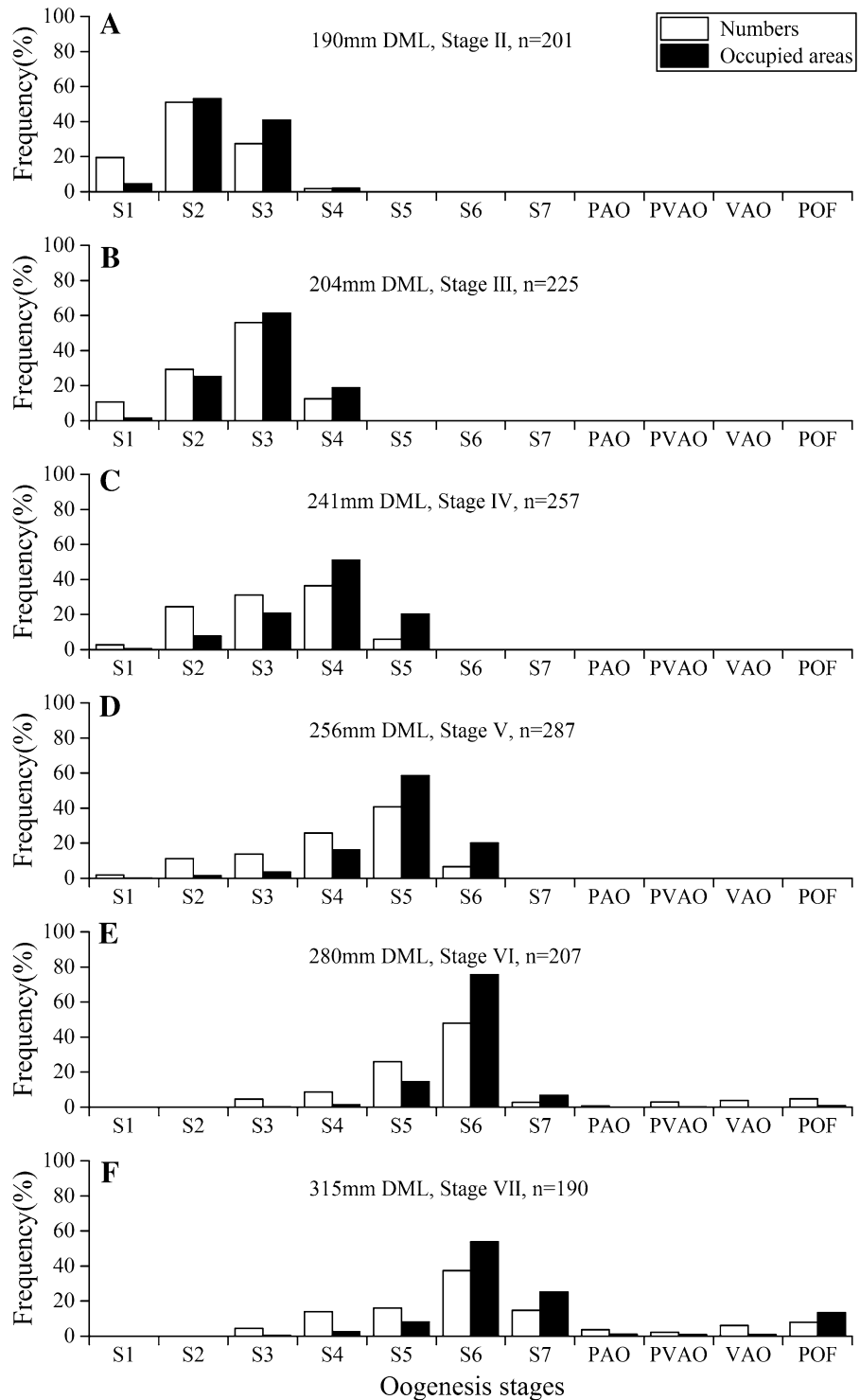
sections. In addition, a small portion of both primary atretic oocytes and previtellogenic atretic oocytes was found in these ovaries, which is indicative of resorption, whereas the oogonia (*S1*) and primary oocytes (*S2*) were absent, possibly indicating that new oocytes had ceased production in these ovaries.

