

UNIVERSIDADE FEDERAL DO RIO GRANDE
PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

**BIOLOGIA REPRODUTIVA DE *Octopus*
tehuelchus, ORBIGNY 1834 (CEPHALOPODA:
OCTOPODIDAE) NO SUL DO BRASIL**

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A Deus e a capacidade humana de
sonhar...

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RESUMO

Octopus tehuelchus é um pequeno polvo bentônico endêmico das águas subtropicais e temperadas da plataforma continental do Atlântico Sul ocidental. Sua biologia reprodutiva foi estudada a partir de 319 exemplares, medindo entre 20 e 79 mm de comprimento do manto (ML), coletados entre 1979 e 2009 na plataforma interna do Sul do Brasil. As fêmeas foram mais numerosas águas menos profundas e alcançaram tamanhos maiores que os machos. Fêmeas e machos totalmente maduros foram observados em todos os meses amostrados e o tamanho médio do manto na maturação sexual foi de 46 mm nas fêmeas e 27 mm nos machos. O número de ovócitos intraováricos variou entre 20 e 448 e se correlacionou positivamente com o tamanho das fêmeas. Nas fêmeas maduras (estágios III e IV) se observou uma grande amplitude de diâmetros nos ovócitos intraováricos, em alguns casos com uma distribuição bimodal. O número de ovos das quatro posturas observadas variou entre 86 e 237 e não foi observada bimodalidade nas suas distribuições de diâmetros. A glândula digestiva cresceu proporcionalmente ao peso corporal nas fêmeas, porém não nos machos, o que sugere uma acumulação de reservas para um longo período de postura e cuidado parental das desovas nas fêmeas e uma priorização da reprodução em detrimento ao crescimento nos machos. A comparação do ciclo reprodutivo de *O. tehuelchus* no Sul do Brasil com o de populações do Norte da Patagônia mostra que a espécie possui o potencial para desovar o ano todo, porém as limitações ecológicas permitem que este potencial se expresse somente nas latitudes mais baixas da sua distribuição.

Palavras-chave: *Octopus tehuelchus*, ciclo reprodutivo, maturação sexual, fecundidade, Atlântico Sul ocidental.

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1 **Reproductive biology of *Octopus tehuelchus*, Orbigny 1834 (Cephalopoda:
2 Octopodidae) in Southern Brazil**

3

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5

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10

11 **RUNNING TITLE:** Reproduction of *Octopus tehuelchus* in southern Brazil

12

13 **SUMMARY**

14 *Octopus tehuelchus* is a small octopus endemic to subtropical and temperate waters of
15 the southwestern Atlantic continental shelf. Its reproductive biology was studied by
16 examining 319 individuals, measuring 20 to 79 mm mantle length (ML), collected between
17 1979 and 2009 along southern Brazil. Females are more numerous in shallower waters and
18 attain larger size than males. Fully mature males and females were observed in all seasons and
19 mean mantle length at maturity was 46 mm for females and 27 mm for males. The number of
20 intraovaric oocytes of maturing females ranged from 20 to 448 and was positively correlated
21 with female size. In mature females, a wide range of intraovaric oocytes diameters was
22 observed, in some cases with a bimodal distribution. The number of eggs of four egg layings
23 ranged from 86 to 237 and no bimodality in the diameters was observed. Digestive gland
24 grew proportionally to body weight along maturation in females but not in males, suggesting
25 accumulation of reserves for spawning and parental care in females and priority for sexual
26 maturation over growth in males. The comparison of the reproductive cycle of *O. tehuelchus*

27 in southern Brazil with populations from northern Patagonia shows that the species has the
28 potential for year round spawning, but ecological constraints only allows it to express this
29 potential in the lower latitudes of its distribution.

30

31 **KEY WORDS:** *Octopus tehuelchus*, reproductive cycle, sexual maturation, fecundity,
32 southwestern Atlantic.

33

34 **RESUMEM**

35 *Octopus tehuelchus* es un pequeño pulpo endémico de las aguas subtropicales y
36 templadas de la plataforma continental del Atlántico Sudoccidental. Su biología reproductiva
37 se estudió examinando 319 ejemplares, entre 20 y 79 mm de longitud del manto (ML),
38 recolectados entre 1979 y 2009 en la plataforma interior del sur de Brasil. Las hembras fueron
39 más numerosas en aguas menos profundas y alcanzaron tamaños mayores que los machos.
40 Machos y hembras totalmente maduros fueron observados en todas las estaciones y la talla
41 media del manto en la madurez sexual fue 46 mm en las hembras y 27 mm en los machos.
42 El número de ovocitos intraováricos osciló entre 20 y 448 y se correlacionó positivamente
43 con el tamaño de las hembras. En las hembras maduras, se observó una amplia gama de
44 diámetros de ovocitos intraováricos, en algunos casos con una distribución bimodal. . El
45 número de huevos de las cuatro puestas observadas osciló entre 86 hasta 237 y no se observó
46 bimodalidad en sus diámetros. La glándula digestiva creció proporcionalmente con el peso
47 corporal en las hembras pero no en los machos, lo que sugiere la acumulación de reservas
48 para la puesta y cuidado de los huevos en las hembras y la priorización de la reproducción
49 sobre el crecimiento en los machos. La comparación del ciclo reproductivo de *O. tehuelchus*
50 en sur de Brasil con poblaciones del norte de la Patagonia muestra que la especie tiene el

51 potencial para desovar todo el año, pero limitaciones ecológicas permiten que este potencial
52 se exprese solo en las bajas latitudes.

53

54 **PALABRAS – CLAVE:** *Octopus tehuelchus*, ciclo reproductivo, maduración sexual,
55 fecundidad, Atlántico Sudoccidental.

56

57 INTRODUCTION

58 Cephalopods have developed a wide array of reproductive strategies, which enable
59 them to occupy all marine habitats (Rocha *et al.*, 2001). Particularly, the family Octopodidae
60 has experienced an intense speciation, occupying coastal benthic environments from the
61 tropics to temperate regions (Norman, 2003). In this genera, a range of reproductive strategies
62 occurs, from species with wide distribution, large body size, small eggs, high fecundity and
63 pelagic hatchlings, such as *Octopus vulgaris* (Guerra, 1975; Mangold, 1987; Rocha *et al.*,
64 2001; Otero *et al.*, 2007; Villanueva and Norman, 2008) to endemic species, with small body
65 size, larger eggs, low fecundity and benthic development, such as *Octopus tehuelchus* (Pujals,
66 1982; Iribarne, 1991; Ré, 1998). This last species occurs from subtropical southeastern Brazil
67 (20°S) (Haimovici and Perez, 1991) to the temperate habitats of San Jorge Gulf, in northern
68 Patagonia, Argentina (43°S) (Ré and Simes, 1992; Ré, 1998).

69 In southern Brazil, *Octopus tehuelchus* occurs over the continental shelf, on sandy and
70 muddy bottoms, up to 100 m depth and usually associated with gastropod shells (Haimovici
71 and Andriguetto, 1986). Due its low abundance in commercial landings, its life cycle and
72 biology are poorly known in Brazil. However, the species is frequently found in stomach
73 contents of demersal teleosts and marine mammals from this region (Santos and Haimovici,
74 2002). In the Patagonian gulfs, the species is commercially exploited on a small artisanal
75 fishery. (Storero, 2010).

