



Reproductive biology of *Parona signata* (Actinopterygii: Carangidae), a valuable economic resource, in the coastal area of Mar del Plata, Buenos Aires, Argentina

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The reproductive biology and life cycle of Parona leatherjacket, *Parona signata*, present in Mar del Plata (38°00'S 57°33'W) coast, was studied. Samples were obtained monthly since January 2018 to February 2019 from the artisanal fishermen and the commercial fleet of Mar del Plata. A histological analysis was carried out and the main biologic-reproductive parameters were estimated: fecundity, oocyte frequency distribution and gonadosomatic index (GSI). Both the macroscopic and microscopic analyses showed reproductive activity in March and November. Mature females were recorded, which showed hydrated oocytes, as was evidenced by the histological procedures. Both, the histological and the oocyte diameter distribution analyses showed the presence of all oocyte maturation stages in ovaries in active-spawning subphase, indicating that *P. signata* is a multiple spawner with indeterminate annual fecundity. Batch fecundity ranged between 36,426 and 126,035 hydrated oocytes/ female. Relative fecundity ranged between 42 and 150 oocytes/ g female ovary free.

Keywords: Fecundity, Gonad histology, Life cycle, Pampo solteiro, Reproductive ecology.

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Se estudió la biología reproductiva y el ciclo de vida de la palometa pintada, *Parona signata* en Mar del Plata (38°00'S 57°33'O). Las muestras fueron obtenidas mensualmente desde enero de 2018, hasta febrero de 2019, provenientes de la pesca artesanal y/o de la flota comercial de la localidad Mar del Plata. Además, se llevó a cabo un análisis histológico y se estimaron los principales parámetros reproductivos tales como: la fecundidad, análisis de la distribución de la frecuencia de diámetros ovocitarios e índice gonadosomático (GSI) mensual. Tanto el análisis macroscópico como el microscópico permitieron afirmar que en los meses de marzo y noviembre existe actividad reproductiva. Se encontraron hembras en puesta, las cuales presentaron ovocitos hidratados en los cortes histológicos. Ambos, los análisis histológicos y de distribución de la frecuencia de diámetros ovocitarios mostraron la presencia de todas las fases de maduración oocitaria en ovarios correspondientes a la sub-fase de desove activo, indicando que *P. signata* es un desovante parcial con fecundidad anual indeterminada. La fecundidad parcial osciló entre 36.426 y 126.035 ovocitos hidratados/hembra. La fecundidad relativa varió entre 42 y 150 ovocitos por gramo de hembra libre de ovario.

Palabras clave: Ciclo de vida, Ecología reproductiva, Fecundidad, Histología gonadal, Palometa.

INTRODUCTION

The pelagic fishes are essential components in the marine ecosystem due to their important biomass in the intermediate levels of the trophic chain. They play an important role in the connection of the upper and lower trophic levels. This means lower level fishes serve as food for other species (Cury *et al.*, 2003). Moreover, pelagic fishes include species of great commercial interest.

The argentine continental shelf bottoms possess a substrate composed mainly of sand, some mud and rocky areas. The waters that cover this region have a sub-Antarctic origin, diluted by continental discharges and modified by exchanges of mass and heat with the atmosphere. These nutrient rich sub-Antarctic currents and their interactions with continental discharges make the Atlantic coast of the province of Buenos Aires an important fish feeding and breeding area (Cousseau, Perrotta, 2013).

The Order Carangiformes (Actinopterygii) is a monophyletic fish group, widely distributed in the Atlantic, Pacific and Indian Oceans, which includes six families, 37 genera, and 160 species (Bannikov, 1987; Nelson *et al.*, 2016). Only one family (Carangidae) occurs in Argentina and includes 140 species, which are extremely variable in body shape (Cousseau, 2010). Three species of this family are commercially important in the argentine sea: *Parona signata* (Jenyns, 1841), *Trachurus lathami* Nichols, 1920 and *Seriola lalandi* Valenciennes, 1833.

