

Research Article

***Paralichthys patagonicus* spawning areas and reproductive potential in the Bonaerense Coastal Zone, Argentina (34°-42°S)**

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ABSTRACT. The reproductive biology of Patagonian flounder (*Paralichthys patagonicus*) was studied in the Bonaerense Coastal Zone (34°-42°S) using samples collected in December 2003 and 2005. According to the literature, the data were analyzed considering two population groups: one in the northern zone (34°-38°S) and another in El Rincón (38°-42°S). The estimated size at first maturity for both sexes in 2003 was 31.57 cm TL for the northern zone and 29.58 cm TL for El Rincón. In 2005, the L_{50} was only estimated for the northern zone, and the value obtained was less than that of 2003 (27.82 cm TL). In December 2003, the greatest reproductive activity took place in El Rincón. However, in December 2005, the highest proportions of hydrated females were observed in the northern area. This could be due to the cooler temperatures recorded for this zone, as compared with 2003. Batch fecundity showed a potential fit to size and a lineal fit to weight and ranged from 14,685 (30 cm TL) to 153,717 (59 cm TL) hydrated oocytes. Relative fecundity oscillated between 24 and 170 hydrated oocytes/g. The values of these parameters differed between years and among population groups. These differences could be attributed in part to the difference in seawater temperature between the two years, especially the high temperatures observed in 2005.

Keywords: *Paralichthys patagonicus*, spawning, fecundity, size at first maturity, Bonaerense Coastal Zone, Argentina.

Áreas de desove y potencial reproductivo de *Paralichthys patagonicus* en la Zona Costera Bonaerense, Argentina (34°-42°S)

RESUMEN. Se estudió la biología reproductiva del lenguado (*Paralichthys patagonicus*) en la Zona Costera Bonaerense (34°-42°S). El material provino de muestreos realizados durante diciembre de 2003 y 2005. En base a la bibliografía los datos fueron analizados teniendo en cuenta dos grupos poblacionales, uno en el norte (34°-38°S) y otro en El Rincón (38°-42°S). La estimación de la talla de primera madurez en 2003 para ambos sexos fue de 31.57 cm LT para la zona norte y de 29.58 cm LT para El Rincón. En 2005 solo se pudo estimar el L_{50} para la zona norte y el valor obtenido fue menor al 2003 (27.82 cm LT). En diciembre del 2003 la mayor actividad reproductiva tuvo lugar en El Rincón. Sin embargo, en diciembre de 2005 las proporciones más altas de hembras hidratadas se localizaron en el sector norte. Esto podría deberse a que se registraron temperaturas más frías para esta zona respecto a 2003. La fecundidad parcial presentó un ajuste potencial con la talla y lineal con el peso y varió entre 14.685 (30cm TL) y 15.3717 (50 cm TL) ovocitos hidratados. La fecundidad relativa varió entre 24 y 170 ovocitos hidratados/g. Los valores de ambos parámetros presentaron diferencias tanto interanuales como entre los grupos poblacionales. Estas diferencias podrían atribuirse en parte a la diferencia en la temperatura del agua entre los dos años, especialmente a las altas temperaturas observadas en 2005.

Palabras clave: *Paralichthys patagonicus*, desove, fecundidad, talla de primera madurez, Zona Costera Bonaerense, Argentina.

INTRODUCTION

Approximately 45 flatfish species from six Families (Pleuronectidae, Achiridae, Bothidae, Paralichthyidae, Cynoglossidae and Achirosetidae) occur in the southwest Atlantic Ocean south of the Amazon River. However, not all of them have commercial importance due to either their small size or low abundance; only the pleuronectids and paralichthyids are commercially important (Díaz de Astarloa, 2002).

Paralichthyid flounders are distributed mainly between the coast and 150 m on the Brazilian, Uruguayan and Argentinean continental shelves (Díaz de Astarloa & Munroe, 1998); however, the main concentrations are located in northern Argentina and Uruguay (34°-41°S) (Díaz de Astarloa & Fabré, 2003). Three paralichthyid species (*Paralichthys patagonicus*, *P. isosceles* and *Xystreureys rasile*) co-occur in two main groups, a northern group between 34°-38°S, and a southern group between 38°-41°S (Díaz de Astarloa, 2002). *P. patagonicus* is the most abundant species in the northern area with relatively high densities in spring (Díaz de Astarloa, 2002) which coincides with the reproductive activity of the species. This flounder spawns between September and February with maximum activity in November (Macchi & Díaz de Astarloa, 1996). The low density observed in summer could be due to reproductive migrations towards shallower areas where it is under sampled (Díaz de Astarloa & Fabré, 2003).