Stage VII, partially spent ovaries Compared to Stage VI, both the ripe oocytes (*S7*) and post-ovulatory follicles had increased greatly in number ($\chi^2 = 279.23$, $P < 0.05$ for Stage 7; $\chi^2 = 77.39$, $P < 0.05$ for *POF*) (Fig. 2F, 3F). Even though the late vitellogenic oocytes (*S6*) were still abundant, there was a significant decrease in number in contrast to those found in the ripe ovaries ($\chi^2 = 1421.68$, $P < 0.05$) (Fig. 3F). Except for both primary and previtellogenic atretic oocytes, vitellogenic atretic oocytes were also found in these ovaries, suggesting that oocytes are destined to mature in their monocyclical life-history.

Oocyte size distribution

In the ovaries at stages II and III, oocytes exhibited a unimodal length–frequency distribution, which was represented by the primary oocytes of ca. 0.10–0.20 mm (Fig. 4A, B). At stage IV, the oocytes also exhibited a unimodal length–frequency distribution, but in an extended size range, where the single mode of oocytes increased to ca. 0.30 mm, and the largest oocytes were already ca. 0.65 mm (Fig. 4C).

After reaching stage V, the length–frequency distribution of oocytes was bimodal (Fig. 4D). Two well-defined groups of oocytes were present simultaneously, with modes of ca. 0.10–0.25 and 0.40–0.50 mm. These oocytes were mostly represented by the multiple follicular oocytes (*S3*) and early vitellogenic oocytes (*S5*).

In the ovaries at stages VI and VII, oocytes exhibited a polymodal length–frequency distribution, along with an absence of oocytes of less than ca. 0.10 mm (Fig. 4E, F). The ripe ovaries contained a further mode of vitellogenic oocytes of ca. 0.80–0.90 mm, while a few vitellogenic oocytes had progressed into the ripe stage, with a major axis length of ca. 0.95–1.15 mm (Fig. 4E). Additionally, the atretic oocytes at the primary and previtellogenic stages were present in these ripe ovaries, with major axis lengths of ca. 0.12–0.19 and ca. 0.21–0.36 mm, respectively. At stage VII, a peak bulk of ripe oocytes (*S7*) had occurred, and another two modes of oocytes were found in the range of ca. 0.30–0.50 and ca. 0.75–0.90 mm, which were represented by previtellogenic oocytes (*S4*) and late vitellogenic oocytes (*S6*), respectively (Fig. 4F).

Discussion

Logistic constraints allowed only to conduct the histological observations on material frozen onboard, then later thawed and fixed. The quality of the histological sections, however, should be suitable to interpret the progress of oogenesis by well demonstrating the appearance and proliferation of follicle cells, the penetration of follicular folds, the formation and shrinkage of follicular syncytium, and the chorion formation and yolk material synthesis. All of such characteristics are the main references to identify oocyte development in cephalopod species (Selman & Arnold, 1977; Sauer & Lipiński, 1990). Moreover, our findings are consistent with the observations of Laptikhovsky & Arkhipkin (2001), Jiang et al. (2007), and ICES (2010) for, respectively, *D. gahi*, *S. maindroni* and *O. vulgaris*, which suggest the appropriateness of our adopted procedures. In particular, the presence and absence of follicle cells around the oocyte periphery as well as the structure of follicular syncytium are the most important features for oogenesis identification, despite the controversy over whether or not these cells engage in yolk synthesis (Bottke, 1974; Bolognari et al., 1976). In fish species, instead, the yolk globules are the most important characteristic for separating stages of cortical alveolar, early vitellogenesis, and late vitellogenesis (McBride et al., 2016), but these globules may look ruptured and coalescenced without adequate preservation of fresh materials, resulting in misinterpretation of histological sections (Mackie & Lewis, 2001).

The present evidence that the frequency distribution of each stage of oocyte development in number or occupied areas is unimodal and that it increases in line with the onset of ovarian development indicates that the ovarian maturation in *I. argentinus* is group-synchronous. It is reasonable to expect that the ovaries grow a major group of oocytes for developing. This conclusion is further supported by the unimodal distribution of oocyte size in the primary growth, multiple follicular, and previtellogenic ovaries, which indicates that only a single major group of oocytes is produced. It is very similar to other group-synchronous species, such as fish *Encrasicholina heteroloba* (Wright, 1992) and *Dicentrarchus labrax* (Asturiano et al., 2002), and sepiolid *Sepiola atlantica* (Rodrigues