76 Most information on the growth and reproductive biology of the species comes from
77 studies conducted in Patagonia, in an environment with the predominance of rocky bottoms,
78 discharge of freshwater creeks and channels, high tidal range (up to 9 m) and large seasonal
79 variation of temperature and luminosity (Pollero and Iribarne, 1988; Iribarne, 1991; Navarte
80 *et al*, 2006; Klaich *et al*, 2008; Storero *et al*, 2010). This environment contrasts with southern
81 Brazil, which presents sandy and muddy bottoms, small tidal range, temperature rarely below
82 12°C, even in the cold months (Haimovici *et al.*, 1996) and lower variation in luminosity
83 between winter and summer (Bakun and Parrish, 1990).

84 The aim of this work is to study the reproductive biology of *Octopus tehuelchus* in the
85 subtropical environment of the continental shelf in southern Brazil, allowing a better
86 understanding of the reproductive strategy along its distribution.

87

88 **MATERIAL AND METHODS**

89

90 **Data Collection**

91 Individuals of *Octopus tehuelchus* were collected from bottom trawl surveys by the
92 R/V Atlântico Sul and from commercial trawling along southern Brazil in the 28°S to 34°S
93 range at depth from 15 to 100 m, between 1979 and 2009. Specimens were fixed in 10%
94 formalin and preserved in 70% ethanol. A total of 125 males and 194 females were examined.

95 All preserved individuals had their Total Length (TL), Dorsal Mantle Length (ML),
96 Total Body Weight (BW) and Digestive Gland Weight (DGW) recorded. Females had their
97 Ovary Weight (OW), Oviducts Weight (including the oviducal glands) (OvW) and maximum
98 diameter of oviducal glands recorded. All developing intraovaric oocytes were counted and
99 the Maximum Diameter of Oocytes (MDO) was recorded. Four egg layings (one of them
100 accompanied by female) were observed, spawned eggs were measured and recently spawned

101 hatchlings were measured and weighed. In males, Testis (TW) and the Spermatophoric Sac
102 (including the glandular system) (SSW) were weighted. Spermatophores in the Needham's
103 sac were counted and measured. All the measurements were taken to 0.1 mm and weights to
104 0.01 g.

105 The reproductive cycle was analyzed by a combination of the monthly frequencies of
106 males and females in each maturity stage and the monthly variations of the maturity and
107 gonadosomatic indices.

108 A Maturity Index (MI) was calculated as $MI = SSW / (TW + SSW)$ for males and $MI =$
109 $OvW / (OW + OvW)$ for females (Hayashi, 1970). The Gonadosomatic Index (GSI) was
110 calculated as $GSI = (SSW / (BW - SSW)) \times 100$ for males and $GSI = (OW / (BW - OW)) \times 100$
111 for females (Otero *et al.*, 2007). Digestive Gland Index (DGI) was calculated as $DGI =$
112 $(DGW / (BW - DGW)) \times 100$, similar to the one used for *Octopus vulgaris* (Otero *et al.*,
113 2007).

114 The maturity scale was modified of Guerra (1975), Pujals (1982) and Perez and
115 Haimovici (1991). For females, five stages were defined based on the size and colour of the
116 oviducts and oviducal glands and the mean diameter of the developing oocytes: *Immature (I)*:
117 translucent oviducts, oviducal glands little differentiated, with diameter usually smaller than 2
118 mm; *Initial Maturity (II)*: whitish oviducal glands between 2 and 3 mm in diameter and
119 developing oocytes 2 to 4 mm long; *Intermediate Maturity (III)*: oviducal glands brown/black,
120 3 to 4.5 mm in diameter, most oocytes between 4 and 7.5 mm long; *Advanced Maturity (IV)*:
121 enlarged oviducts, sometimes with oocytes being released, mean diameter of the larger
122 oocytes over 7.5 mm; *Post-Spawning (V)*: ovary clearly flaccid with reduced size and few
123 eggs in it, oviducts dilated and small oviducal glands.

124 The maturity scale for males included four stages: *Immature (I)*: small and whitish
125 testis, glandular system slightly differentiated and absence of spermatophores in the

126 Needham's sac; *Initial Maturity (II)*: testicle in development, usually heavier than the
127 glandular system and Needham's sac with few (<20) spermatophores; *Advanced Maturity*
128 (*III*): testicle weight lighter than glandular system and Needham' s sac full; *Post-liberation of*
129 *spermatophores (IV)*: glandular system still bulky, Needham's sac partially or totally empty,
130 with spermatophores being released and testicle relatively small, striped and usually lighter
131 than the glandular system.

132 Potential fecundity was defined as the number of developing oocytes with the largest
133 diameter above 4 mm in ovaries of stages III and IV females.

134

135 **Data analysis**

136 Reproductive indices were compared with the non-parametric Kruskal-Wallis test for
137 multiple comparisons, because assumptions for normality and homogeneity of variance were
138 not satisfied.

139 Sex ratio was calculated monthly, for 15 mm dorsal mantle length classes (ML 15
140 mm) and for 30 m depth classes. Significant deviations from the 1:1 proportion were tested
141 using the χ^2 test, adjusted to Yates correction (Zar, 1984).

142 Length-weight relationships were estimated for the total sample and according to sex.
143 Data were adjusted to power model ($y = ax^b$), where $y= BW$; $x= ML$; $a=$ the y-intercept; and
144 $b=$ the slope. The goodness of fit was expressed by r^2 and the analysis of covariance
145 (ANCOVA) (Zar, 1984) was used to test for differences in the slope of log-transformed
146 relationships.

147 The mean mantle length at maturity (ML50%) was estimated starting from the
148 proportion (P_i) of stages III and IV individuals, grouped in 6 mm ML classes, adjusted to the
149 logistic model: $P_i = 1 - \{1 / [1 + \exp \{-(\alpha + \beta ML_i)\}]\}$

150 The Bhattacharya method (Bhattacharya, 1967; King, 2007) was used to discriminate
151 normal components in the diameter frequency distribution of intraovaric oocytes.

152

153 **RESULTS**

154

155 **Sex Ratio**

156 In total, 174 females and 125 males were sampled. The sex ratio did not differ
157 significantly among sampling months (Table 1) but the proportion of females was
158 significantly higher among the specimens over 45 mm ML ($\chi^2 = 13.89$ and 5.18; $p < 0.05$)
159 (Table 2). Regarding the depth of capture, the proportion of females was higher in all depth
160 ranges. However, the differences were significant only under 30 m depth ($\chi^2 = 4.17$; p
161 < 0.05) (Table 3). These results indicate that females grow larger than males and that
162 spawning may occur mainly in shallow waters.

163

164 **Length-weight relationships**

165 Females *O. tehuelchus* ranged from 21 to 79 mm ML (mean 47.2) and from 8.3 to
166 228.5 g BW (mean 69.5) and males ranged from 20 to 76 mm ML (mean 42.6) and 4.7 to
167 125.1 g BW (mean 46.0). . The dorsal mantle length/total body weight relationships
168 (ML/BW) were calculated only for individuals caught in 2009 (n= 64), which were less
169 affected by the dehydration observed in specimens preserved in alcohol for long periods.
170 Relationships were best described by the power equations (Table 4): females: $BW = 0.0211 \times$
171 $ML^{2.2098}$, males: $BW = 0.0113 \times ML^{2.3337}$ and both sexes combined: $BW = 0.0072 \times ML^{2.4772}$.
172 Slope comparisons did not show heterogeneity between sexes (ANCOVA, $p = 0.634$).
173 However, these results should be considered with care by the low number of individuals,
174 mainly males (Table 4).

175 **Maturation and size-at-maturity**

176 Fully mature males and females were observed in all seasons. Therefore, the
177 mean size and weight at maturity were calculated including specimens collected year
178 round.