P. patagonicus occurs from shallow waters up to 200 m depth and is commonly captured between 70 and 100 m (Carneiro, 1995; Díaz de Astarloa & Munroe, 1998); however, Díaz de Astarloa & Fabré (2003) observed that it mainly occurs between 41 and 70 m depth. This species is the most commercially important paralichthyid flounder in the southwest Atlantic mainly because of its high abundance and large size as compared to other flatfishes in the area (Díaz de Astarloa, 1994). It is also the main species of flounder landed from commercial bottom trawl fisheries on the continental shelf and coastal shallow-waters in southern Brazil (Carneiro, 1995).

Due to the intense exploitation of this flounder and the scarcity of information in the literature, it is important to improve our understanding of the biology of this species. The aim of the present study is to provide information about maturation and spawning of *P. patagonicus* in the Buenos Aires coastal area and the Argentinean–Uruguayan Common Fishing Zone (AUCFZ) from macroscopic and histological analyses of gonads. In addition, length at first sexual maturity and batch and relative fecundity are also estimated. This information, although preliminary, is critical for

the sustainable management of this marine resource, particularly for establishing seasonal or space fishing prohibitions.

MATERIALS AND METHODS

P. patagonicus specimens were obtained from bottom trawls done during two spring fisheries evaluation cruises from Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) in 2003 (from 28 November to 21 December 2003) and 2005 (from 12 November to 21 December 2005) (Fig. 1). Biological samples were taken by using a bottom net with a mouth width of about 20 m, a height of about 4 m, and with 20 mm mesh at the inner cover of the cod end. In addition, oceanographic sampling was done at each trawl station with a Sea-Bird 19 CTD (conductivity-temperature-depth profiler), with a lowering speed of 0.5 m s⁻¹.

All individuals sampled (N = 662) were sexed, measured to the nearest centimeter (total length, TL) and weighed to the nearest gram (total weight, TW). The maturity stage for males and females was determined macroscopically following a maturity key designed for fishery studies (Macchi & Pájaro, 2003) which includes the following stages: 1) immature, 2) developing and partially spent, 3) gravid (with hydrated oocytes) or running, 4) spent, and 5) resting.

Based on the literature, biological data were separated into two groups according to their origin: a northern group between 34°-38°S corresponding to the Argentinean–Uruguayan Common Fishing Zone

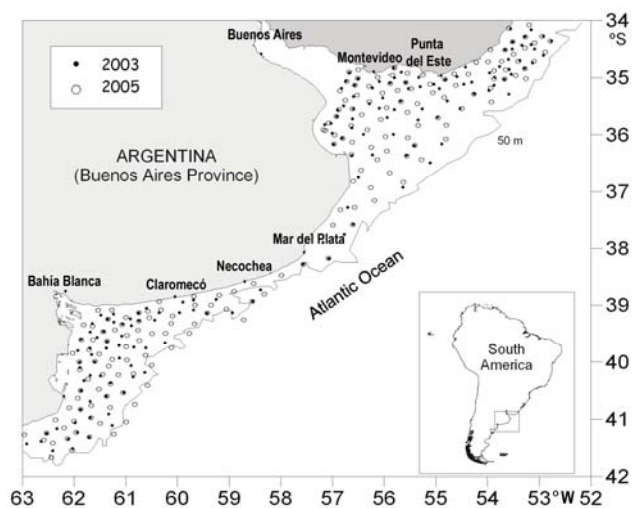


Figure 1. Spatial distribution of demersal trawl stations during December 2003 and 2005.

Figura 1. Distribución espacial de los lances de pesca durante diciembre 2003 y 2005.