Parona signata (locally called as “Pampo-solteiro” or “Pampo-macho” (Brazil), “Palometa pintada” (Argentine) and “Parona leatherjacket” (global), is a medium size, coastal pelagic fish that occurs in South America, from Rio de Janeiro (22°35'S) to the

Estrecho de Magallanes and Islas Malvinas (Cousseau, Perrotta, 2013). Adult specimens of Parona leatherjacket can reach a maximum of 600 mm of total length, and the highest age recorded was 6 years old (San Román, 1976). Due to its commercial value and wide distribution, this species results an important economic resource for the region. It is commercialized in the domestic market, whole, fresh. It has been exported frozen whole (Cousseau, Perrotta, 2013). The landings statistics for Argentina ranged from 350 to 1123 tons (mean= 740 tons/year), between 2012 and 2016 (Navarro *et al.*, 2019). Parona leatherjacket was recently evaluated by the IUCN and included in Red List of Threatened Species 2019 (Carpenter *et al.*, 2019). As Carpenter *et al.* (2019) indicates: ... “This pelagic, nearshore species can be common and abundant in several parts of its range. It is commercially exploited in a large portion of its range (Argentina and Uruguay), and catch has declined by about 76% over the past decade. However, more information is needed on fishing effort trends over time before catch decline can be attributed to actual population decline. Given its broad overall range and the current lack of data to infer or suspect population decline, this species is listed as Least Concern. Research into fishing effort trends in Argentina and Uruguay should be a priority, moving forward”.

Although the previous assessments point out that this resource must be a “hot spot” in research with conservation purposes, biology reproductive studies (including life cycle and estimates of reproductive parameters) of *P. signata* are scarce. At respect, an analysis of trends across studies of age and size at maturation support that fishing pressure is playing a major role in the life history changes observed in commercial fish stocks (Sharpe, Hendry, 2009). At present, the biological studies performed in *P. signata* include: age estimations and length–age relationships (San Román, 1976), ovarian development at macroscopic level (Macchi, Acha 1998), estimations of first maturity size (Perrotta *et al.*, 2006). Finally, although a spring–summer spawning was suggested (Cousseau, Perrotta, 2013) no reliable histological data that support it, including epoch and area, was published at present. So far, no studies related to the life cycle (based on monthly samples during an annual cycle) of adults *P. signata* have been carried out. Moreover, neither estimation of monthly GSI means values nor fecundity has been previously reported for this species.

Taking into account the above-mentioned information, the aim of the present work was to study the life cycle of the Parona leatherjacket, *P. signata*, in Mar del Plata coast (38°00'S 57°33'W), Buenos Aires, Argentine. The objectives were: (1) to perform an histological analysis of ovarian development during an annual cycle and describe the stages of oocyte development; (2) to characterize the seasons and spawning areas, if there were any in the area under study; (3) to estimate the main biologic reproductive parameters for the stock of *P. signata* that inhabits in Mar del Plata (batch fecundity, frequency of oocyte diameter distribution, and gonadosomatic index).

MATERIAL AND METHODS

Study area and fish sampling: Samples were collected monthly, from January 2018 to February 2019, from artisanal fishermen and the commercial fleet of Mar del Plata (38°00'S 57°33'W), Buenos Aires province, Argentine. These fleets usually employed

pelagic bottom-trawls with gates. A total of 111 females were collected and analyzed during the sampled period.

Additionally, temperature and salinity were retrieved from Servicio de Hidrografía Naval and then compared with the estimated biological parameters (http://www.hidro.gov.ar/ceado/Ef/EF_FIJAS.asp?op=8). Spawning epoch of fishes are usually defined by bounded ranges of these two environmental variables.

After collected, fishes were transported immediately to the Laboratorio de Biotaxonomía Morfológica y Molecular de Peces (BIMOPE), at Instituto de Investigaciones Marinas y Costeras (IIMyC) (UNMdP – CONICET). The specimens were taxonomically identified following Cousseau, Perrotta (2013). Total length (TL) and standard length (SL) were recorded to the nearest mm. Total weight (TW) and ovary weight (OW) were recorded to 0.1g with an analytic balance. Specimens were sexed. A macroscopic and microscopic maturity scale was employed based on five phases according to Brown-Peterson *et al.* (2011), as follows: 1-immature, 2-developing, 3-spawning capable (which include a sub-phase of active spawning), 4-regressing and 5-regenerating. Three voucher specimens were deposited in the Fish Collection of the IIMyC (UNMdP 5162, 5163 and 5164). Each individual was represented by a digital photograph and a tissue sample, stored in alcohol 96°.