179 Females in stages I and II (n = 76) were observed in all size range including seven
180 females over 60 mm ML (Fig 1). Females in stages III and IV (n= 95) measured over 24 mm
181 ML and weighed over 30 g. Although the small variation, the mean ML increase significantly
182 along maturation ($p <0.05$) (Table 5). The maturity curve of females showed a good fit to the
183 logistic model ($r^2 = 0.980$) and $ML_{50\%}$ calculated was 45.9 mm (Fig. 2).

184 Males in stages I and II (n= 58) were observed in all sizes including five individuals
185 over 60 mm (Fig. 1). All males in stages III and IV (n = 65) were over 30 mm ML and 15 g
186 BW. The $ML_{50\%}$ was 27.4 mm, however, the maturity curve of males did not show a good fit
187 to the logistic model ($r^2 = 0.129$) (Fig 2).

188

189 **Seasonality**

190 Females in stages I or II were observed in all months sampled. Stage III individuals
191 were caught more frequently in April, June and November and stage IV individuals were
192 caught in all months sampled, mainly in January, August and September (Fig. 3). A single
193 stage V female with an egg laying was observed in July. Three other egg layings without the
194 spawned female were found in the same month and a fourth was observed in November.
195 Reproductive indices were not homogeneous throughout all months ($p <0.05$) (Fig 4). Higher
196 (GSI) and lower (MI) associated to sexual maturity were observed in January (summer) and
197 June, August and September (late autumn to late winter).

198 Immature and initial maturity males (stages I and II) occurred year round, but more
199 frequently in April, June and December. Stages III and IV also occurred in all sampled

200 months, more frequently in January (summer), September, October and November (late
201 winter and early spring) (Fig. 3). There were no significant differences in the monthly means
202 of IM and GSI ($p > 0.05$), indicating mature males in all seasons (Fig.4).

203 In both sexes, maturity stages and reproductive indices support a year round sexual
204 maturation cycle.

205

206 **Pre-spawning oocytes and spermatophores**

207 The mean number of oocytes counts in the ovaries of 67 maturing females (stages III
208 and IV) was 246.8 oocytes (ranging from 20 to 448). In these ovaries, the diameter of the
209 oocytes ranged from 1.8 to 13.9 mm (Table 5). A wide range of oocytes diameters was
210 observed, in some cases with a bimodal distribution (Fig 5).The number of oocytes (ON)
211 increased significantly with female ML (ON = $1.4889 \times (\text{ML})^{1.3094}$; $r = 0.524$ $n = 67$) (Fig.
212 6). Immature and initial maturity females (stages I and II, $n = 68$) had 185 oocytes in average
213 (50 to 514), most of them with diameter under 4 mm (Table 5).

214 In stages III and IV males ($n = 67$), the number of developing spermatophores ranged
215 from 1 to 62 (21.2 ± 12.3), with maximum length ranging from 1.7 to 57.4 mm (21.2 ± 8.9).
216 Non-significant correlation was found between the number of spermatophores and the male
217 size ($r = 0.086$) (Fig. 6).

218

219 **Spawning and hatchlings**

220 All the egg layings were observed in gastropod shells of *Tonna galea* and *Adelomenon*
221 *brasiliiana*. The number of eggs attached to the shells in the layings ranged from 86 to 237
222 (165.2 ± 60.5 ; $n = 4$). The maximum diameter of these eggs ranged from 8.1 to 14.4 mm and
223 their diameter distribution were unimodal.

224 Recently spawned juveniles ($n = 16$) resembled small adults, and measured from 5.0 to
225 6.40 mm ML and from 10.5 to 15.0 mm TL and weighed from 0.07 to 0.12 g (Table 6).

226

227 **Digestive gland index and reproductive investment**

228 The Digestive Gland Weight (DGW) of females increased significantly along the
229 sexual maturation ($p < 0.05$). The Digestive Gland Index (DGI) was significantly lower in
230 stage I ($p < 0.05$), and remained constant in the others stages (Fig. 7). Monthly DGI of females
231 did not follow a regular pattern: the lowest values were observed in April and May and the
232 highest ones, in January, March, August and December (Fig. 8).

233 Digestive gland weight and index of males did not show significant changes along
234 maturation ($p > 0.05$), although small decrease in DGI means along maturation was observed
235 (Fig. 7). Seasonally, higher DGI values were observed in spring (Fig. 8).

236

237 **DISCUSSION**

238 *Octopus tehuelchus* is a small species with large eggs and low fecundity, endemic of
239 the subtropical and temperate waters of the southwestern Atlantic continental shelf
240 (Haimovici and Perez, 1991; Ré, 1998). This study shows that its reproductive biology can
241 adapt to both environments, with seasonal spawning in temperate waters and year round
242 spawning in subtropical environments. Temperature and daily photoperiod can influence
243 growth and reproduction in cephalopods (Richard, 1967; Van Heukelom, 1973; Mangold,
244 1987). Seasonal differences in the day length and water temperature are smaller in the
245 subtropical environment of southern Brazil (32°S), where daylight ranges from 10 to 14 h
246 (Bakun and Parrish, 1990) and bottom temperatures on the continental shelf range between
247 12°C and 24°C (Haimovici *et al.*, 1996). In contrast, in northern Patagonia (40 to 42°S),
248 daylight ranges from 9 to 15 h and air temperatures along San Antonio Bay coast range

249 between 6°C and 24°C (Iribarne, 1991) (Fig. 9). Despite not discriminating the effects of each
250 factor, Iribarne (1991) observed that high intensity of light and temperature were associated to
251 the intensification of growth and sexual maturation of *Octopus tehuelchus* in northern
252 Patagonia, where sexual maturation occurs between December and May and spawning occurs
253 from June to November (Pujals, 1982; Ré 1989). Storero *et al* (2010) observed two distinct
254 sub-annual cohorts in the mantle length distributions within San Antonio Bay, suggesting that
255 *O. tehuelchus* can have a more extended spawning season even in the higher latitudes of its
256 distribution. In contrast, in southern Brazil, mature males and females were observed year
257 round and egg broods were sampled both in cold and warm months.

258 *Octopus tehuelchus* uses a wide variety of bottoms types to deposit eggs. On the rocky
259 bottoms of San Matias Gulf (41° to 42°S), the eggs are attached directly to the substrata (Ré,
260 1998). On the sandy bottoms of the San Antonio Bay (40° 40'S), the eggs are attached to
261 shelters, mainly bivalve shells: *Ostrea puelchana*, *Ammiantis purpurata*, *Mytilus edulis*
262 *platensis*, *Chlamys tehuelchus* and *Pitar rostratus* and gastropods: *Buccinanops gradatum*,
263 *Odontocymbiola subnodososa* e *Zidona dufresnei* (Iribarne, 1990). Although most of these
264 mollusks also occur in southern Brazil (Rios, 2009), egg laying of *Octopus tehuelchus* in this
265 region was found only inside shells of large sized gastropods *Tonna galea* and *Adelomenon*
266 *brasiliiana*. Iribarne (1990) notes that the abundance of small shells in San Antonio Bay could
267 favor the selection of smaller octopuses. However, the availability of larger shells in southern
268 Brazil does not seem to have favored larger individuals in this region.

