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Aggregations and reproductive events of the narrownose smooth-hound shark, Mustelus schmitti, in relation to temperature and depth in coastal waters of the southwestern Atlantic Ocean (38-42° S)

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Manuscripts

1   **Aggregations and reproductive events of the narrownose smooth-hound shark,**

2   *Mustelus schmitti***, in relation to temperature and depth in coastal waters of the**

3   **southwestern Atlantic Ocean (38-42° S)**

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14

15   **Abstract**

16   Knowledge of environmental influence on reproductive dynamics in

17   chondrichthyans is critical for effective management. This study assessed the potential

18   influences of temperature and depth on aggregations and reproductive events of the

19   narrownose smooth-hound shark (*Mustelus schmitti*), using an analysis criterion based

20   on the co-occurrence of different reproductive stages. Data were collected from 363

21   sample sites during six research surveys, covering an area of approximately 13,920 nm^2

22   along the coast (38–42º S and depths < 50 m). *M. schmitti* adult aggregations were

23   mostly associated with the warmer springs surveys, being the aggregation sites

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24 significantly warmer than those of non aggregation. Pupping and ovulation evidences

25 were observed, almost simultaneously, during all spring surveys. However, the

26 occurrence of these reproductive events showed a clear interannual variability pattern

27 significantly explained by water temperature. Particularly, the ovulation occurrence was

28 associated with temperatures above 16-17 °C. These results suggest that *M. schmitti*

29 adults may follow the warming gradient along the environment and thus they aggregate

30 in shallow and productive waters during the course of spring. Their reproductive events

31 <u>could</u> be induced by temperature increase. This scenario suggests that changes in bottom

32 temperature patterns may be important drivers of the reproductive dynamics of *M.*

33 *schmitti.*

34 **Additional keywords:** reproductive aggregations, environmental cues, elasmobranchs,

35 coastal ecosystem

36 **Running head:** Reproductive dynamics of *Mustelus schmitti* shark.

37

38 **Introduction**

39   Most cartilaginous fishes (chondrichthyans) possess low reproductive potential

40 characterized by late sexual maturity, reduced fecundity and long gestation period

41 (Cortés, 2000; Stevens *et al*., 2000). Therefore, their populations are very vulnerable

42 and have low resilience to intense fishing levels (Stevens *et al*., 2000, Dulvy *et al*.,

43 2014). As a consequence, different conservation and management programs have been

44 developed and implemented to avoid the decline of their populations (Stevens *et al*.,

45 2000; Musick, 2005). For example, marine protected areas (MAPs) and seasonal fishing

46 restrictions are often used to promote reproductive success of several chondrichthyans

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47 (Hall, 2009; Speed *et al*., 2010; Colonello *et al*., 2014). However, the efficiency of these

48 management strategies largely depends on the adequate selection of the protected spatial

49 and temporal boundaries according to the reproductive dynamics of each particular

50 species (Speed *et al*., 2010).

51   Many chondrichthyans perform seasonal movements and aggregations

52 throughout their reproductive cycles (reviewed by Bres, 1993; Speed *et al*., 2010).

53 Different environmental variables such as temperature, depth, salinity, tide and current

54 have been proposed as important factors related to this behavior (Speed *et al*., 2010).

55 Among them, temperature has been considered important for many chondrichthyans

56 species (Crawshaw and Hammel, 1973; Casterlin and Reynolds, 1979; Wallman and

57 Bennet, 2006; Hight and Lowe, 2007; Di Santo and Bennet, 2011; Speed *et al*. 2012),

58 leading to suggest that water temperature variations can be a key environmental cue for

59 triggering seasonal migrations and local aggregations of elasmobranchs (Jacoby *et al*.,

60 2011; Schlaff *et al*., 2014). It must be noted that temperature not only affects metabolic

61 processes including gestation time (Economakis and Lobel, 1998; Wallman and

62 Bennett, 2006; Hight and Lowe, 2007; Robbins, 2007; Jirik and Lowe, 2012), but also

63 gonadal maturation and reproductive events (Dobson and Dodd, 1977; Mull *et al*., 2008;

64 Waltrick *et al*., 2014).

65   The narrownose smooth-hound *Mustelus schmitti* is one of the most abundant

66 chondrichthyans along the southwestern Atlantic Ocean (Massa *et al*., 2004a),

67 inhabiting coastal waters down to 200 m depth from Rio de Janeiro (22°S Brazil) to

68 Puerto Deseado (47°47´S Argentina, Menni *et al*., 2010). This species is a small

69 aplacental viviparous shark (reaching 108.5 cm of maximum total length) with an

70 annual reproductive cycle in which pupping occurs during spring/early summer, just

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71 prior to mating (Menni *et al*., 1986; Oddone *et al*., 2005; Cortés, 2007; Colautti *et al*.,