(AUCFZ) and a southern group between 38°-41°S, in El Rincón (Díaz de Astarloa, 2002). This information was used to analyze the length distribution at different sampling sites and to determine the length at first maturity (L_{50}) of *P. patagonicus*. To estimate this parameter, a logistic model was fitted to the proportion of mature individuals (stage 2 to 5) by length class by using the maximum likelihood method (Kendall & Stuart, 1967). Coefficients of regression obtained for males and females from both sampled years were compared using a χ^2 test (Aubone & Wöhler, 2000).

Because flounder ovaries present a low number of hydrated oocytes during the spawning phase and the mature components are of reduced diameter, it is difficult to differentiate the spawning stage from advanced maturation, making it necessary to do histological analyses of the gonads for classification (Macchi & Díaz de Astarloa, 1996).

For histological examinations, 266 paired ovaries of varying maturity stage were removed after capture and preserved in 10% neutral buffered formalin. Fixed gonads were weighed (GW) to the nearest 0.1 g and a portion of tissue was removed from the centre of each ovary, dehydrated in methanol, cleared in xylol and embedded in paraffin. Tissues were sectioned at approximately 4 μ m thick and stained with Harri's haematoxylin followed by eosin counterstain.

Batch fecundity (BF, number of oocytes released per spawning) was estimated gravimetrically with the hydrated oocyte method (Hunter *et al.*, 1985). Samples were examined histologically to determine the presence of postovulatory follicles (POFs) and hydrated oocytes. To avoid biases when estimating batch fecundity, only ovaries with hydrated oocytes and without recent POFs were used. A total of 42 mature females (16 in 2003 and 26 in 2005) were selected to estimate batch fecundity. Three pieces of ovary approximately 0.1 g each were sampled from the anterior, middle, and posterior sections of each gonad, weighed (0.1 mg), and the number of hydrated oocytes counted. Batch fecundity was estimated as the product of the mean number of hydrated oocytes per unit ovarian weight and total ovarian weight (GW). Relative fecundity (RF, number of hydrated oocytes per gram of ovary-free body weight) was estimated as the batch fecundity divided by female weight (without ovaries). The relationships between BF and the variables TL and TW (ovary-free) were described using simple standard regression (Draper & Smith, 1981). A power model and a linear model were fitted to the relationships of BF *versus* TL and BF *versus* TW (ovary-free), respectively. Comparisons between the mean BF estimated from each spawning area

during both years were done using ANOVA (Sokal & Rohlf, 1969).

During December 2003 and 2005, it was possible to collect samples of hydrated oocytes to estimate oocyte dry weight of females (DW) as an index of egg quality from different sampling groups. For this, 100 hydrated oocytes were removed from gravid ovaries of 51 females (17 from 2003 and 34 from 2005). These oocytes were rinsed in distilled water, dried for 20 h at 60°C and weighed (\pm 0.1 mg). An ANOVA test was used to compare DW mean values obtained from each sampling area and year. Taking into account that yolk reserves could be associated with the female nutritional state, we estimated the condition factor ($K = TW/TL^3 * 100$) for each female and compared the mean value obtained from the different years using a Student t-test for independent samples.

RESULTS

Length at first maturity

Length at first maturity (L_{50}) estimated for males and females of *Paralichthys patagonicus* in December 2003 and 2005 for the northern zone revealed high significant differences between sexes (t-test: $t_{03} = 16.6$, $n = 145$ $P < 0.01$; $t_{05} = 15.02$, $n = 360$ $P < 0.01$). Females reached sexual maturity at a larger size than males. In addition, during 2003 L_{50} obtained by taking both sexes together (31.6 cm TL) was significantly higher than in 2005 (27.82 cm TL) ($P < 0.01$) (Table 1).

Due to the low number of samples ($n = 69$) in particular the scarcity of juveniles ($n = 9$) obtained in El Rincón during 2005, L_{50} was only estimated for 2003. In this area, the mean value obtained for both sexes combined (29.6 cm TL) was significantly lower than in the northern zone ($P < 0.01$) (Table 1).

Spawning areas

During December 2003, the greater percentages of *P. patagonicus* spawning females (with hydrated oocytes) were detected in the El Rincón area (south off Buenos Aires Province) (Fig. 2a). This area was characterized by a bottom temperature front with values ranging between 13 and 17°C. On the other hand, in December 2005 spawning females were mainly located in the external sector of the Río de la Plata estuary (Fig. 2b), where water temperature was generally colder than that in 2003; nevertheless, the concentrations of reproductive females detected in the northern zone also coincided with bottom temperatures between 13 and 17°C.