269 In southern Brazil, a wide range of sizes of developing intraovarian oocytes (Fig. 5) and
270 of egg sizes in the spawning (Fig. 10) were observed. In northern Patagonia, some females
271 also showed oocytes at different stages of development (Pujals, 1982). These wide ranges in
272 oocytes size may decrease competition among siblings. However there are differences in the
273 evolution of the DGI between regions, that suggest that the individual spawning period may

274 be longer in southern Brazil: in northern Patagonia, the DGI decreases along sexual
275 maturation and can be associated to intense reserve mobilization along a short spawning
276 season (Pujals, 1982; Iribarne, 1991); in southern Brazil, the DGI remains high along
277 maturation, suggesting the accumulation of reserves for a longer period of spawning and
278 parental care of the eggs. However, it is not consensual that the digestive gland has an
279 important role in energy storage in cephalopods, as many authors also consider the reserves in
280 the muscle and gonads (Moltchaniwskyj and Semmens, 2000; Rosa *et al.*, 2004; Semmens *et*
281 *al.*, 2004).

282 In males, the DGI decreases in both regions, characterizing a larger mobilization of
283 energy reserves for the reproduction and anticipation of sexual maturation rather than growth
284 (Iribarne, 1991). Moreover, males mature at smaller sizes than females in both areas (Ré,
285 1989). In the San Matias Gulf, females mature up to three months after males. After
286 copulation, the sperm is stored in the oviducal glands of females (Ré, 1998).

287 The lack of seasonality in the spawning in southern Brazil may be associated to year
288 round availability of food for hatchlings. Productivity on the inner shelf of southern Brazil is
289 relatively high (Ciotti *et al.*, 1995) mainly as a consequence of the runoff of nutrient-rich
290 waters, carried from the La Plata River and Patos Lagoon (Piola *et al.*, 2005). In this region,
291 other neritic cephalopods such as *Doryteuthis(Loligo) sanpaulensis* spawn year round
292 (Andriguetto and Haimovici, 1991; Haimovici, 1998a) and many bony fishes are multiple
293 spawners (Haimovici, 1998b). In the Patagonian gulfs, productivity is dependent of the tidal
294 fronts and shows a strong seasonal variation, where higher productivity rates are concentrated
295 in spring and summer (Acha *et al.*, 2004). In temperate environments, such as the northern
296 Patagonian gulfs, seasonality in the productivity makes availability of food and consequent
297 survival for young octopus in the cold season more difficult. Furthermore, according to
298 Klaich *et al.* (2006), food intake, growth and food conversion of *O. tehuelchus* in

299 experimental conditions were lower at 10°C when compared to 15°C. Therefore, the
300 conclusion is that *O. tehuelchus* has the potential for year round spawning, but ecological
301 constrains only enable it to express this potential in the lowest latitudes of its distribution
302 range.

303

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311

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410 **TABLES**

411

412 Table 1: Monthly variation in sex ratio of *Octopus tehuelchus* in southern Brazil (*significant413 χ^2 departures from the 1:1 sex ratio p <0.05).

414

Month	Number	Females	Males	χ^2
Jan	49	59%	41%	1.67
Mar	5	40%	60%	0.40
Apr	14	50%	50%	0.07
May	18	67%	33%	2.00
Jun	43	51%	49%	0.05
Aug	14	64%	36%	1.21
Sep	44	64%	36%	3.30
Oct	14	57%	43%	0.36
Nov	56	63%	38%	3.52
Dec	30	40%	60%	1.23

415 Table 2: Sex ratio variation of *Octopus tehuelchus* according to ML 15 mm classes in
 416 southern Brazil (*significant χ^2 departures from the 1:1 sex ratio $p < 0.05$).

ML Classes range (mm)	Number	Females	Males	χ^2
15 – 29.9	17	35%	65%	1.53
30 – 44.9	140	51%	49%	0.04
45 – 59.9	104	68%	32%	13.89*
> 60	38	68%	32%	5.18*

417 Table 3: Sex ratio variation of *Octopus tehuelchus* according to 30 m depth classes in
418 southern Brazil (*significant χ^2 departures from the 1:1 sex ratio $p < 0.05$).

Depth range (m)	Number	Females	Males	χ^2
< 30	24	71%	29%	4.21*
30 – 59.9	171	56%	44%	2.58
> 60	58	60%	40%	2.50

419 Table 4: Range of Mantle Length (ML) and Total Body Weight (BW) and power
 420 regression parameters of length/weight relationships for females and males of *Octopus*
 421 *tehuwelchus* caught in southern Brazil in 2009.

	n	Min-Max (Mean) ML	Min-Max (Mean) BW	A	B	r^2
Females	47	30-65 (49.9) mm	21.9-228.5 (123.4) g	0.0211	2.2098	0.7240
Males	17	28-52 (40.1) mm	32.5-119.4 (65.1) g	0.0113	2.3337	0.8290
Both sexes	64			0.0072	2.4772	0.8347

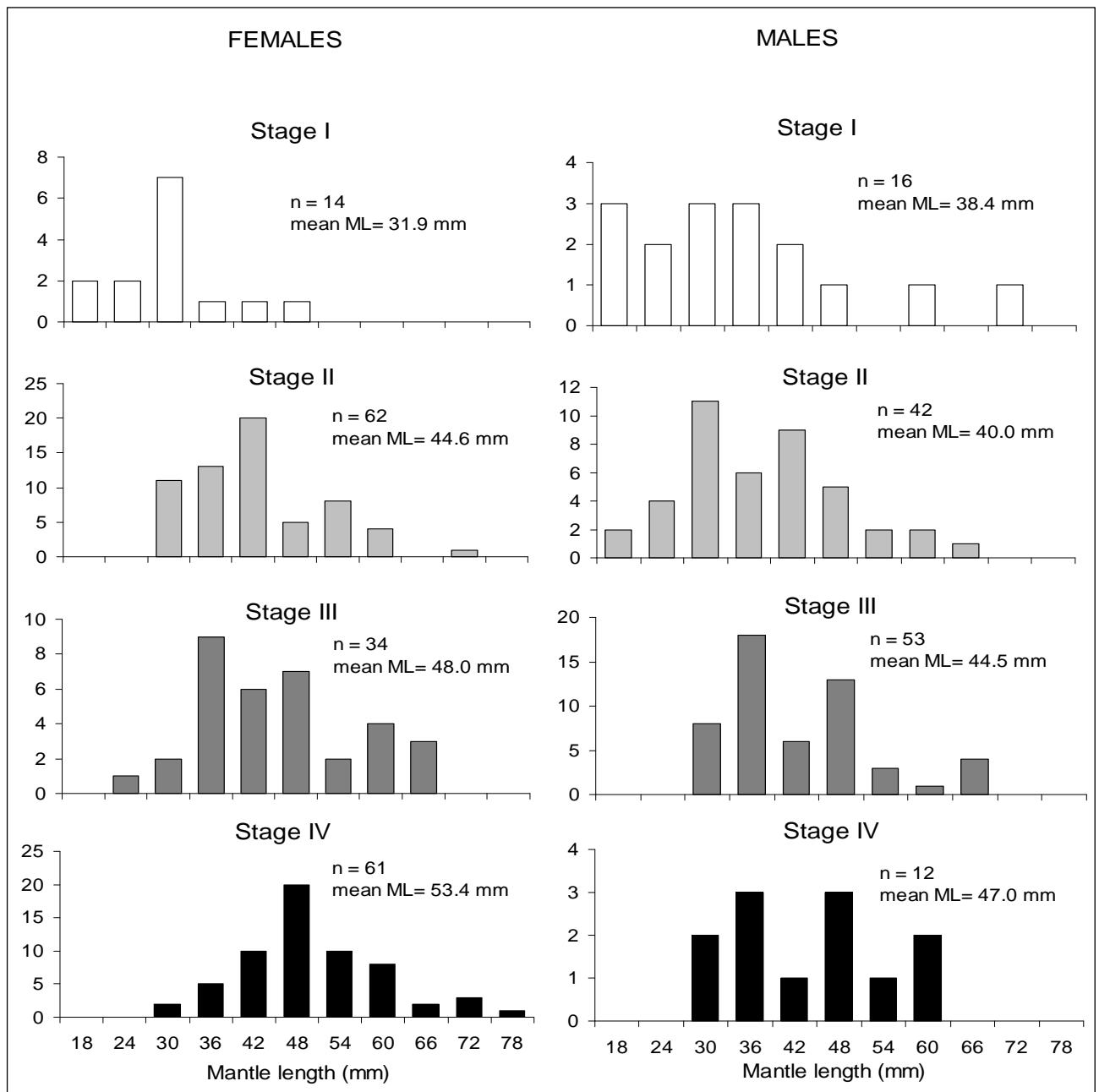
422 Table 5: Variation in the number and Maximum Diameter (MDO) of intraovaric
 423 oocytes in females of *Octopus tehuelchus* in different maturation stage (Stages I, II, III
 424 and IV) in southern Brazil.