72 2010). As several chondrichthyans inhabiting the southwestern Atlantic coast (Lucifora

73 *et al*., 2002, 2005; Mabragaña *et al*., 2002; Colonello *et al*., 2007a; Vögler *et al*., 2008),

74 this shark performs seasonal reproductive movements (Massa *et al*., 2004a; Oddone *et*

75 *al*., 2007; Colautti *et al*., 2010; Cortés *et al*., 2011). Two main coastal areas within the

76 southwestern Atlantic Ocean, specifically Río de la Plata (34-38°S) and El Rincón (38-

77 42°S), have been characterized as breeding ground for *M. schmitti* (Cousseau, 1986;

78 Oddone *et al*., 2007; Colautti *et al*., 2010; Cortés *et al*., 2011). The abundance of adults

79 in these last areas increases significantly during spring-summer when temperature rise

80 and decreases during winter when temperature cools (Cousseau, 1986; Jaureguizar *et*

81 *al*., 2004; Colautti *et al*., 2010; Cortés *et al*., 2011). Although the spatial and temporal

82 migratory pattern of this species is unknown, the increase in abundance of larger-size

83 *M. schimitti* in deeper waters during autumn/winter (Pereyra *et al*., 2008; Cortés *et al*.,

84 2011) suggest that adults undertake seasonal migrations from shallow to deeper waters

85 (Cortés *et al*., 2011).

86   *Mustelus schmitti* is exploited by different fisheries in southern Brazil, Uruguay

87 and Argentina, and is one of the most landed shark species in this last country (Massa *et*

88 *al*., 2004b; Oddone *et al*., 2005). Based on the population decline trend and the

89 continuous fishing intensity over the past years, the IUCN Red List of Threatened

90 Species has categorized this species as endangered (Massa *et al*., 2006). An important

91 fisheries management policy encompassing the protection of *M. schmitti* populations in

92 the southwestern Atlantic coast involves the seasonal delimitation of closed areas to

93 trawling that include, among other features, areas considered essential for reproduction

94 of different chondrichthyans species (Colonello *et al*., 2014). However, the relative

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95   importance of the protected spatial and temporal boundaries for the conservation of *M.*

96   *schmitti* (and probably other species) could change annually if the environmental

97   variability affects its reproductive dynamics. Thus, the detailed understanding of how

98   environmental variables control the *M. schmitti* aggregations and reproductive events

99   will be required to optimize the current management programs.

100   Although previous studies have already assessed the influence of different

101   environmental variables on the distribution of *M. schmitti* (Massa, 1998; Cousseau *et*

102   *al*., 1998; Colautti *et al*., 2010; Cortés *et al*., 2011), the environmental cues triggering

103   adult aggregations and reproductive events remain unknown. In this context, the aim of

104   this study was to assess the occurrence of *M. schmitti* adult aggregations and

105   reproductive events in relation to temperature and depth in coastal waters of the

106   southwestern Atlantic Ocean.

107

108   **Materials and Methods**

109   *Data*

110   Data were collected from six research surveys carried out using Research

111   Vessels "*Dr. Eduardo L. Holmberg"* and "*Cap. Oca Balda"* of the Instituto Nacional de

112   Investigación y Desarrollo Pesquero (National Institute for Fisheries Research and

113   Development). Surveys were carried out during late spring (between November, 04 and

114   December, 21) from 2003, 2005, 2008, 2011 and 2012, and during winter (August)

115   from 2004. This last survey was used as a non-reproductive control scenario (NRC).

116   Each survey covered an area of approximately 13,920 nm^2, in coastal waters of the

117   Buenos Aires province, Argentina (38–42º S < 50 m.), using a stratified random or in

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118   transect perpendicular to the coast sampling design (Fig. 1). A total of 363 sample sites

119   were surveyed. A fishing trawling was conducted in each sample site using a standard

120   bottom trawl net (Engel type net, 200 mm inner mesh-size with a vertical height of 5 m

121   and a horizontal opening of 20 m) at 4 knots, during 15 or 30 minutes. Depth (m) and

122   bottom temperature (ºC) value were registered for each sample site using a Sea-Bird

123   Electronics conductivity-temperature-depth (CTD) unit.

124   *M. schmitti* was identified in 273 sample sites. Total length (TL, cm), sex and

125   reproductive stage of each specimen were registered on board. Reproductive stages

126   were identified according to the criteria established by Colonello *et al*. (2007b), but only

127   the adult specimens were considered in this study: Adult females at stage III (**FIII**):

128   ovaries with a leading batch of vitellogenic follicles, developed oviductal glands and

129   narrow uteri with thick walls; Females at stage IV (**FIV**): uteri with eggs inside;

130   Females at stage V (**FV**): ovaries with a leading batch of vitellogenic follicles and uteri

131   with embryos inside; Adult males: Testis with large spermatic lobes developed and

132   scarce epigonal organ, claspers calcified surpassing the pelvic fins.