Table 1. Length at first maturity (L_{50} cm) estimated for *Paralichthys patagonicus*. b: coefficient of the logistic model, N: number of fish analysed.

Tabla 1. Talla de primera madurez (L_{50} cm) estimada para *Paralichthys patagonicus*. b: coeficiente del modelo logístico, N: número de peces analizados.

Date	Region		Males	Females	Males and females
December 2003	Northern zone	L_{50} (cm)	27.00	36.00	31.6
		b	0.54	0.40	0.25
		N	54	91	145
December 2003	El Rincón	L_{50} (cm)	27.05	32	29.6
		b	0.36	0.63	0.42
		N	42	46	88
December 2005	Northern zone	L_{50} (cm)	22.78	30.28	27.82
		b	0.33	0.38	0.32
		N	161	199	360

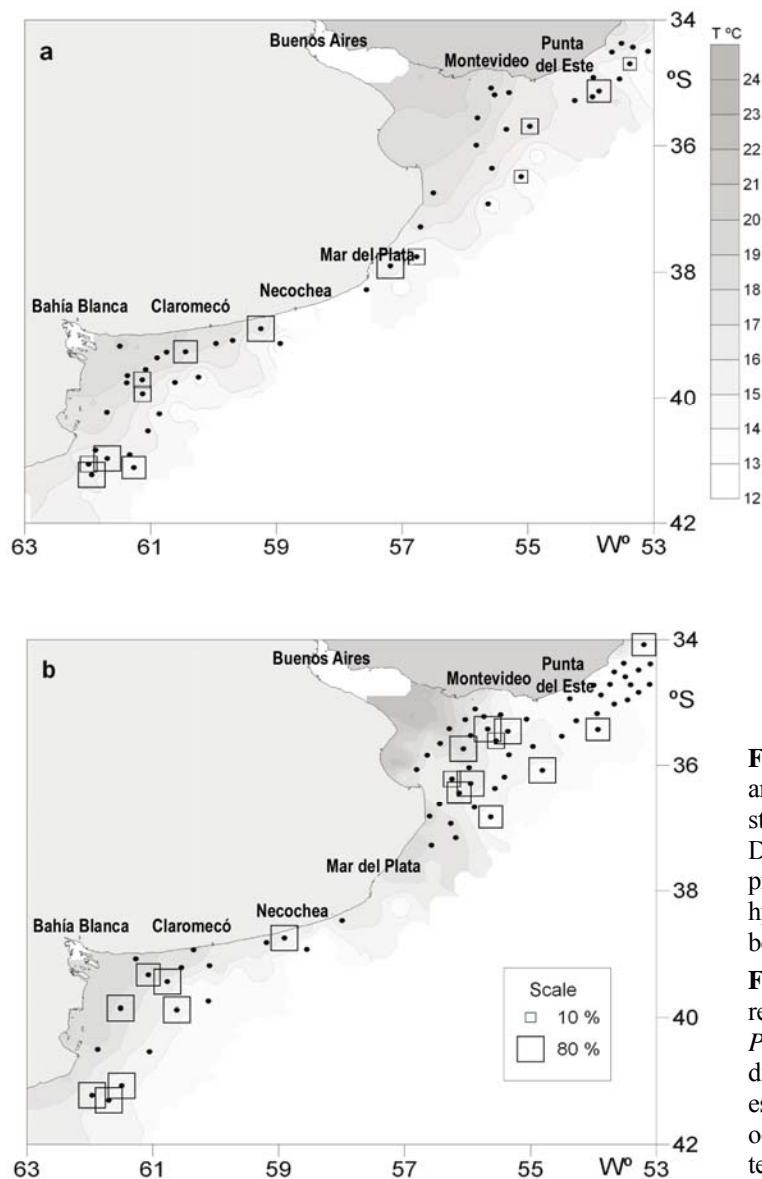


Figure 2. Presence of activity females (dots) (stage 2 and 3) and percentages of *P. patagonicus* in spawning stage (squares) during a) December 2003 and b) December 2005. The size of the squares is proportional to the percentage of gravid females (with hydrated oocytes). The isotherms, (°C), represent the bottom temperature field.