	Stage I	Stage II	Stage III	Stage IV
Number of females	12	56	22	45
Mean Female ML (\pm SD) (mm)	32.8 ± 7.4	43.9 ± 9.5	46.6 ± 9.1	48.5 ± 8.4
Range oocytes number	50 to 350	65 to 514	20 to 410	112 to 448
Mean oocytes number (\pm SD)	139.7 ± 76.6	226.9 ± 108.0	205.1 ± 93.3	267.1 ± 83.5
Range MDO (mm)	0.2 to 3.0	1.0 to 7.4	1.8 to 10.8	1.5 to 13.9
Mean MDO (\pm SD) (mm)	1.4 ± 0.7	3.1 ± 0.8	5.5 ± 1.8	8.0 ± 2.2

425 Table 6: Variation in the number and Maximum Diameter of Eggs (MDE) and in the Mantle
 426 Length (ML), Total Length (TL) and Total Body Weight (BW) of hatchling on four eggs
 427 laying of *Octopus tehuelchus* in southern Brazil in comparison with northern Patagonia. (1)
 428 Iribarne, 1991; (2) Ré, 1998.

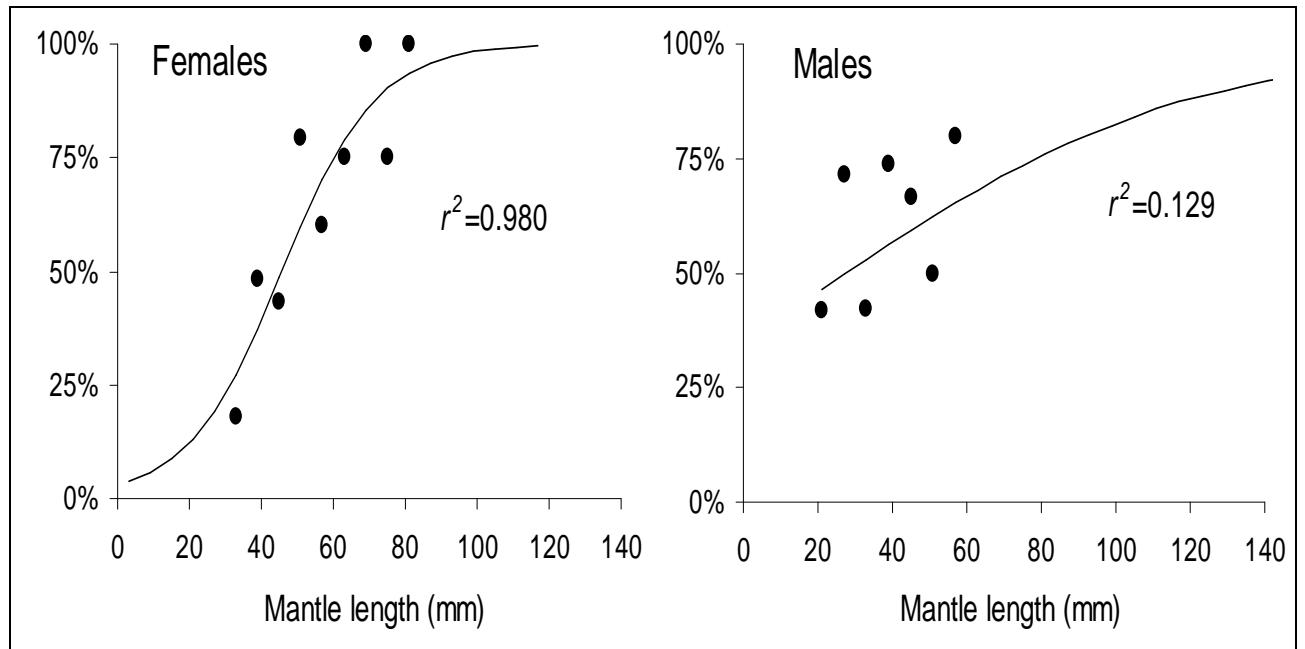
	Southern Brazil	Northern Patagonia
Maximum of eggs per laying	237	227 (2)
mean egg laying fecundity (\pm SD)	165.15 ± 60.84	No data
Spawning egg measured	445	300 (1)
range MDE (mm)	8.13 to 14.44	9.0 to 12.0 (1)
mean MDE (\pm SD) (mm)	10.33 ± 1.09	9.87 ± 0.61 (1)
Juveniles sampled	16	280 (1)
mean ML (\pm SD) (mm)	5.77 ± 0.46	6.64 ± 0.38 (1)
mean TL (\pm SD) (mm)	12.06 ± 1.06	14.23 ± 0.83 (1)
mean BW (\pm SD) (g)	0.095 ± 0.014	0.139 ± 0.019 (1)