133   The relative abundance (expressed as individuals per nm^2, ind/nm^2) of each

134   reproductive stage was estimated in each sample site by the swept area method

135   (Alverson and Pereyra, 1969). The sizes (TL) distribution of **FIII** and **FIV** were

136   assessed and the presence of lengths larger than 75 cm (length from which 100% of the

137   females were pregnant during the NRC survey) in a sample site was considered as post-

138   partum evidence.

139   *Assessment of the aggregation scenarios*

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140   Based on the abundance of *M. schmitti* adult males and females per sample site

141 during spring, the following aggregation states were defined: non aggregated adults (up

142 to 524 ind/nm^2, corresponding this value to the 50% percentile of the abundance

143 distribution), moderate aggregation (up to 1321 ind/nm^2, corresponding this value to the

144 75% percentile of the abundance distribution), and high aggregation (more than 1321

145 ind/nm^2). Each sex was separately considered for the above abundance states.

146   Depending on the occurrence of adult male and/or female aggregation in each

147 sample site, the following sexual aggregation scenarios were considered. **Only male**

148 **aggregations**: occurrence of male aggregations with non-aggregated or absent females.

149 **Only female aggregations**: occurrence of female aggregations with non-aggregated or

150 absent males. **Male and female aggregations**: occurrence of both sexes aggregated.

151   According to the occurrence of the reproductive stages in sites where females

152 were aggregated (moderate or high aggregation), the following aggregation scenarios

153 were proposed in order to assess the *M. schmitti* reproductive dynamics. **Pre-**

154 **reproductive females**: occurrence of adult female aggregations with no **FIV**. The

155 eventual occurrence of **FIII** larger than 75 cm with no **FV** was considered within this

156 scenario. **Pupping**: occurrence of **FV** and **FIII** aggregations with no **FIV**. **FIII**

157 including animals larger than 75 cm were present. **Pupping-ovulation**: occurrence of

158 **FV**, **FIII** and **FIV** aggregations. **FIII** and/or **FIV** larger than 75 cm were present.

159 **Ovulation**: occurrence of **FIII** and **FIV** aggregations with no **FV**. The aggregation

160 scenarios with ovulation evidences (**pupping-ovulation** and **ovulation** scenarios) were

161 associated with active or recent mating and considered as reproductive aggregations.

162 **Post-mating-ovulation**: occurrence of **FIV** aggregations with no **FV** and **FIII**.

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163   *Spatial distribution of the M. schmitti aggregation scenarios in relation to the*

164   *environmental conditions*

165   Each sample site was associated with an aggregation scenario according to the

166   criteria previously established. The spatial distribution and the proportion of sites with

167   absence of *M. schmitti* adults, non-aggregated adults and aggregated adults was assessed

168   for each research survey. Also, this analysis was performed considering only the

169   different aggregation scenarios.

170   In order to compare the temperature patterns between different years and

171   seasons, linear regression models between bottom temperature and depth were adjusted

172   for each research survey performed. Ordinary Least Squares algorithm and Fisher´s test

173   were used to evaluate the slopes of the linear regressions. The linear regression models

174   obtained for the different years during spring were compared by a likelihood ratio test.

175   In order to inquire the environmental factors triggering the *M. schmitti* adult

176   aggregations and the occurrence of their reproductive events during spring (reproductive

177   aggregations), the bottom temperature and depth conditions associated with the

178   following set of scenarios were compared: Adult absence, no aggregated adults, **only**

179   **male aggregations**, **only female aggregations**, and **male and female aggregations**.

180   Aggregation scenarios with no ovulation evidence (**pre-reproductive females** and only

181   **pupping** scenarios) and with ovulation evidence or reproductive aggregations

182   (**pupping-ovulation** and **ovulation** scenarios).

183   Also, in order to assess the reproductive aggregation size depending on the

184   environmental condition, the male and female relative abundance in sites associated

185   with **pupping-ovulation** and **ovulation** scenarios was compared between different

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186 temperature and depth registered. For this analysis, temperature and depth were

187 considered as discrete variables (factors), binned as follow. Temperature, >15: less than

188 15.5; 16: [15.5-16.5); 17: [16.5-17.5); 18: [17.5-18.5); 18-20 [18.5-20.5). Depth, 10: [5-

189 15); 20: [15-25); 30: [25-35); 40-50: [35-50].

190 All comparisons mentioned above were performed using Kruskal-Wallis test

191 followed by the Dunn´s multiple comparisons or Mann-Whitney test.