Fig. 4 Size–frequency distribution of ovarian oocytes in *Illex argentinus* showing the process of oocyte development and resorption at maturation
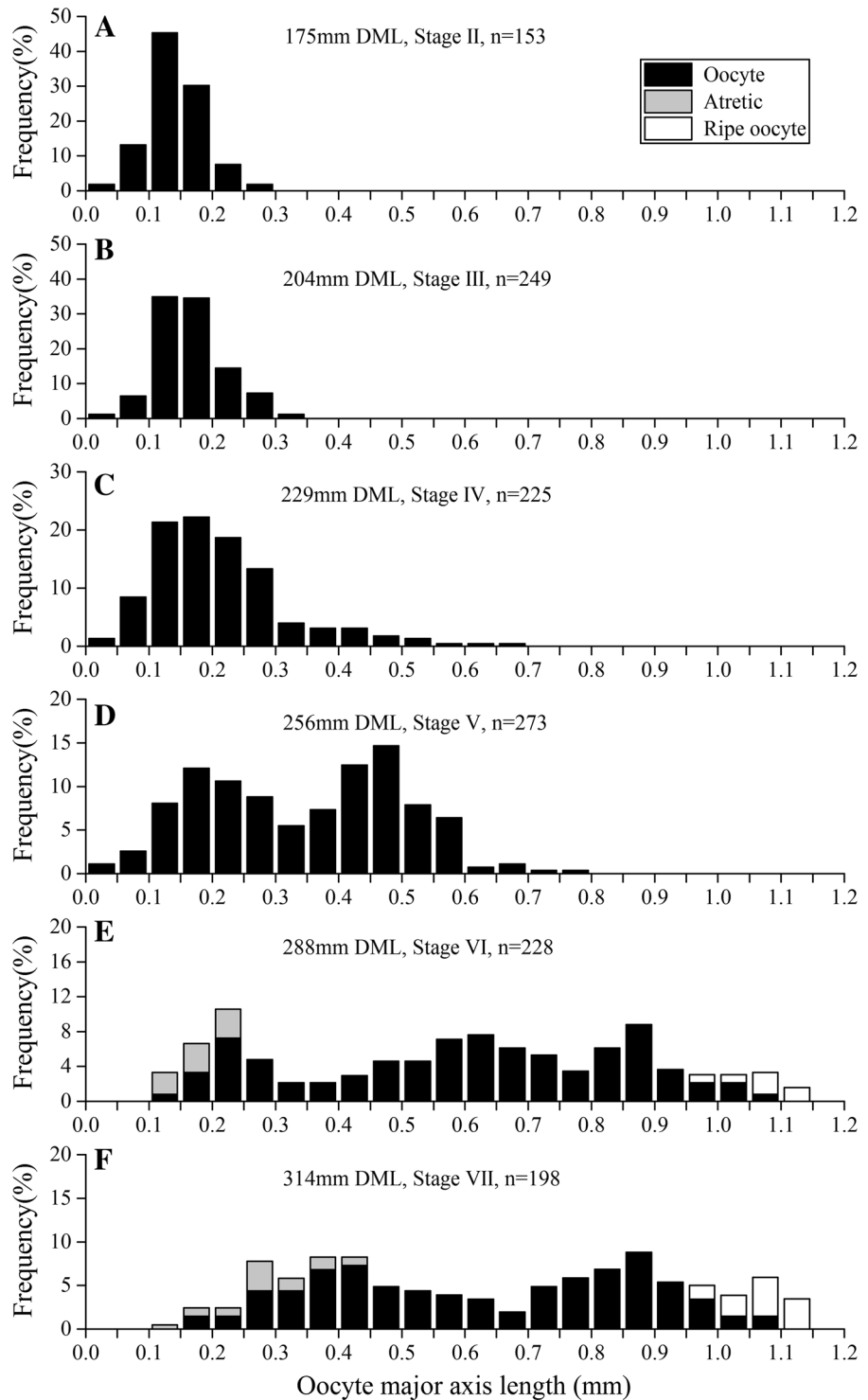
et al., 2011, 2012), which all produce a dominant oocyte stock for development. However, it should be noted that the oocyte stock is not destined for total release in this semelparous animal, as only about 70% of potential fecundity is released (Laptikhovsky & Nigmatullin, 1992, 1993).