429 **FIGURES**



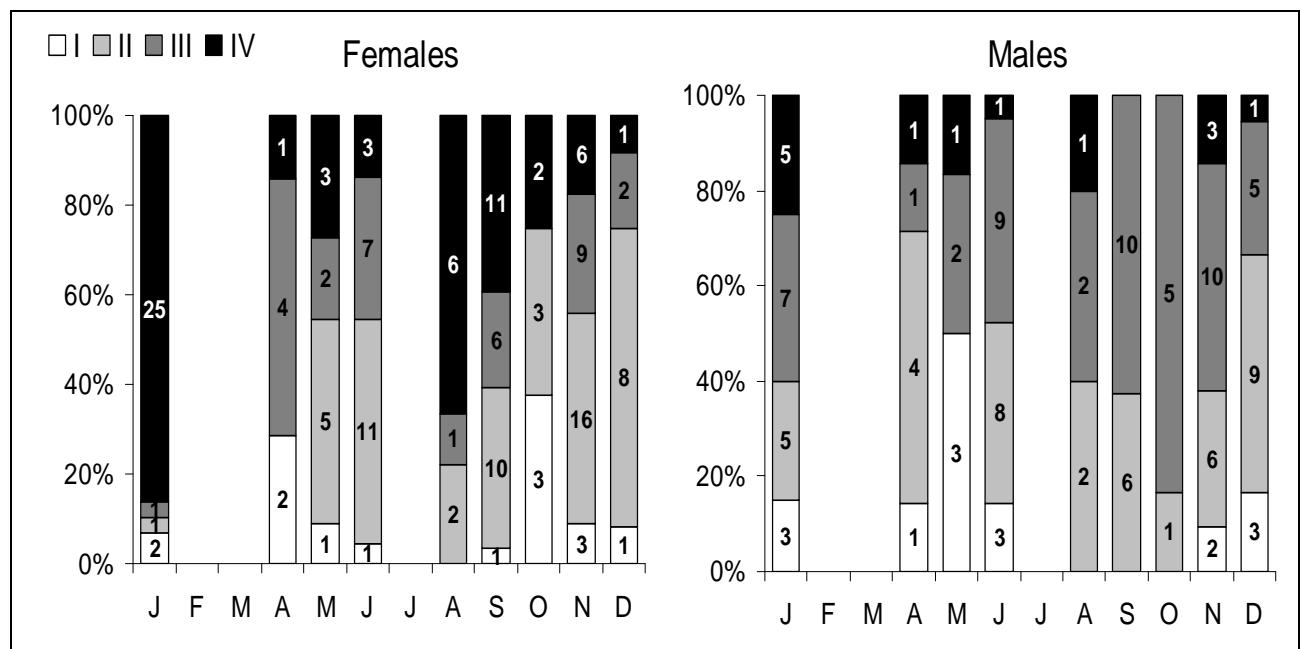
430

431 Fig. 1: Frequency of Mantle Length (ML) classes in each maturity stage of females and
432 males of *Octopus tehuelchus* in southern Brazil.



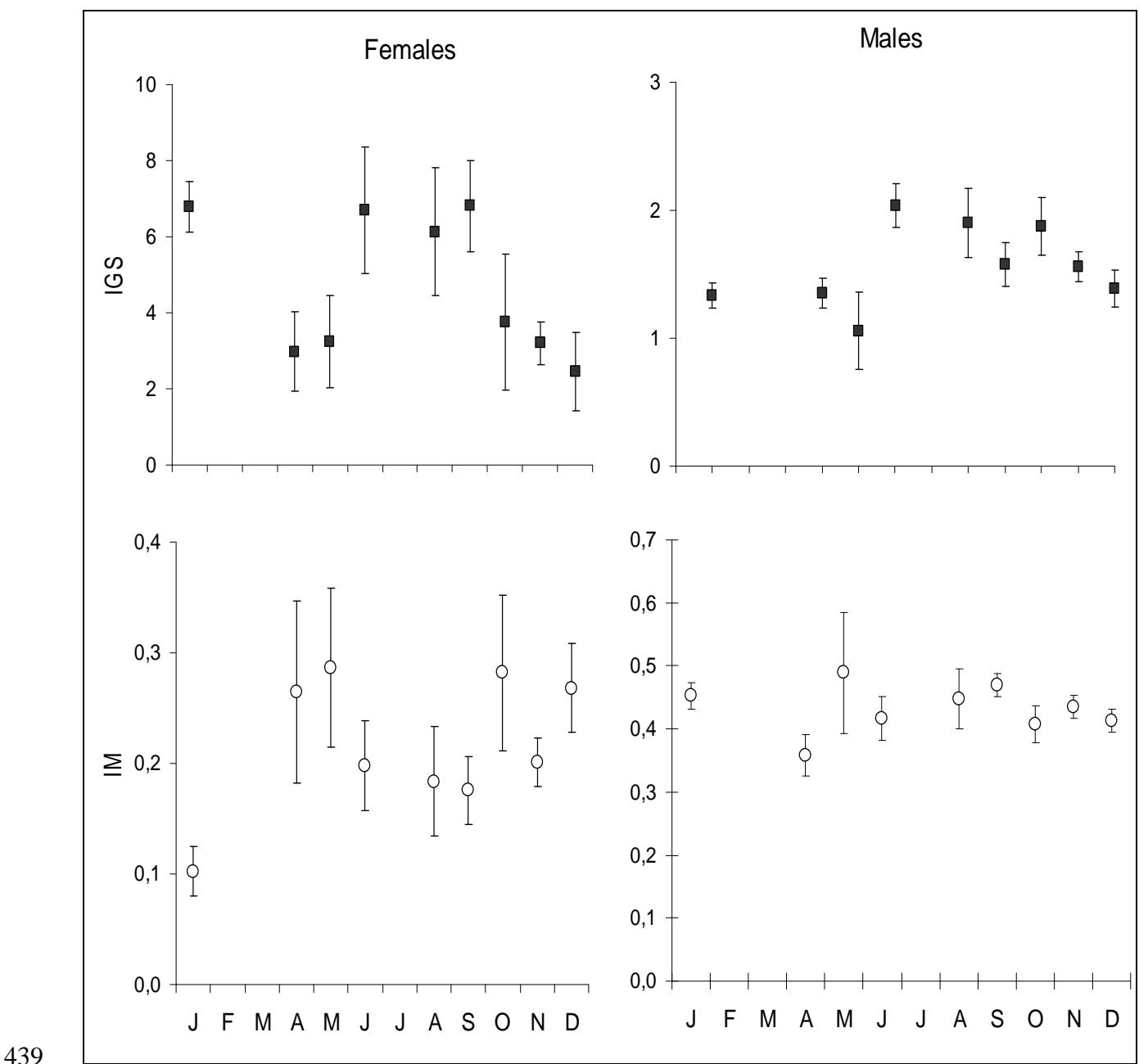
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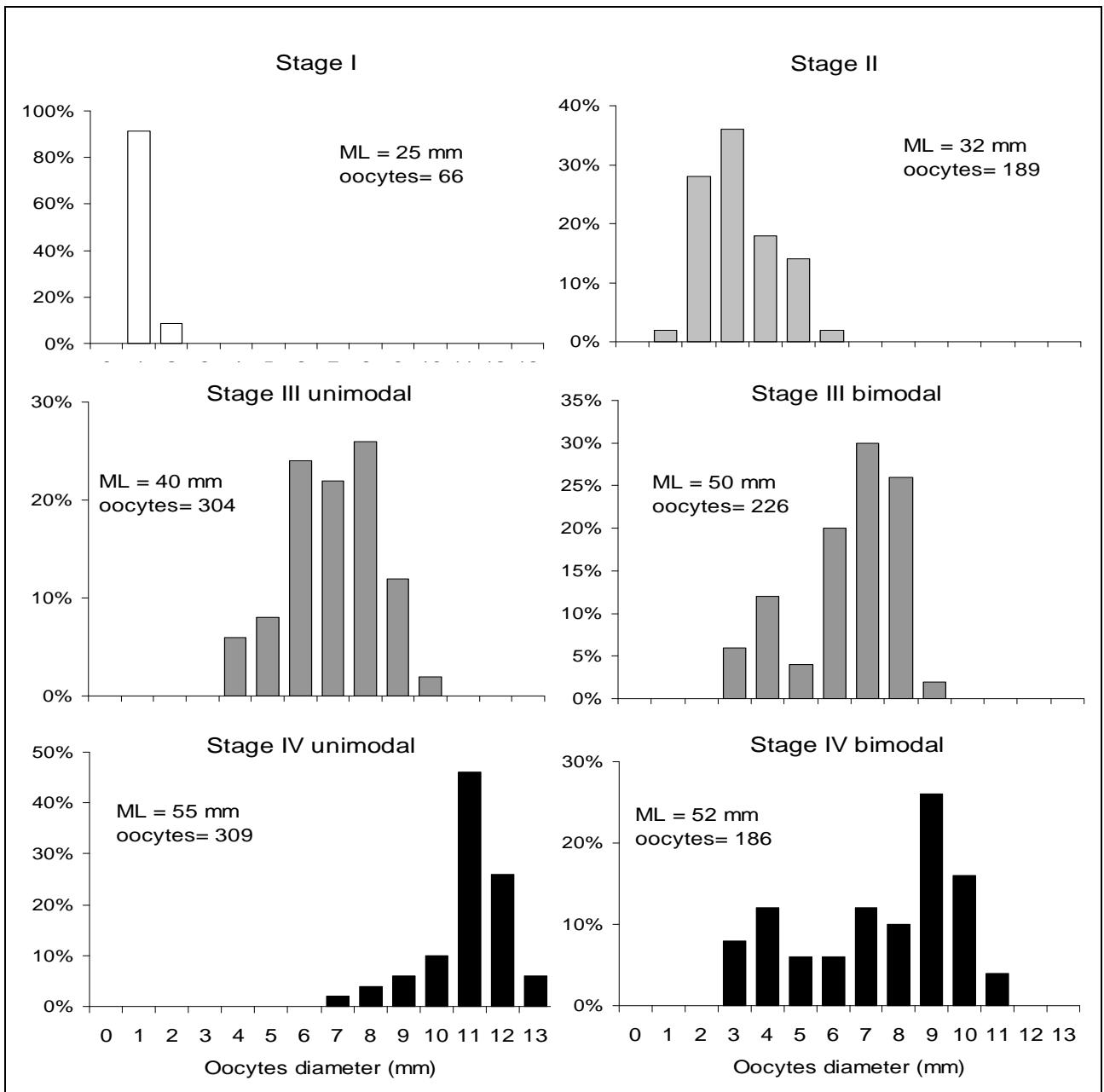
434 Fig. 2: Relative frequency curve of mature individuals by Mantle Length (ML) classes (mm),
 435 of females and males of *Octopus tehuelchus* in southern Brazil.