192 Since reproductive aggregations represent very important events for

193 reproductive success, the probability to perform a fishing trawl during spring on a *M.*

194 *schmitti* reproductive aggregation site (**pupping-ovulation** or **ovulation** scenarios) was

195 assessed in relation to depth and temperature. Generalized linear models (GLMs) were

196 developed to analyze the occurrence probability of the above condition depending on

197 depth or bottom temperature registered in the fishing site. Since depth and temperature

198 were significantly correlated, a GLM was performed separately for each variable. A

199 binomial distribution for response variable and a *logit* link function were used to

200 develop the models. The adjustment of GLMs parameters was evaluated using the

201 Student´s t test.

202 Results were considered statistically significant at p<0.05. Statistical

203 analyses were performed using SPSS 17.0, GraphPad Prism 5.0 and R 3.1.0 Software.

204

205 **Results**

206 *M. schmitti aggregations and reproductive events in relation to temperature and depth*

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207   The occurrence and spatial distribution of *M. schmitti* aggregations and their

208   reproductive events showed differences depending on the survey assessed (Fig. 1). Only

209   the 10% of total sites during the NRC survey (carried out on winter 2004) showed

210   evidences of adult aggregations, and no evidences of reproductive events were observed

211   (Fig. 1a). The water temperature during this last survey showed a significant and linear

212   increase with depth (Fig 1a). In contrast, the proportion of sites with adult aggregations

213   during spring surveys was higher (ranging between 17 and 72%), and evidences of

214   reproductive and/or post-reproductive events were always observed (Fig. 1b-d). The

215   temperature and depth linear relationships observed for all these last surveys were

216   opposite to the observed in the NRC survey (winter), showing significant and negative

217   slopes. However, the estimated values for this last parameter and also for the intercept

218   depended significantly on the year considered (Fig. 1b-d). It must be noted that the

219   higher proportions of adult aggregations during spring surveys were observed, in

220   general, when water temperature values over the studied area were higher (Fig. 1b-d).

221   The 83.3% of the scarce sample sites in which adult aggregations were found

222   during the NRC survey (winter) was characterized by male aggregations, whereas the

223   50% was characterized by pre-reproductive female aggregations (Table 1 and Fig. 2a).

224   Females at stage IV (characterizing the ovulation scenarios) were absent during this last

225   survey. During spring, a large proportion of total aggregation sites (45.4%) were

226   characterized by **only male aggregations** (Table 1), which in general appeared

227   southward between 20 and 40 m depths (Figs 2b-f). The remaining 54.6% of adult

228   aggregation sites (in which only females or both sexes were aggregated), appeared

229   distributed in general at the most coastal regions, and most of them (44.6%) were

230   associated with the reproductive events (Table 1 and Figs 2b-f).

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231   A clear inter-annual variability pattern in the progress of the reproductive

232   dynamics (**pre-reproductive females**, **pupping**, **pupping-ovulation**, **post-mating-**

233   **ovulation**) was observed during spring surveys. For instance, this variability was clearly

234   reflected by the higher proportion of the most advanced reproductive scenarios

235   (**ovulation** and **post-mating-ovulation** scenarios) observed during 2003 and 2005

236   surveys (Figs 2b and c) in contrast to the almost infrequent or early reproductive

237   evidences observed during the 2008 and 2011 surveys (Table 1, Figs 2d and e).

238   **Pupping** events (with no ovulation evidences) were infrequent and observed only on

239   this last survey, southward between 20 and 50 m depths (Table 1, Fig. 2e). It must be

240   noted that most aggregation scenarios with ovulation evidences (more than 68%) were

241   characterized by high female aggregations co-occurring with males moderately or

242   highly aggregated (Figs 2b-f). On the other hand, most **only male aggregations** sites

243   were associated with the surveys in which **post-mating-ovulation** evidences were

244   observed (Figs 2b, c and f).

245   The bottom temperature in sites in which adults were aggregated during spring

246   was significantly higher than the registered in sites where adults were no aggregated or

247   absent (Fig. 3a). In turn, this difference was larger for the sites associated with female

248   aggregations compared to the **only male aggregation** sites (Fig. 3a). On the other hand,

249   only the depths associated with **male and female aggregations** were statistically

250   different to the depths in which adults were no aggregated or absent (Fig. 3b).

251   On the other hand, the water temperature in sites associated with female

252   aggregations with ovulation evidences (reproductive aggregations), including **pupping-**

253   **ovulation** and **ovulation** scenarios, was significantly higher than the registered in sites

254   in which females were aggregated but no ovulation evidences were registered (Fig. 4a).

255   While more than the 75% of aggregation sites associated with the ovulation scenarios

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256   were observed at temperatures higher than 16 °C, approximately the 50% of female

257   aggregations with no ovulation evidences were observed at temperatures lesser than this

258   value (Fig. 4a). Conversely, no statistically significant differences between the scenarios

259   previously compared were observed for the depth conditions (Fig. 4b).