Histological processing: Weighed ovaries were stored in Davidson solution for 12 hours. After that, ovaries were preserved in Ethanol 50°. A small tissue portion was removed from the fixed ovary and then it was histologically processed according to González-Castro *et al.* (2011) and López *et al.* (2013). Sections were cut at 5µm and stained with hematoxylin/eosin (H/E) technique.

Frequency distribution analysis of oocyte diameters: After the fixation, a small ovary portion was removed and rehydrated. A total of 220 oocytes per ovary were measured by the longest axis with a graduated ocular according to González-Castro *et al.* (2011). The oocyte diameter frequency distribution of six hydrated ovaries was plotted.

Fecundity estimation and Gonadosomatic Index (GSI): Batch fecundity (number of oocytes released per spawning) was estimated employing five ovaries in spawning capable maturation phase (active spawning sub-phase), stored in ethanol 50° after fixation. These ovaries contained hydrated oocytes and they showed no evidence of post-ovulatory follicles or atresic follicle. Three portions (of approximately 1 g) of the anterior, medial and posterior parts of the gonad were rehydrated, weighed with an analytic balance (± 0.0001 g) and all hydrated oocytes were counted. Batch fecundity was calculated according to Hunter *et al.* (1985). Relative fecundity (number of hydrated oocytes per gram of ovary-free body weight) was calculated as the batch fecundity divided by female weight (ovary-free) (Hunter *et al.*, 1985). The GSI was calculated as the ovary weight divided body weight ($\times 100$). It was analyzed in relation of the annual cycle and maturity phases. In order to test homogeneity of variances (Homocedasticity) a Levene Test was performed. As this assumption was not met, a Welch ANOVA followed by the Games-Howell Post-Hoc Test ($\alpha=0.05$) were performed, to test if exist differences in the females GSI among the sampled months. The Games-Howell Post-Hoc test is employed when equal variances are not assumed.

Ovarian Cycle: From macroscopic and microscopic ovary analysis, the gonadal cycle was recognized.

RESULTS

Gonadosomatic index (GSI), sea temperature and salinity: Two peaks corresponding to March (GSI= 6.89 ± 6.22 SD) and November (GSI= 10.67 ± 2.64 SD) (where the maximum value was reached) were showed in the monthly average GSI values. These peaks corresponded to the reproductive period of *P. signata*. The GSI showed lower values in December (1.76 ± 0.6 SD). Moreover, the winter season is associated with small GSI values (Fig. 1). There were differences among groups, Welch ANOVA ($F=24.727$, $p < 0.001$) and Games-Howell Post-Hoc test revealed that the GSI was higher for October (mean GSI = 6.20 ± 2.51 SD) and November (mean GSI= 10.67 ± 2.64 SD) when compared to the others months (data not shown), with the exception of March (mean GSI= 6.87 ± 6.22 SD). There were no differences among the months of October, November and March (Oct-Nov $p=0.74$; Mar-Oct $p=0.90$; Mar-Nov $p=1.00$).

Sea temperature rounded $\sim 10^\circ\text{C}$ in the winter season, while ranged between 17 to 21°C in the spring-summer season. Highest values corresponded to the two GSI peaks in March and November (20 and 17°C , respectively). Accordingly, the lowest GSI mean value was obtained in August (1.34 ± 0.11 SD) and corresponded to the lower temperature mean values (10°C). The salinity of 34 ups did not present fluctuations during the sample period.