436

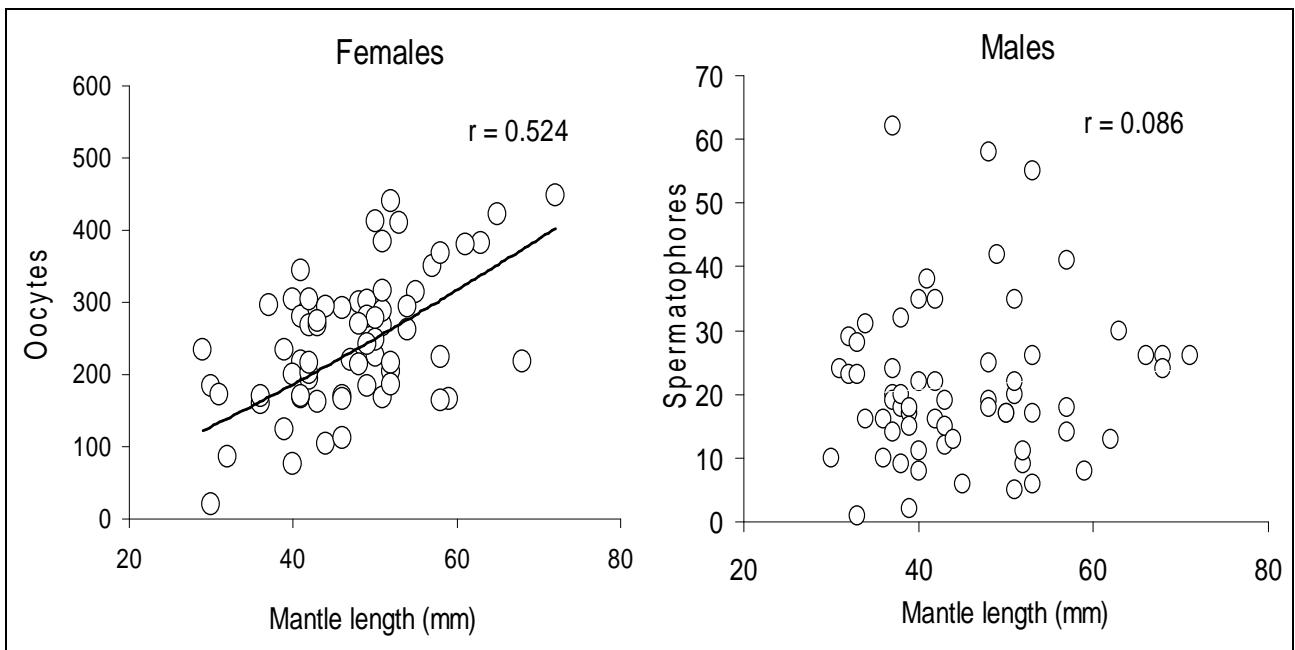
437 Fig. 3: Monthly frequency (%) of the maturity stages of *Octopus tehuelchus* females and
438 males in southern Brazil (values in the bars indicate the number in each stage).





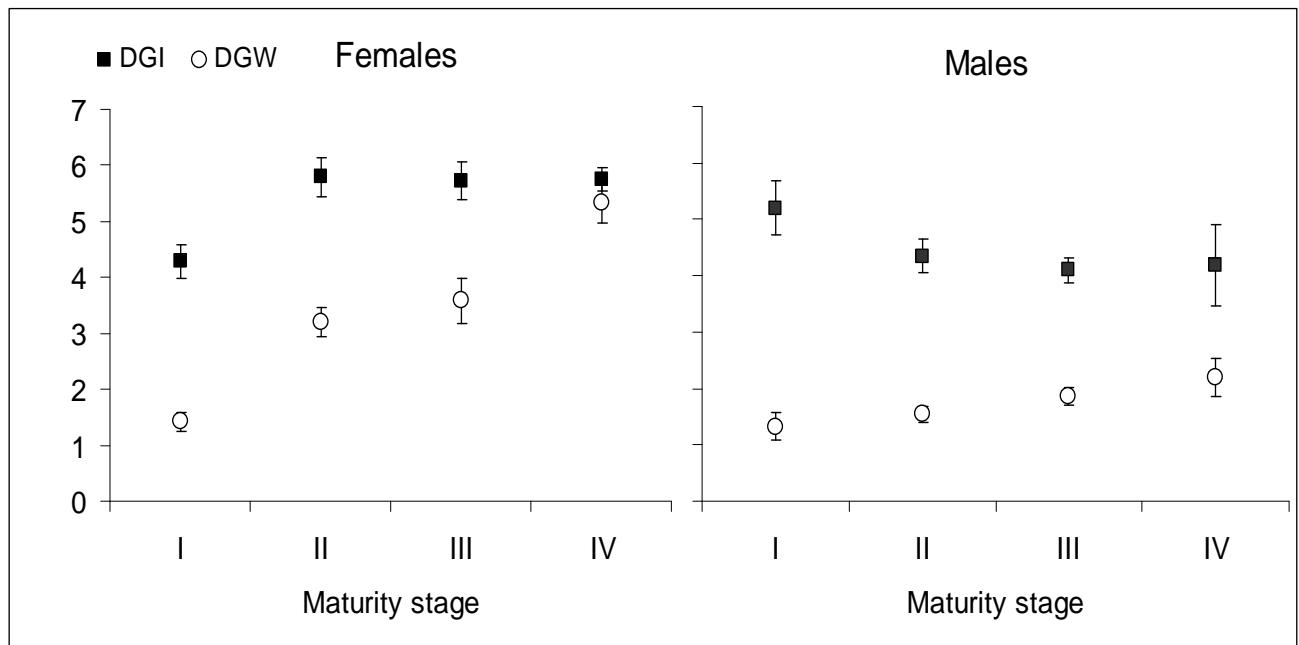
442

443 Fig. 5: Frequency of oocytes length classes of *Octopus tehuelchus* females at different
 444 maturity stages in southern Brazil. The Mantle Length (ML) and Total Fecundity
 445 (oocytes) of each individual were specified.