260   Male and female relative abundances associated with the reproductive

261   aggregations was also different depending on the environmental condition registered,

262   being in general larger when temperature was higher or depth lesser (Fig. 5). However,

263   these differences were statistically significant only for females and mainly in sites

264   where temperature registered was higher than 17.5 °C or depth lesser than 15 m (Fig. 5).

265   It must be noted that the maximum values of male relative abundances in the

266   reproductive aggregation sites were lesser than those observed for females (Fig. 5).

267   *Probability to perform a fishing trawl on a Mustelus schmitti reproductive aggregation*

268   *depending on the environmental conditions*

269   The GLMs developed in the present study showed that the probability to

270   perform a fishing trawl on a *M. schmitti* reproductive aggregation (**pupping-ovulation**

271   or **ovulation** scenarios) was significantly dependent on the temperature or the depth

272   condition registered (Fig. 6). However, the model based on water temperature explained

273   a higher percentage of the variability in this probability compared to that based on depth

274   (Fig. 6). The models showed that the probability to perform a fishing trawl on a *M.*

275   *schmitti* reproductive aggregation increased in warmer waters (at least up to 20 °C), with

276   a maximum probability value of 0.75 (Fig. 6a), or as depth decreased, with a maximum

277   probability value of 0.6 (Fig. 6b). It must be noted that the fit of models resulted almost

278   linear when temperature was higher than 17 °C (Fig. 6a), or depth lesser than 20 m (Fig.

279   6b).

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280

Discussion

282   This study describes, for the first time, the <u>potential</u> influences of temperature

283 and depth on the aggregations and reproductive events of *M. schmitti*. These results,

284 together with previous ecological information give a clear picture of the reproductive

285 aggregation patterns of this shark in coastal waters of Argentina.

286   The coastal aggregations of *M. schmitti* adults observed during spring in this

287 study is in agreement with the reported in other southwestern Atlantic Ocean areas

288 (Oddone *et al*., 2007; Hozbor and Massa, 2008; Pereyra *et al*., 2008; Colautti *et al*.,

289 2010; Cortés *et al*., 2011). These aggregations, which would be associated with

290 reproduction, have also been observed in other smooth-hound sharks (*M. lenticulatus*,

291 Francis and Mace, 1980; *M. canis*, Conrath and Musick, 2002; *M. henlei*, Hopkins and

292 Cech, 2003; *M. californicus*, Espinoza *et al*., 2011), leading to suggest that species

293 belonging to this genus have a consistent coastal aggregation pattern during its

294 reproductive cycle.

295   The full understanding of the mechanisms triggering reproductive movements

296 and aggregations among chondrichthyans represents an important goal for its proper

297 management (Speed *et al*., 2010). In this sense, *M. schmitti* adult aggregations <u>tended to</u>

298 <u>be</u> more frequent in the spring surveys with the higher temperature patterns, and the

299 sites with adult aggregations were significantly warmer than those in which adults were

300 non-aggregated or absent. Although far to be conclusive, these results suggest that this

301 species may have a thermal selection behavior similarly to that already demonstrated in

302 other chondrichthyans (Crawshaw and Hammel, 1973; Casterlin and Reynolds, 1979;

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303   Wallman and Bennet, 2006; Hight and Lowe, 2007; Di Santo and Bennet, 2011; Speed

304   *et al*. 2012). Thus, *M. schmitti* adults could follow the warming gradients along the

305   environment, involving as it was shown changes in depth. More information,

306   encompassing all seasons, is necessary in order to assess if this hypothetical frame

307   explains in part the *M. schmitti* adult aggregation patterns throughout the year.

308   Particularly, it would be interesting to evaluate if the seasonal changes in the

309   temperature-depth pattern observed in the studied region (Cortés *et al*., 2011; Cortés,

310   2012; present study) are associated with the timing of the coastal aggregations of this

311   species.

312   The *M. schmitti* reproductive events were found during all spring surveys and

313   were represented mostly by aggregations associated with **pupping-ovulation** and

314   **ovulation** scenarios. **Pupping** scenarios were infrequent and observed only in one

315   survey. This result suggest that pupping event occurs in a very short period of time and

316   probably almost simultaneously with ovulation and mating, as it was previously

317   suggested in other studies (Menni *et al*., 1986; Oddone *et al*., 2005; Cortés, 2007).