The dominant cohort of oocytes at the early ovary stages in *I. argentinus* is clearly designed to develop in several batches afterwards, since the oocyte size distribution in vitellogenic ovaries was bimodal and polymodal in ripe and partially spent ovaries coupled with the presence of oocytes at various stages of development. This feature has commonly been suggested as a clear indication that oocytes develop in several batches for group-synchronous ovulation during the spawning season (Wright, 1992; Boyle et al., 1995; Mylonas & Zohar, 2007). Based on the morphological analysis of oocytes, Laptikhovsky & Nigmatullin (1992) also reported a similar mode of oocyte size distribution and suggested that this species grows the oocyte stock through multiple batches. Such patterns of oogenesis could allow *I. argentinus* to ovulate numerous egg batches intermittently over an extended period of time, which could be one to two months (Brunetti et al., 1991; Arkhipkin & Laptikhovsky, 1994; Nigmatullin & Laptikhovsky, 1994; Schwarz & Perez, 2013). This appears to be a common feature for the group-synchronous species *Loligo forbesii* (Rocha & Guerra, 1996), *Sepiola atlantica* (Rodrigues et al., 2011, 2012), and *Lycoteuthis lorigera* (Hoving et al., 2014), where several batches of oocytes were detected in the ovaries. This characteristic would enable individuals to generate a clutch of oocytes from the dominant population of earlier stages into any of the subsequent stages for intermittent ovulation (Wallace & Selman, 1981; Asturiano et al., 2002; Mylonas & Zohar, 2007).

Furthermore, this group-synchronous ovarian development found for *I. argentinus* is characterized as a consistent peak mode of vitellogenic oocytes along with a bulk of ripe oocytes in the ripe and partially spent ovaries, indicating that the oocytes were developing in a slow and steady process. This characteristic could be explained by intermittent spawning (Rocha et al., 2001; Jereb & Roper, 2005), as a similar observation was found in confirmed intermittent spawners of cephalopod species, e.g., *Thysanoteuthis rhombus* (Nigmatullin et al., 1995), *Loligo vulgaris* (Rocha & Guerra, 1996), and *Loligo forbesii* (Collins et al., 1995; Rocha & Guerra, 1996). However, this is in contrast to the synchronous ovarian development for a single ovulation at the end of their lifetime, as exemplified by the species *Onykia ingens* (Jackson & Mladenov, 1994; Laptikhovsky et al., 2007), *Gonatus antarcticus* (Laptikhovsky et al.,

2007), and histioteuthids (Laptikhovsky, 2001; Hoving & Lipiński, 2009), where all are assumed to adopt the strategy of simultaneous terminal spawning. Also, the group-synchrony could be different to asynchronous (or quasi-asynchronous) ovaries, where oocytes of all stages are present without dominant populations as found in species *Sthenoteuthis oualaniensis* (Harman et al., 1989) and bobtail squid genera *Rossia* and *Neorossia* (Laptikhovsky et al., 2008).

The production of new oocytes in the ovaries of *I. argentinus* appears to stop when the ovaries reach their maturity, since both the oogonia and primary oocytes disappear in those specimens with mature and partially spent ovaries. Laptikhovsky & Nigmatullin (1993) also found that in the genus *Illex* species oocytes <0.05 mm in diameter were absent after reaching morphological maturing stage, which was expected to be the cessation of oocyte production prior to the onset of vitellogenesis. The same has been documented for two other Ommastrephid *Sthenoteuthis pteropus* (Laptikhovsky & Nigmatullin, 2005) and *D. gigas* (Nigmatullin & Markaida, 2009), but these species continue active feeding and substantial somatic growth after the beginning of spawning (Nigmatullin, 2011). In contrast, *I. argentinus* is found to decrease feeding activity and practically ceases growing once spawning commences (Arkhipkin, 1993; Laptikhovsky & Nigmatullin, 1993), which in turn results in the end of oocyte production due to the limited energy reserve as suggested by Harman et al. (1989). Meanwhile, it is reasonable to expect that the number of ova produced for each batch decreases during the progress of spawning, due to the end of oocyte production coupled with the occurrence of oocyte resorption. Specifically, oocyte resorption is an alternative energy source allowing oocyte maturation to proceed with limit energy reserves (McBride et al., 2013; Mendo et al., 2016). The fact that a decreasing volume of eggs occurs at each release means that the species is thought to be a "descending" spawning type, which is a typical strategy of the slope-shelf and neritic-oceanic ommastrephids (Nigmatullin & Laptikhovsky, 1994; Laptikhovsky & Nigmatullin, 1999; Nigmatullin, 2011), in particular the genus *Illex* (Laptikhovsky & Nigmatullin, 1993).