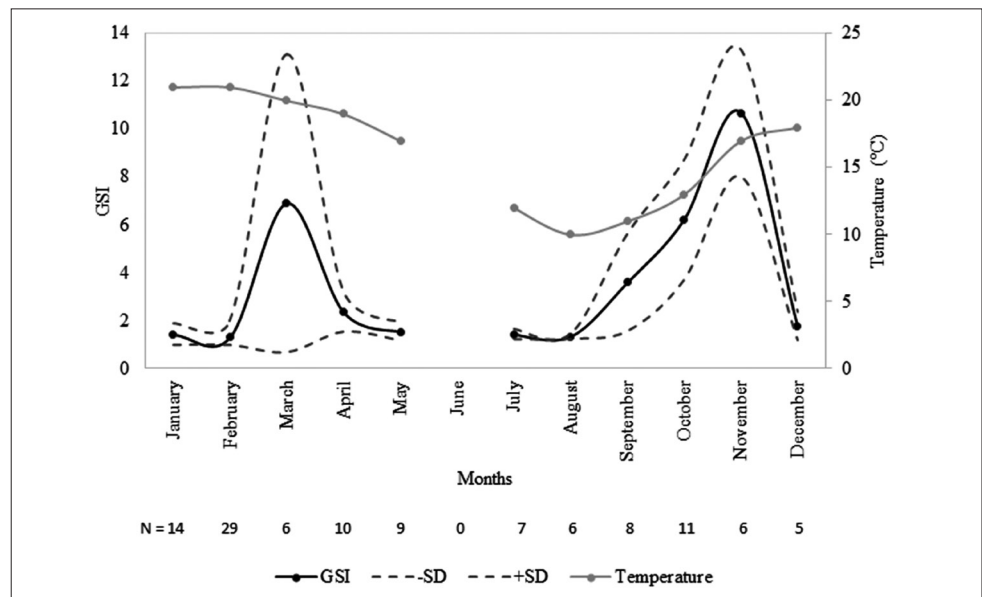


FIGURE 1 | Monthly variation of the gonadosomatic index (GSI) of females (black continuous line), standard deviation (dashed lines) and temperature (in Celsius degrees) (gray continuous line) based on an annual cycle.

Ovarian cycle: Five ovarian phases were recorded. Phase 1 (immature individuals) (n=18) was recorded mostly between April, July and August. Phase 2 (development) (n=35) and 3 (spawning capable) (n=26) were recorded in almost every month, except for December. The most advanced maturity phase (active spawning sub-phase) was recorded in March and November. Phase 4 (regressing) (n=15) was recorded in summer season and a few individuals in May. Finally, Phase 5 (regenerating) (n=17) was recorded in February, April and May after the reproductive events (Fig. 2).

Microscopic description of the ovarian maturity phases: In the analyzed period, five phases were recorded:

1) Immature: in this phase individuals presented small pink ovaries, with a thin tunic. Ovaries contained only oogonias and oocytes in primary growth. Ovarian lamellas were arranged in order. This stage was observed between April and October.

2) Development: an increase in the ovarian size/weight characterized this phase. The oocytes and blood vessels become visible. The ovaries showed primary growth, cortical alveoli, and early vitelogenesis oocytes. This stage was observed in almost every month.

3) Spawning capable: the ovaries approximately occupied half of the abdominal cavity and they were yellow. Ovarian arteries and its ramifications were evident. The oocytes were macroscopically visible (mostly yolk oocytes). This stage was observed in all annual cycle months, without winter ones. In the active spawning sub-phase the ovaries occupied almost the total abdominal cavity and they were orange, with a thick tunic. Ovaries contained very big hydrated oocytes (approximately 1,250 μm). This stage was observed in March and November.