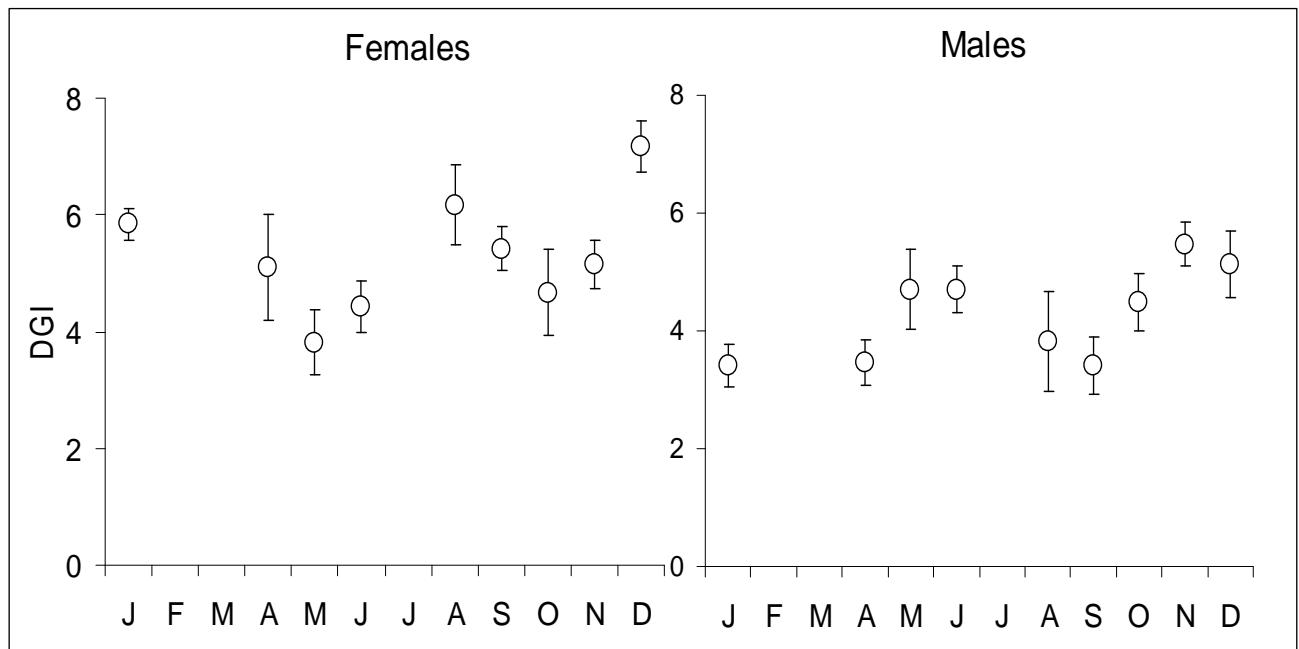


446

447 Fig. 6: Relationship between Mantle Length (ML) and Potential Fecundity (number of
 448 developing oocytes and spermatophores) of mature females and males of *Octopus tehuelchus*
 449 in southern Brazil.

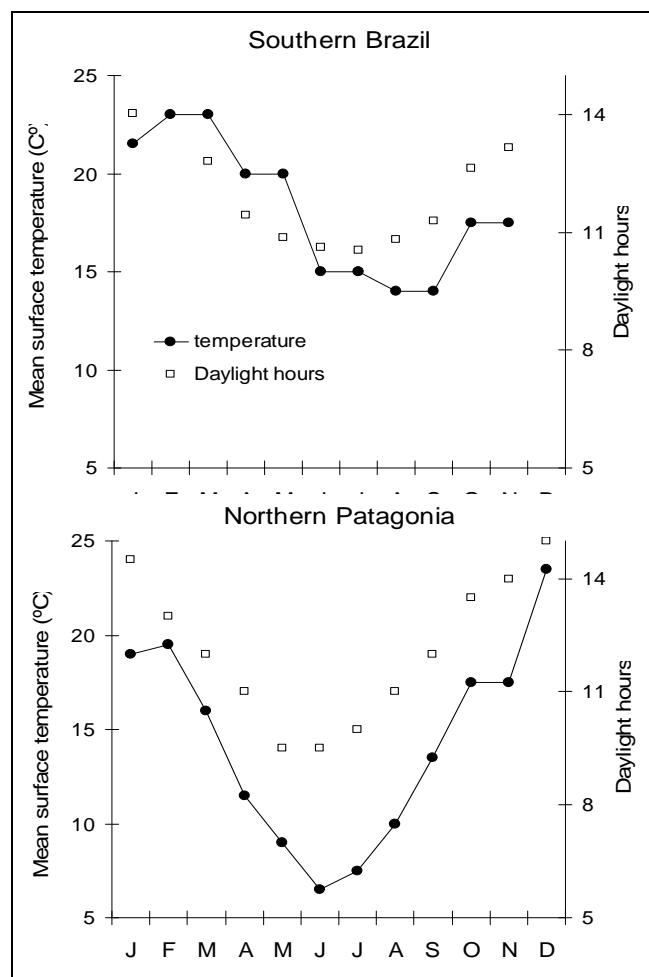


450 Fig. 7: Mean (\pm SE) of Digestive Gland Weight (DGW) and Digestive Gland Index (DGI) in
 451 each maturity stages of *Octopus tehuelchus* females and males in southern Brazil.



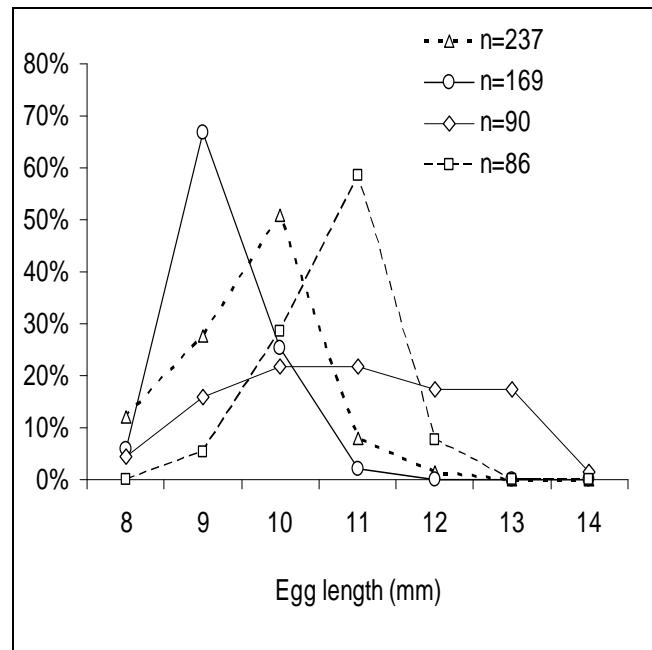
452 Fig. 8: Monthly trends of mean (\pm SE) values of the Digestive Gland Index (DGI) of *Octopus*
453 *tehuelchus* females and males in southern Brazil.

454



455 Fig. 9: Comparison of surface temperatures (Bakun and Parrish, 1990) and daylight hours in
456 southern Brazil and San Antonio Bay, northern Patagonia (Iribarne, 1991).

457



458 Fig. 10: Frequency of spawning eggs size in four egg layings of *Octopus tehuelchus* in
459 southern Brazil.

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