Ultimately, this study provides an insight into the ovarian growth of *I. argentinus* based on the histological observation of the females' ovaries, in which

the ovarian development is group-synchrony as being evidenced by only a dominant cohort of oocytes produced to develop in several batches. And such pattern of ovary development provides further evidence that this species undergoes intermittent spawning strategy, and releases eggs in several batches from the single dominant stock of oocytes, which is estimated over 70 thousands in potential (Laptikhovsky & Nigmatullin, 1993). In addition, although the deep-frozen specimens here are sufficient to allow adequate interpretation of the main histological characteristics of oogenesis and of group-synchronous ovarian development, adequate preservation of fresh gonads should be recommended in future researches due to distortions that occur in some oocytes after frozen. Meanwhile, further investigations need to address the extent of impact on reproduction from fluctuating environmental conditions in the southwest Atlantic (Gan et al., 1998; Acha et al., 2004), due to the underlying phenotypic characteristics of highly environment-sensitive growth for this species (Boyle & Rodhouse, 2005; Jereb & Roper, 2010). This observation can provide a deeper insight into how this species effectively adapts to its living surroundings in environments likely to initiate oocyte maturation and hence group-synchronous ovulation.

References

Acha, E. M., H. W. Mianzan, R. A. Guerrero, M. Favero & J. Bava, 2004. Marine fronts at the continental shelves of austral South America: physical and ecological processes. Journal of Marine Systems 44: 83–105.

Adcock, G. J., P. W. Shaw, P. G. Rodhouse & G. R. Carvalho, 1999. Microsatellite analysis of genetic diversity in the squid *Illex argentinus* during a period of intensive fishing. Marine Ecology Progress Series 187: 171–178.

Anderson, C. I. & P. G. Rodhouse, 2001. Life cycles, oceanography and variability: ommastrephid squid in variable oceanographic environments. Fisheries Research 54: 133–143.

Arizmendi-Rodríguez, D. I., C. Rodríguez-Jaramillo, C. Quiñonez-Velázquez & C. A. Salinas-Zavala, 2012. Reproductive indicators and gonad development of the Panama brief squid *Lolliguncula panamensis* (Berry 1911) in the Gulf of California, Mexico. Journal of Shellfish Research 31: 817–826.

Arkhipkin, A., 1993. Age, growth, stock structure and migratory rate of pre-spawning short-finned squid *Illex argentinus* based on statolith ageing investigations. Fisheries Research 16: 313–338.

Arkhipkin, A. I., 2012. Squid as nutrient vectors linking Southwest Atlantic marine ecosystems. Deep-Sea Research Part II: Topical Studies in Oceanography 95: 7–20.

Arkhipkin, A. & V. Laptikhovsky, 1994. Seasonal and inter-annual variability in growth and maturation of winter-spawning *Illex argentinus* (Cephalopoda, Ommastrephidae) in the Southwest Atlantic. Aquatic Living Resources 7: 221–232.

Asturiano, J. F., L. A. Sorbera, J. Ramos, D. E. Kime, M. Carrillo & S. Zanuy, 2002. Group-synchronous ovarian development, spawning and spermiation in the European sea bass (*Dicentrarchus labrax* L.) could be regulated by shifts in gonadal steroidogenesis. Scientia Marina 66: 273–282.

Bainy, M. C. R. S. & M. Haimovici, 2012. Seasonality in Growth and Hatching of the Argentine Short-Finned Squid *Illex argentinus* (Cephalopoda: Ommastrephidae) Inferred from Aging on Statoliths in Southern Brazil. Journal of Shellfish Research 31: 135–143.

Bolognari, A., M. P. A. Carmignani & G. Zaccone, 1976. A cytochemical analysis of the follicular cells and the yolk in the growing oocytes of *Octopus vulgaris* (Cephalopoda, Mollusca). Acta Histochemica 55: 167–175.

Bottke, W., 1974. The fine structure of the ovarian follicle of *Alloteuthis subulata* Lam. (Mollusca, Cephalopoda). Cell and Tissue Research 150: 463–479.

Boyle, P. & P. Rodhouse, 2005. Cephalopods: Ecology and Fisheries. Wiley-Blackwell, Oxford.