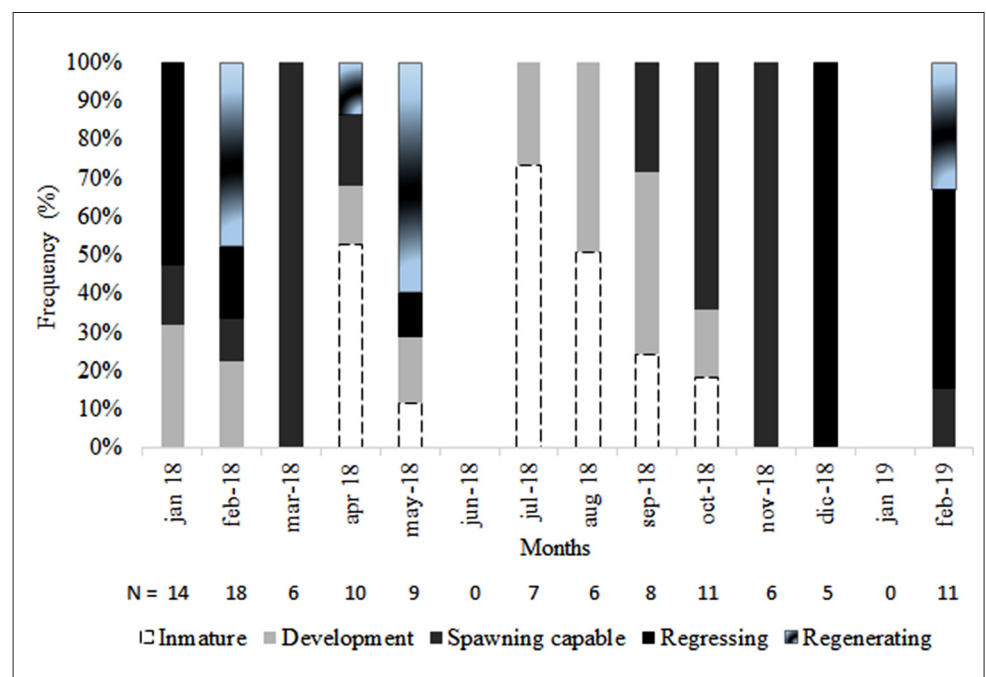


FIGURE 2 | Monthly relative frequency of the different gonadal development stages observed in females of *Parona signata* on the annual cycle.

4) Regressing ovaries: this phase is characterized by flaccid ovaries with thick tunics, displaying prominent blood vessel due to recent spawning activity. Atresia and post-ovulatory follicles are typically present. This stage was observed in January, February, May and December.

5) Regenerating: the ovaries, in yellow, decreased their size. The tunic remained thick and the ovaries recovered the turgidity. Predominantly primary growth oocytes presented. This stage was observed in February, April and May.

Microscopic description of the development and growth of oocyte phases: During the analyzed period, the followings stages were observed: Primary growth oocytes; Cortical alveolus stage; Yolke oocytes; Hydrated oocytes; Atretic follicle; Post-ovulatory follicles (POFs) (Tab. 1, Fig. 3).

Frequency distribution of oocyte diameters. The size distribution of formalin-preserved oocytes (spawning capable phase) of *P. signata* is presented in the Fig. 4. The smallest group was composed mainly of primary growth oocytes (smaller than 150 μm). The second group was composed of cortical alveolus stage oocytes ranging from 200 to 300 μm . A third group included yolke oocytes between 350 and 750 μm . The fourth

TABLE 1 | Description of stages of oocyte development, atretic follicles and post-ovulatory follicles of *Parona signata* from the present study.

Stages	Microscopic characteristics
Primary growth oocytes	Small cells with a diameter between 50-200 μm , showing a big nucleus. Between 4 -16 nucleolus were found in their periphery (in some cases rounding 18 nucleolus). These cells have a basophil cytoplasm.
Cortical alveoli	With a size between 200-300 μm , with a big nucleus. Small vesicles, the cortical alveoli, characterized this phase. They located in the cell periphery. In this stage the cytoplasm is basophilic, the radiate zone is visible (highly eosinophilic), as well as the follicular layer (granulosa and theca cells).
Yolke oocytes	Oocytes with diameters from 350 to 750 μm . Yolk granules (with eosinophilic affinity) present in the cytoplasm, due to the exogenous vitellogenesis. Although less frequent, lipid vesicles are observed dispersed between the yolk granules. Radiated zone (highly eosinophilic) and the follicular cells reach their maximum development. The nucleus continues in central position, with an irregular shape due to the cytoplasm growth. It is remarkable the presence of lipid vessels in the yolke oocytes cytoplasm.
Hydrated oocytes	Hydrated oocytes; with a size between 1000 and 1500 μm . Germinal vesicle migration (GVM), and break down (GVBD) of nuclear membrane are the previous steps to the cytoplasmic hydration, where the cellular size increase notoriously. Hydrated oocytes displayed an irregular shape, due to the alcohol dehydration performed during the histological procedures.
Atretic follicles	Disintegration and reabsorption of the oocytes by the follicular cells, from the periphery to the center. Observed mainly at the end of the reproductive cycle. Characterized by nucleus disintegration and autolysis of yolk oocytes.
Post-ovulatory follicles	After ovulation, the remaining follicular cells form an irregular shape in the ovary tissue. The follicular lumen size decreased and the degradation increased according to the time since ovulation. Characterized by irregular shape and advanced degradation. With a small follicular lumen.