Figura 2. Presencia de hembras en actividad reproductiva (puntos) (estadio 2 y 3) y porcentajes de *P. patagonicus* en desove (cuadrados) durante a) diciembre 2003 y b) 2005. El tamaño de los cuadrados es proporcional al porcentaje de hembras grávidas (con oocitos hidratados). Las isotermas (°C), representan la temperatura de fondo.

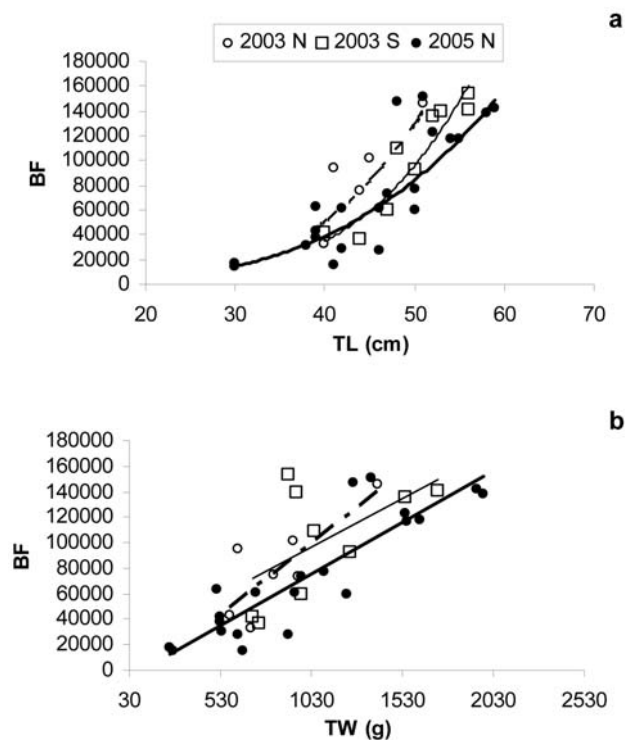


Figure 3. Batch fecundity (BF) of *P. patagonicus* as a function of a) total length (TL) and b) total weight (TW) (without ovary) obtained from December 2003 for northern zone (white dots and dashed line), December 2003 for El Rincón (squares and fill line) and December 2005 for northern zone (black dots and fill bold line).

Figura 3. Fecundidad parcial (BF) de *P. patagonicus* en función de a) la longitud total (TL) y b) el peso total (TW) (sin ovario) obtenidos en diciembre de 2003 en la zona norte (puntos blancos y línea punteada), diciembre de 2003 en El Rincón (cuadrados y línea continua) y diciembre de 2005 en la zona norte (puntos negro y línea en negrita).

Fecundity

P. patagonicus batch fecundity (BF) estimated for December 2003 and December 2005 showed a potential relationship with length and a linear relationship with weight of the individuals (Figure 3). Values of BF obtained for December 2003 ranged between 32,485 (39 cm TL) and 145,877 (51 cm TL) hydrated oocytes for the northern zone with an average of 80,830 ($\pm 28,148$) hydrated oocytes. ANOVA test showed that these estimates were significantly higher ($P < 0.05$) than those obtained for El Rincón, where fecundity values ranged between 36,215 (44 cm TL) and 153,717 (56 cm TL) hydrated oocytes with an average of 101,202 ($\pm 29,755$).

In 2005 only four mature females from El Rincon were selected for estimates of BF, with values ranging between 79,422 (46 cm TL) and 111,586 (50 cm TL)

hydrated oocytes. For the northern zone, BF values obtained in 2005 were significantly lower than in 2003 ($P < 0.01$) and ranged between 14,685 (30 cm TL) and 150,730 (59 cm TL) hydrated oocytes, with a mean value of 76,398 ($\pm 16,742$) hydrated oocytes.