318   Although reproductive events were observed in all spring surveys, a significant

319   interannual variability was evident in the progress of the reproductive dynamics, which

320   was also coincident with differences in the sexual aggregation pattern. Noteworthy, a

321   high number of **only male aggregations** were observed southward between 20-50 m

322   depths in the surveys in which reproductive progress appeared to be more advanced

323   (**post-mating-ovulation** evidences). This result is consistent with the sexual segregation

324   pattern previously observed in adults of this species (Menni, 1985; Cosseau, 1986;

325   Cortés *et al*., 2011), and similarly to the reported by Francis (1988) for *M. lenticulatus*.

326   Since temperatures associated with **only male aggregation** scenarios were different to

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327   those associated with sites in which most females were aggregated, it would be

328   interesting to assess if a sex-dependent thermal selection behavior is related to the

329   sexual segregation in *M. schmitti.* Sexual segregation is common among elasmobranchs

330   (Wearmouth and Sims, 2010) and could lead to a differential exploitation between sexes

331   affecting consequently populations structure (Mucientes *et al.*, 2009). Thus, sexual

332   segregation in *M. schmitti* needs to be carefully assessed in future studies and

333   considered for successful management and conservation of their populations.

334   The interannual variability in the occurrence and spatial distribution of the *M.*

335   *schmitti* reproductive scenarios were significantly associated with the bottom

336   temperature condition. The ovulation event in sites with female aggregation was

337   significantly explained by the increase in temperature, but no by the change in depth.

338   The aggregations with ovulation evidences (reproductive aggregations) were in general

339   associated with temperatures surpassing 16 °C, and the size of these aggregations

340   increased significantly in fishing sites warmer than 17.5 °C. These results suggest that

341   temperature increase may trigger and synchronize the *M. schmitti* ovulation/mating and

342   probably also pupping, as has been proposed in other chondrichthyans (Waltrick *et al.*,

343   2014). This thermal synchronization of the reproductive events could be explained in

344   part by the thermal dependence on gonadal steroidogenesis pathways that control the

345   different gametogenesis stages (Dobson and Dodd, 1977; Heupel *et al.*, 1999; Mull *et*

346   *al.*, 2008, 2010; Waltrick *et al.*, 2014). It must be noted that this scenario of

347   reproductive activity driven by temperature rise would be in accordance with the use of

348   shallow and warmer areas reported in several chondrichthyans species during the

349   breeding season (Francis and Mace, 1980; Snelson *et al.*, 1998; Schwartz, 1990;

350   Conrath and Musick, 2002; Mabragaña *et al.*, 2002; Hopkins and Cech, 2003; Lucifora

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351   *et al*., 2004; Collins *et al*., 2007; Vögler *et al*., 2008; Carlisle and Starr, 2009; Espinoza

352   *et al*., 2011).

353   In accordance with the results previously discussed, the models developed in the

354   present study showed that the probability to perform a fishing trawl on a *M. schimitti*

355   reproductive aggregation during spring resulted significantly explained by the bottom

356   temperature and, although in a lesser extent, by the depth registered in the fishing site.

357   Based on these models it is possible to suggest that the probability to trawl on a *M.*

358   *schimitti* reproductive aggregation during spring increase considerably and almost

359   linearly with an increase in temperature (at least up to 20 °C) or a decrease in depth.

360   However, the significance of depth as predictive variable could be partially explained

361   by the correlation with temperature. It is important to mention that these models

362   summarize all data previously analyzed providing important and practical information

363   for management of *M. schmitti* populations.

364   The limited data available during the winter season and the complete lack of data

365   during summer and autumn make it difficult to assess and understand the dynamics of

366   *M. schmitti* adult population throughout its whole reproductive cycle. Thus, more

367   research surveys covering all seasons in a same year and across different years,

368   considering further other factors such as salinity and trophic ecology, have to be carried

369   out in order to get a better understanding of the reproductive dynamics of this species.

370   Also, tagging/tracking studies should be performed in order to contribute to this last

371   purpose. Finally, ecophysiological studies assessing the influences of temperature

372   variations on sex steroidogenesis and gonadal maturation in *M. schmitti* are extremely

373   necessary to elucidate the hypothesis of reproductive thermo-synchronization presented

374   in this work.

16

375   In conclusion, this study suggests that *M. schmitti* adults aggregate in shallow

376   coastal areas during the course of spring by following the warming gradients along the

377   environment. Once adults aggregate in coastal areas, the gradual increase of water

378   temperature above 16-17 ℃ <u>could</u> trigger synchronously the reproductive events.

379   Sexual segregation appears to be evident in relation with the reproductive dynamics.

380   This environmental control scenario of adult aggregations and their reproductive events

381   appears to be consistent with the observed in many other coastal chondrichthyans.

382   Lastly, it is important to consider that the closed areas to trawling currently adopted in

383   the studied region during spring and summer (see Fig. 1 in Colonello *et al.*, 2014)

384   would be large enough to encompass the spatial distribution of most *M. schmitti*

385   reproductive aggregations observed in this study. However, the above relationship

386   between temperature variations, depth dependents, and the timing of reproductive

387   aggregations would implicate that the interannual variability in the spatial and temporal

388   patterns of water temperature should be considered to optimize the management

389   strategies adopted for the conservation of this and probably other species.