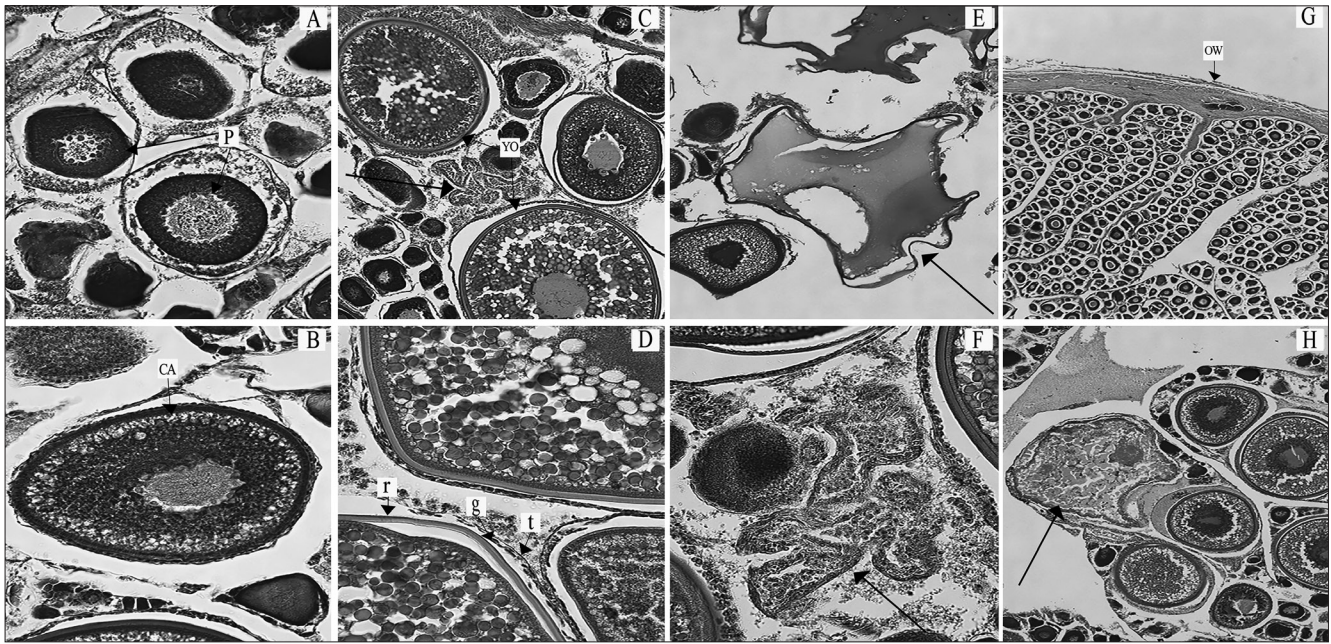


FIGURE 3 | Photomicrographs of *Parona signata*. **A**: primary growth oocytes (P); **B**: cortical alveoli oocytes (CA); **C**: yolked oocytes (YO) and a post-ovulatory follicle (large arrow); **D**: details of a yolked oocyte (r: radiate zone; g: granulosa cells; t: theca cells); **E**: hydrated oocyte (arrow); **F**: details of a post-ovulatory follicle (arrow); **G**: primary growth oocytes in an adult ovary (notice the thick wall of the ovary – OW); **H**: atresic follicle (arrow).

group included hydrated oocytes ranging from 800 to 1500 μm . Ovaries displayed a continuous distribution from primary growth to hydrated oocytes as revealed by the histological analysis. This indicates a constant recruitment of pre vitellogenic oocytes to the hydrated group during the reproductive season, which implies this species present an indeterminate fecundity.

Fecundity: Batch fecundity estimates ranged between 34,246 and 126,035 hydrated oocytes, with an average of 69,834 ($\pm 42,228$). Batch fecundity estimated corresponding to females between 460 and 480 mm TL respectively. Relative fecundity estimates ranged between 42 and 150 oocytes per gram of female, for 490 and 460 mm TL, respectively.