Boyle, P. R., G. J. Pierce & L. C. Hastie, 1995. Flexible reproductive strategies in the squid *Loligo forbesi*. Marine Biology 121: 501–508.

Brunetti, N., M. Ivanovic, E. Louge & H. Christiansen, 1991. Estudio de la biología reproductiva y de la fecundidad en dos subpoblaciones del calamar (*Illex argentinus*) [Reproductive biology and fecundity of two stocks of the squid (*Illex argentinus*)]. Frente Marítimo 8: 73–84.

Brunetti, N. E., B. Elena, G. R. Rossi, M. L. Ivanovic, A. Aubone, R. Guerrero & H. Benavides, 1998a. Summer distribution, abundance and population structure of *Illex argentinus* on the Argentine shelf in relation to environmental features. South African Journal of Marine Science 20: 175–186.

Brunetti, N. E., M. Ivanovic, G. Rossi, B. Elena & S. Pineda, 1998b. Fishery biology and life history of *Illex argentinus*. In Okutani, T. (ed.), Contributed Papers to International Symposium on Large Pelagic Squids, July 18–19, 1996, for JAMARC's 25th Anniversary of Its Foundation. Japan Marine Fishery Resources Research Center (JAMARC), Tokyo: 217–231.

Carvalho, G. R., A. Thompson & A. L. Stoner, 1992. Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. Journal of Experimental Marine Biology and Ecology 158: 105–121.

Collins, M. A., G. M. Burnell & P. G. Rodhouse, 1995. Reproductive strategies of male and female *Loligo forbesi* (Cephalopoda: Loliginidae). Journal of the Marine Biological Association of the United Kingdom 75: 621–634.

Cornwell, R. E., M. J. Law Smith, L. G. Boothroyd, F. R. Moore, H. P. Davis, M. Stirrat, B. Tiddeman & D. I. Perrett, 2006. Reproductive strategy, sexual development and attraction to facial characteristics. Philosophical Transactions of the Royal Society of London B: Biological Sciences 361: 2143–2154.

Crespi-Abril, A. C., E. M. Morsan, G. N. Williams & D. A. Gagliardini, 2013. Spatial distribution of *Illex argentinus* in San Matias Gulf (Northern Patagonia, Argentina) in relation to environmental variables: a contribution to the new interpretation of the population structuring. Journal of Sea Research 77: 22–31.

FAO, 2014. The State of World Fisheries and Aquaculture 2014. Opportunities and Challenges. FAO, Rome.

Gan, J., L. A. Mysak & D. N. Straub, 1998. Simulation of the South Atlantic Ocean circulation and its seasonal variability. Journal of Geophysical Research: Oceans 103: 10241–10251.

Haimovici, M., N. Brunetti, P. Rodhouse, J. Csirke & R. Leta, 1998. *Illex argentinus*. In Rodhouse, P. G., E. G. Dawe & R. K. O'Dor (eds), Squid Recruitment Dynamics: The Genus *Illex* as A model, The Commercial *Illex* Species and Influences on Variability, Vol. 376. FAO, Rome: 27–58.

Hanlon, R. T., N. Kangas & J. W. Forsythe, 2004. Egg-capsule deposition and how behavioral interactions influence spawning rate in the squid *Loligo opalescens* in Monterey Bay, California. Marine Biology 145: 923–930.

Harman, R. F., R. E. Young, S. B. Reid, K. M. Mangold, T. Suzuki & R. F. Hixon, 1989. Evidence for multiple spawning in the tropical oceanic squid *Stenoteuthis oualaniensis* (Teuthoidea: Ommastrephidae). Marine Biology 101: 513–519.

Hernández-Muñoz, A. T., C. Rodríguez-Jaramillo, A. Mejía-Rebollo & C. A. Salinas-Zavala, 2016. Reproductive strategy in jumbo squid *Dosidicus gigas* (D'Orbigny, 1835): a new perspective. Fisheries Research 173: 145–150.

Hoving, H. J. T. & M. R. Lipiński, 2009. Female reproductive biology, and age of deep-sea squid *Histioteuthis miranda* from southern Africa. ICES Journal of Marine Science: Journal du Conseil 66: 1868–1872.