390   **Acknowledgements**

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599   **Figures**

600   **Fig. 1.** Geographic distribution of *Mustelus schmitti* adult aggregations in the NRC

601   survey carried out in winter (**a**) and in the different spring research surveys (**b-f**). The

602   sites in which absence of adults was observed are also shown. Adult aggregations

603   associated with the reproductive or post-reproductive events are identified for each case.

604   The linear regression models between bottom temperature and depth (showing also the

605   parameter values estimated) are shown for each research survey.

606   **Fig. 2.** Geographic distribution of the *Mustelus schmitti* aggregation scenarios in the

607   NRC survey carried out in winter (**a**) and in the different spring research surveys (**b-f**).

608   Male and female aggregations are represented separately.

609   **Fig. 3.** Bottom temperatures (**a**) and depths (**b**) registered in the sample sites associated

610   with absence of *Mustelus schmitti* adults, non aggregated adults, only male

611   aggregations, only female aggregations and male and female aggregations. Different

612   letters indicate significant differences in the environmental variables between the

613   different aggregation conditions. The numbers in brackets below each column indicate

614   the sample size. The whiskers and box plots indicate minimum, 25% percentile, median,

615   75% percentile and minimum value. The cross on each box plot indicates the average

616   value.

27

617   **Fig. 4.** Bottom temperatures (**a**) and depths (**b**) registered in the sample sites associated

618   with *Mustelus schmitti* female aggregations with no ovulation evidences and with

619   ovulation evidences. Asterisk indicates significant differences in the environmental

620   variables between the different female aggregation conditions. The numbers in brackets

621   below each column indicate the sample size. The whiskers and box plots indicate

622   minimum, 25% percentile, median, 75% percentile and minimum value. The cross on

623   each box plot indicates the average value.

624   **Fig. 5.** Relative abundance (individuals per nm^2) of *Mustelus schmitti* male and females

625   in sample sites associated with the reproductive aggregations (**pupping-ovulation** or

626   **ovulation** scenarios) at different temperature (**a**) and depth (**b**) conditions. Different

627   letters indicate significant differences in the relative abundance between the different

628   environmental conditions. The numbers in brackets below each column indicate the

629   sample size. The whiskers and box plots indicate minimum, 25% percentile, median,

630   75% percentile and minimum value.

631   **Fig. 6.** Probability to perform a fishing trawl during spring on a *Mustelus schmitti*

632   reproductive aggregation site (**pupping-ovulation** or **ovulation** scenario) depending on

633   the bottom temperature (**a**) and the depth (**b**) condition registered. Black line indicates

634   the average values of probabilities for the predictor variable range evaluated. The

635   dashed lines indicate confidence intervals to 95%. Empty circles indicate the fishing

636   sites in which reproductive aggregations were present (1) and absent (0) at the different

637   environmental conditions.

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Table 1. **Proportion (%) of** *M. schmitti* **adult aggregation sites at the different scenarios (see Fig. 2 for spatial distribution)**