The relationships between BF and TL and TW (ovary-free) for December 2003 and 2005 were described by:

$$\text{BF } 03' \text{ N} = 9 \text{ E-}3 \text{ TL}^{4.2187} \quad (r^2 = 0.62)$$

$$\text{BF } 03' \text{ N} = 110.43 \text{ TW} - 14,097 \quad (r^2 = 0.65)$$

$$\text{BF } 03' \text{ S} = 1.8 \text{ E-}3 \text{ TL}^{4.555} \quad (r^2 = 0.84)$$

$$\text{BF } 03' \text{ S} = 76.19 \text{ TW} + 17,790 \quad (r^2 = 0.34)$$

$$\text{BF } 05' \text{ N} = 0.118 \text{ TL}^{3.445} \quad (r^2 = 0.72)$$

$$\text{BF } 05' \text{ N} = 80.31 \text{ TW} - 6927 \quad (r^2 = 0.78)$$

P. patagonicus relative fecundity (RF) varied from 24 to 170 hydrated oocytes per gram of female (ovary-free). RF showed great dispersion based on the length of the females and it did not show any trends in relation to the size of the spawners. The values estimated in 2003 did not reveal significant differences between the sampling areas ($P > 0.05$). However, RF estimated in 2005 for the northern zone was significantly lower ($P < 0.01$) than that for 2003. The mean RF for both years was 93 ± 18 and 71 ± 11 oocytes per gram of female (ovary-free) respectively.

Oocytes dry weight

The oocyte dry weight showed no significant relationship with size of females and the female condition factor (K). The mean value obtained for 2003 (1.24 ± 0.15 mg per 100 eggs) showed no significant differences between the sampling areas and the mean value estimated for 2005 (1.28 ± 0.06 mg per 100 eggs) ($P > 0.05$).

DISCUSSION

Length at first maturity (L_{50}) estimated for *P. patagonicus* flatfish in December 2003 and 2005 showed significant differences between sexes, sampling areas, and years. In December 2003 the L_{50} obtained for the northern zone (31.57 cm TL) was significantly higher than in 2005 (27.82 cm TL), and than that estimated for El Rincón (29.58 cm TL). These values represent the first estimates of length at first maturity for this species, thus estimates should be continued in coming years to examine whether differences are the result of a trend, natural variability, or differences in population groups.

The spatial distribution of females of *P. patagonicus* with hydrated oocytes indicates that in December 2003, the major reproductive activity took

place in El Rincón, southern Buenos Aires province. These aggregations of spawning females were observed in association with a bottom temperature front parallel to the coast between 38.5°S and 41°S at depths shallower than 50 m. These results agree with the comments made by Macchi & Acha (1998) who observed the highest concentrations of *P. patagonicus* in the southern area of its distribution during November 1994. However, in December 2005 the highest proportion of spawning females was observed in the external sector of the Rio de la Plata, possibly due to the colder temperatures observed in the northern area relative to 2003.

P. patagonicus batch fecundity (BF) showed a potential relationship with length and a linear relationship with weight of the individuals. The BF values are similar to the estimates made by Macchi & Díaz de Astarloa (1996) for this species; however, these authors found that fecundity was linearly related to both parameters.

The number of hydrated oocytes per unit weight showed a similar pattern to BF, with differences between years but not between sampling areas in 2003. The mean relative fecundity for *P. patagonicus* during December 2003 (93 ± 18 oocytes) was higher than that obtained for December 2005 (73 ± 9 oocytes).

Because the hydrated oocyte diameter depends on the hydration degree, we considered the oocyte dry weight as a better index of egg quality. Dry weight of hydrated oocytes showed no relationship with the size or the nutritional condition of *P. patagonicus* females. This suggests that although the fecundity in 2005 was lower than in 2003, the quality of the oocytes was similar, which in turn indicates that egg yolk reserves were probably similar in 2003 and 2005.

Populations of *P. patagonicus* in the Buenos Aires shelf showed high interannual variability in reproductive activity between 2003 and 2005. These differences could be attributed in part to the difference in seawater temperature between the two years, especially at high temperatures observed in 2005. In addition to this, it was also possible to detect, from satellite images, high positive anomalies in chlorophyll concentrations for 2003 and negative ones for 2005 (Marrari *et al.*, 2010). These observations reinforce the theory of oceanographic anomalies observed in the two sampled years. These results highlight the need for future studies of the reproductive biology of this species in order to elucidate the factors that influence the variability in reproductive activity.

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