Hoving, H. J. T., V. V. Laptikhovsky, M. R. Lipinski & E. Jürgens, 2014. Fecundity, oogenesis, and ovulation pattern of southern African *Lycoteuthis lorigera* (Steenstrup, 1875). Hydrobiologia 725: 23–32.

ICES, 2010. Report of the Workshop on Sexual Maturity Staging of Cephalopods, 8–11 November 2010, Livorno, Italy. ICES CM 2010/ACOM: 49.

Jackson, G. D. & P. V. Mladenov, 1994. Terminal spawning in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae). Journal of Zoology 234: 189–201.

Jereb, P. & C. Roper, 2005. Cephalopods of the World, an Annotated and Illustrated Catalogue of Cephalopod Species Known to Date. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae), Vol. 1. FAO, Rome.

Jereb, P. & C. F. E. Roper, 2010. Cephalopods of the World. An Annotated and Illustrated Catalogue of Cephalopod Species Known to Date. Myopsid and Oegopsid Squids, Vol. 2. FAO, Rome.

Jiang, X. M., F. Y. Fu, Z. Li & X. D. Feng, 2007. Study on the oogenesis and ovarial development of *Sepiella maindroni*. Journal of Fisheries of China 31: 607–617.

Laptikhovsky, V., 2001. First data on ovary maturation and fecundity in the squid family Histioteuthidae. Scientia Marina 65: 127–129.

Laptikhovsky, V. V. & A. I. Arkhipkin, 2001. Oogenesis and gonad development in the cold water loliginid squid *Loligo gahi* (Cephalopoda: Myopsida) on the Falkland shelf. Journal of Molluscan Studies 67: 475–482.

Laptikhovsky, V. V. & C. M. Nigmatullin, 1992. Caracteristicas reproductivas de machos y hembras del calamar (*Illex argentinus*). Frente Marítimo 12: 23–37.

Laptikhovsky, V. V. & C. M. Nigmatullin, 1993. Egg size, fecundity, and spawning in females of the genus *Illex* (Cephalopoda: Ommastrephidae). ICES Journal of Marine Science: Journal du Conseil 50: 393–403.

Laptikhovsky, V. V. & C. M. Nigmatullin, 1999. Egg size and fecundity in females of the subfamilies Todaropsinae and Todarodinae (Cephalopoda: Ommastrephidae). Journal of the Marine Biological Association of the United Kingdom 79: 569–570.

Laptikhovsky, V. V. & C. M. Nigmatullin, 2005. Aspects of female reproductive biology of the orange-back squid, *Sthenoteuthis pteropus* (Steenstup) (Oegopsina: Ommastrephidae) in the eastern tropical Atlantic. Scientia Marina 69: 383–390.

Laptikhovsky, V. V., A. I. Arkhipkin & H. J. T. Hoving, 2007. Reproductive biology in two species of deep-sea squids. Marine Biology 152: 981–990.

Laptikhovsky, V., C. M. Nigmatullin, H. Hoving, B. Onsoy, A. Salman, K. Zumholz & G. Shevtsov, 2008. Reproductive strategies in female polar and deep-sea bobtail squid genera *Rossia* and *Neorossia* (Cephalopoda: Sepiolidae). Polar Biology 31: 1499–1507.

Lu, H. J. & X. J. Chen, 2012. Age, growth and population structure of *Illex argentinus* based on statolith microstructure in Southwest Atlantic Ocean. Journal of Fisheries of China 36: 1049–1056.

Mackie, M. & P. Lewis, 2001. Assessment of gonad staging systems and other methods used in the study of the reproductive biology of narrow-barred Spanish mackerel, *Scomberomorus commerson*, in Western Australia. Department of Fisheries Perth, Western Australia 136: 1–32.

McBride, R. S., S. Somarakis, G. R. Fitzhugh, A. Albert, N. A. Yaragina, M. J. Wuenschel, A. Alonso-Fernández & G. Basilone, 2013. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish and Fisheries 15: 1–35.

McBride, R. S., R. Ferreri, E. K. Towle, J. M. Boucher & G. Basilone, 2016. Yolked oocyte dynamics support agreement between determinate- and indeterminate-method estimates of annual fecundity for a northeastern United States Population of American Shad. PLoS ONE 11: e0164203.

Melo, Y. C. & W. H. H. Sauer, 1998. Ovarian atresia in cephalopods. South African Journal of Marine Science 20: 143–151.