DISCUSSION

This study constitutes the first contribution on the reproductive cycle of *P. signata*, based on a one-year complete monthly sampling, with a histological overview. The highest GSI values were obtained in November and March (10.67 ± 2.64 SD and 6.89 ± 6.22 SD, respectively). During these months, the specimens were in spawning capable phase. Moreover, during this period females in the active spawning sub-phase were recorded. In December, a low GSI value (1.76 ± 0.6 SD) was observed, which was due to females in the regressing ovarian phase. Similar results were found in others carangid

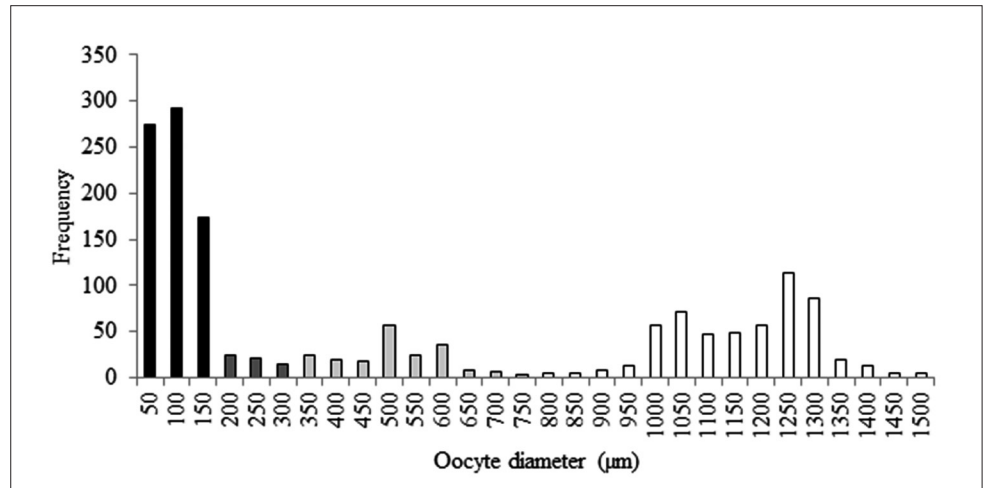


FIGURE 4 | Frequency distribution of oocyte diameters (n=3,000 oocytes measured of *Parona signata*). From black bars to white bars: primary growth oocyte, cortical alveoli, yolked oocytes and hydrated oocytes, respectively.

species, where the species showed two spawning peaks during its annual reproductive season. For example, *Caranx ruber* (Bloch, 1793), a native Cuban species exposed two spawning peaks in summer (García-Arteaga, Reshetnikov, 1986). In addition, *Scomberoides lysan* (Fabricius, 1775) showed a reproductive activity during June and November (Thulasitha, Sivashanthini, 2013). However, other authors postulated that reproductive biology can be highly variable between carangids (McBride *et al.*, 2002).

During the studied period, *P. signata* was present in all months, except on June 2018, when no records were obtained. This could imply that *P. signata* would make an offshore migration, where the temperature would be warmer than in the coast. Another explanation would be to understand it (no *P. signata* catches on June) as a fortuity event derived from the sampling logistic. Radonic (1997) reported the presence of *P. signata* from January to June in Mar del Plata's shores. In the same way, Lagos (2001) recorded this species in Cabo San Antonio's (Buenos Aires Province, Argentina) shores during all year long. Our results indicated that the reproductive events of *P. signata* were performed at temperatures between 21 and 17°C (March and November, respectively), and salinity was ~34 ups. These agree with Lagos (2001), which reported gravid females in Cabo San Antonio shores (36°17'35" S 56°46'37" W) during the spring-summer season. Moreover, Acha (2012) recorded eggs and larvae with densities between 0.01 and 10 larvae/m³ in the surface of El Rincón (Buenos Aires Province, Argentina), during November 2008. Finally, Perrotta *et al.* (2006) and González-Castro *et al.* (2009) recorded the presence of immatures specimens of *P. signata* during the autumn-winter season in estuary areas. These results suggest that *P. signata* could be using some estuaries of Buenos Aires Province with feeding/growing purposes.

The histological analysis and the oocyte diameter frequency distribution showed the presence of all oocyte maturation stages in ovaries in the active-spawning subphase, according to Brown-Peterson *et al.* (2011). This fact indicates that *P. signata* is a multiple spawner with indeterminate annual fecundity. This species displayed continuous oocyte

recruitment throughout the reproductive season (November, March). The oocyte diameter frequent distribution of *P. signata* evidenced four clutches/ groups of oocytes easily differentiable in histological slides (between 50 and 1500 μ m) and ovaries displayed a continuous distribution from primary growth to advanced yolked oocytes. These four clutches showed a continuous distribution and were composed of oocytes in primary growth, cortical alveolus stage, yolked oocytes and finally the hydrated oocytes. It was remarkable the presence of lipid vessels in the yolked oocytes cytoplasm. They would form an oil droplet in the hydrated oocytes, which is vital for the eggs' buoyancy and larvae survival and development.

In this study, hydrated oocytes were employed for the fecundity estimation. The batch fecundity showed a range between 36,426 to 126,035 oocytes/ female (with an average of 69,834 \pm 42,228 SD oocytes), corresponding to gravid females with a ranged length between 460 to 540 mm (TL). This estimation of fecundity was higher when compared with those of the most important species with similar reproductive strategies. For example, the batch fecundity of *Anchoa marmorata* Hildebrand, 1943 ranged from 749 to 3,207, for individuals of 76-114mm TL (López *et al.*, 2013). Rodrigues *et al.* (2008) reported batch fecundity estimation of 13,675 oocytes (\pm 856) for *Engraulis anchoita* Hubbs, Marini, 1935. On the other hand, when comparing the relative fecundity of *P. signata* with the other mentioned species, the values estimated in the present work were much lower. They ranged from 42-150 hydrated oocytes/ female gram (free ovary), compared with 127-422 oocytes for *A. marmorata* (López *et al.*, 2013); and 574 oocytes for *E. anchoita* (Pájaro *et al.*, 1997). These differences could be due to the largest size of the hydrated oocytes of *P. signata* (800 to 1500 μ m), in comparison with other local species (*A. marmorata* and *E. anchoita*), which are less than 1000 μ m (Pájaro *et al.*, 1997; López *et al.*, 2013). The size and number of eggs produced vary greatly among species and all of these traits have important consequences for survival. Egg is positively correlated with the duration of the incubation period and the size of the larva. The greater amount of yolk in large eggs provides more total energy for growth, resulting in larger larvae at hatching and at first feeding. This yields several vital benefits: (a) larvae emerge with a better fitness of behavioral and physiological capabilities than less developed larvae from smaller eggs; (b) they are more resistant to starvation because weight-specific metabolic rates are lower and bodily energy stores are greater; and (c) the larval period is shorter (Fuiman, 2002).

It is necessary to take care of this important economic resource, mainly because is an endemic South West Atlantic Ocean species, but also represent a valuable protein source. An exploited stock is renewed by means of recruitment through reproduction. If fishing pressure on this resource doesn't have any management measure, its populations could be seriously threatened by mature losses. One way of mitigating this risk is to ensure that minimal fishing pressure applied to the populations before the fish reach maturity. So, given that *P. signata* reach maturity at 30.5 cm these fish should not be caught until getting such size. Moreover, as the spawning peaks were recorded in March and November, the gravid females of *P. signata* should be protected from fishing pressure during this period, in order to maintain a sustainable fishery and a proper intraspecific genetic variability. A seasonal closure would be a convenient measure, in order to protect the key life stages of this species. Following these steps, *P. signata* long-term viability might be safe.

This is the first integrative approach of the reproductive biology of *P. signata*, an important food resource for Argentina, Uruguay and Brazil. Our results exposed above strongly suggest that *P. signata* reproduce in the Mar del Plata coastal zone, and the adult specimens would occur during all year long. Future studies should be focused to obtain more specimens in the nearest areas to determine if there exists any correlation with the spawning season and to corroborate the fecundity estimates. Moreover, it will be necessary to expand the sampling area, to infer the plausible movements of this species and hypothesize a life-history model.

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AUTHOR'S CONTRIBUTION

Santiago Julián Bianchi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing.

Mariano González-Castro: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing.

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The authors declare no competing interests.

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