Melo, Y. C. & W. H. H. Sauer, 1999. Confirmation of serial spawning in the chokka squid *Loligo vulgaris reynaudii* off the coast of South Africa. Marine Biology 135: 307–313.

Mendo, T., J. M. Semmens, J. M. Lyle, S. R. Tracey & N. Moltschaniwskyj, 2016. Reproductive strategies and energy sources fuelling reproductive growth in a protracted spawner. Marine Biology 163: 1–11.

Murua, H. & F. Saborido-Rey, 2003. Female reproductive strategies of marine fish species of the North Atlantic. Journal of Northwest Atlantic Fishery Science 31: 23–31.

Mylonas, C. & Y. Zohar, 2007. Promoting oocyte maturation, ovulation and spawning in farmed fish. In Babin, P., J. Cerdà & E. Lubzens (eds), The Fish Oocyte: From Basic Studies to Biotechnological Applications. Springer Netherlands, Dordrecht: 437–474.

Nigmatullin, C. M., 2011. Two spawning patterns in ommastrephid squids and other cephalopods. In 4th International Symposium "Coleoid Cephalopods Through Time". Museum fur Naturkunde Stuttgart, Stuttgart, Abstract volume: 48.

Nigmatullin, C. M. & V. Laptikhovsky, 1994. Reproductive strategies in the squids of the family Ommastrephidae (preliminary report). Ruthenica 4: 79–82.

Nigmatullin, C. M. & U. Markaida, 2009. Oocyte development, fecundity and spawning strategy of large sized jumbo squid *Dosidicus gigas* (Oegopsida: Ommastrephinae). Journal of the Marine Biological Association of the United Kingdom 89: 789–801.

Nigmatullin, C. M., A. Arkhipkin & R. Sabirov, 1995. Age, growth and reproductive biology of diamond-shaped squid *Thysanoteuthis rhombus* (Oegopsida: Thysanoteuthidae). Marine Ecology Progress Series 43: 73–87.

Ortiz, N., 2013. Validation of macroscopic maturity stages of the Patagonian red octopus *Enteroctopus megalocyathus*. Journal of the Marine Biological Association of the United Kingdom 93: 833–842.

Pecl, G., 2001. Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids. Marine Biology 138: 93–101.

Rocha, F. & A. Guerra, 1996. Signs of an extended and intermittent terminal spawning in the squids *Loligo vulgaris* Lamarck and *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae). Journal of Experimental Marine Biology and Ecology 207: 177–189.

Rocha, F., Á. Guerra & Á. F. González, 2001. A review of reproductive strategies in cephalopods. Biological Reviews 76: 291–304.

Rodrigues, M., M. Garcí, J. Troncoso & Á. Guerra, 2011. Spawning strategy in Atlantic bobtail squid *Sepiola atlantica* (Cephalopoda: Sepiolidae). Helgoland Marine Research 65: 43–49.

Rodrigues, M., Á. Guerra & J. S. Troncoso, 2012. Reproduction of the Atlantic bobtail squid *Sepiola atlantica* (Cephalopoda: Sepiolidae) in northwest Spain. Invertebrate Biology 131: 30–39.

Sauer, W. H. & M. R. Lipiński, 1990. Histological validation of morphological stages of sexual maturity in chokker squid *Loligo vulgaris reynaudii* D'Orb (Cephalopoda: Loliginidae). South African Journal of Marine Science 9: 189–200.

Schwarz, R. & J. A. A. Perez, 2013. Age structure and life cycles of the Argentine shortfin squid *Illex argentinus* (Cephalopoda: Ommastrephidae) in southern Brazil. Journal of the Marine Biological Association of the United Kingdom 93: 557–565.

Selman, K. & J. M. Arnold, 1977. An ultrastructural and cytochemical analysis of oogenesis in the squid, *Loligo pealei*. Journal of Morphology 152: 381–400.

Sieiro, P., J. Otero & Á. Guerra, 2014. Contrasting macroscopic maturity staging with histological characteristics of the gonads in female *Octopus vulgaris*. Hydrobiologia 730: 113–125.

Wallace, R. A. & K. Selman, 1981. Cellular and dynamic aspects of oocyte growth in Teleosts. American Zoologist 21: 325–343.

Wright, P. J., 1992. Ovarian development, spawning frequency and batch fecundity in *Encrasicholina heteroloba* (Ruppell, 1858). Journal of Fish Biology 40: 833–844.