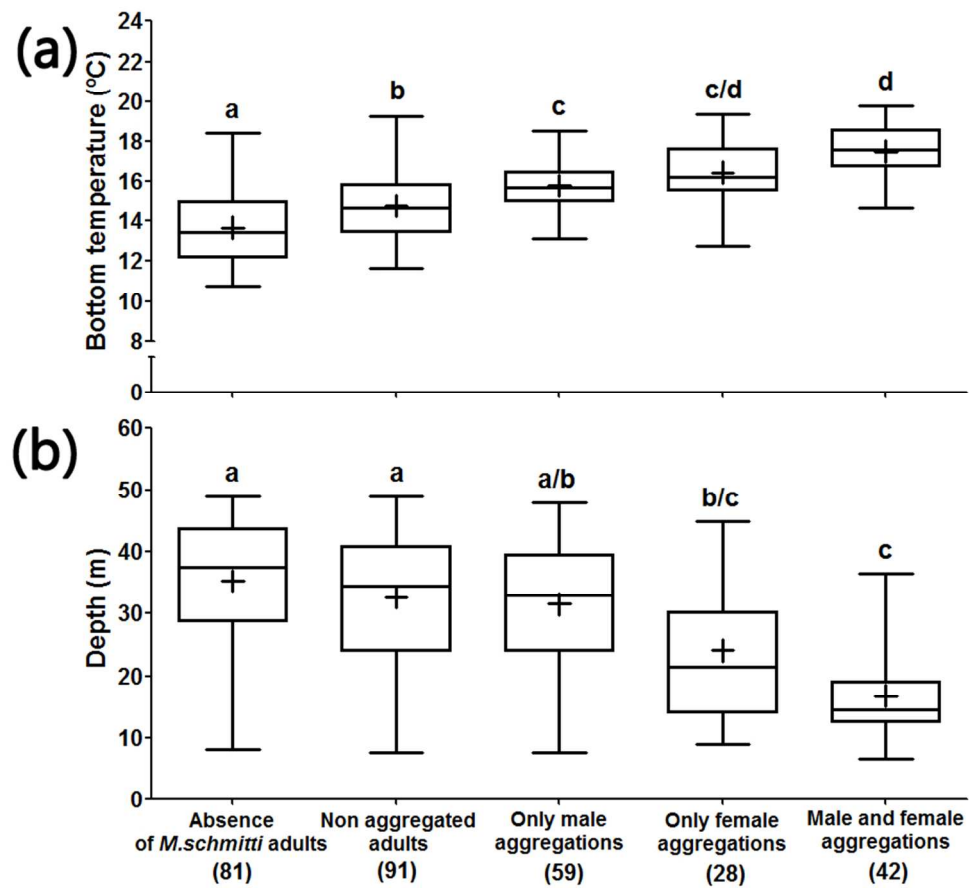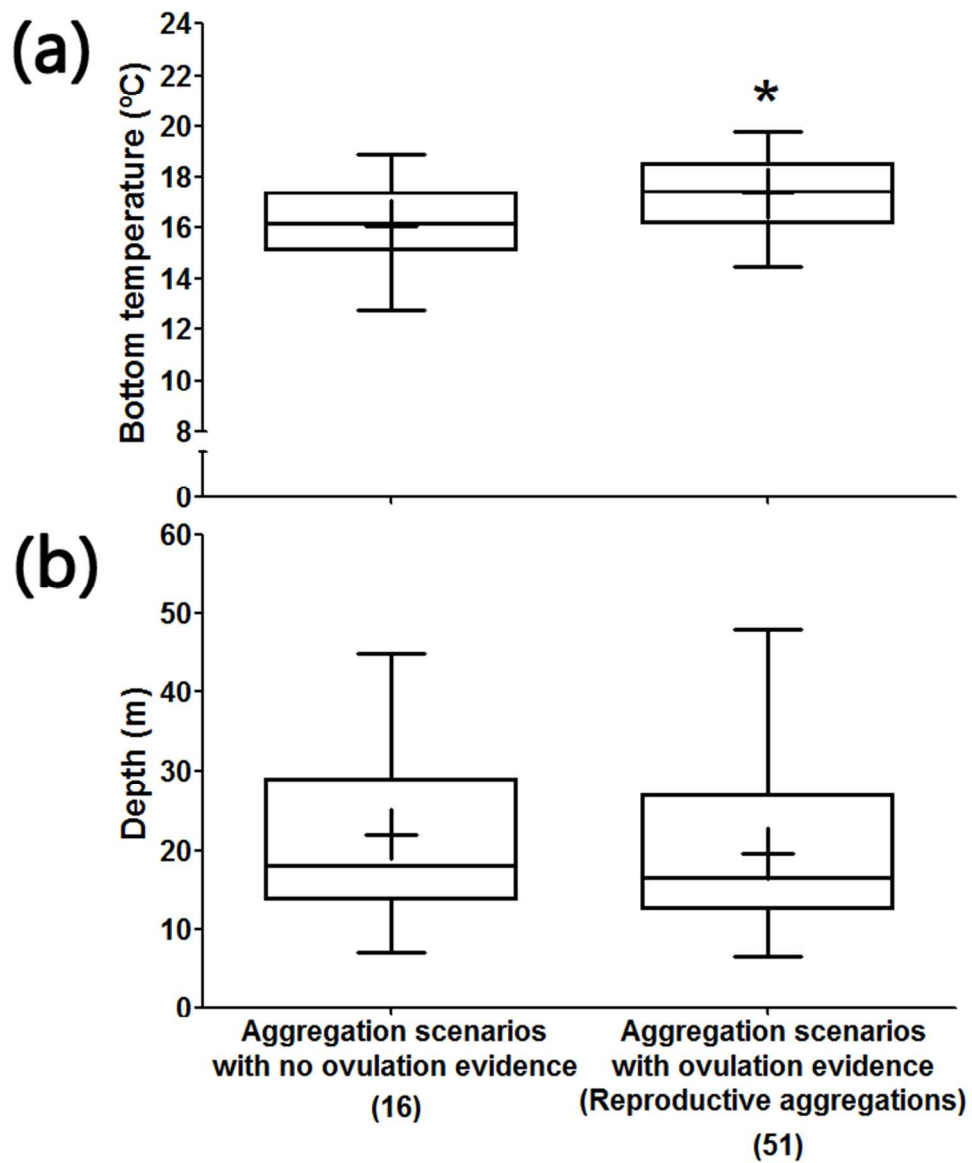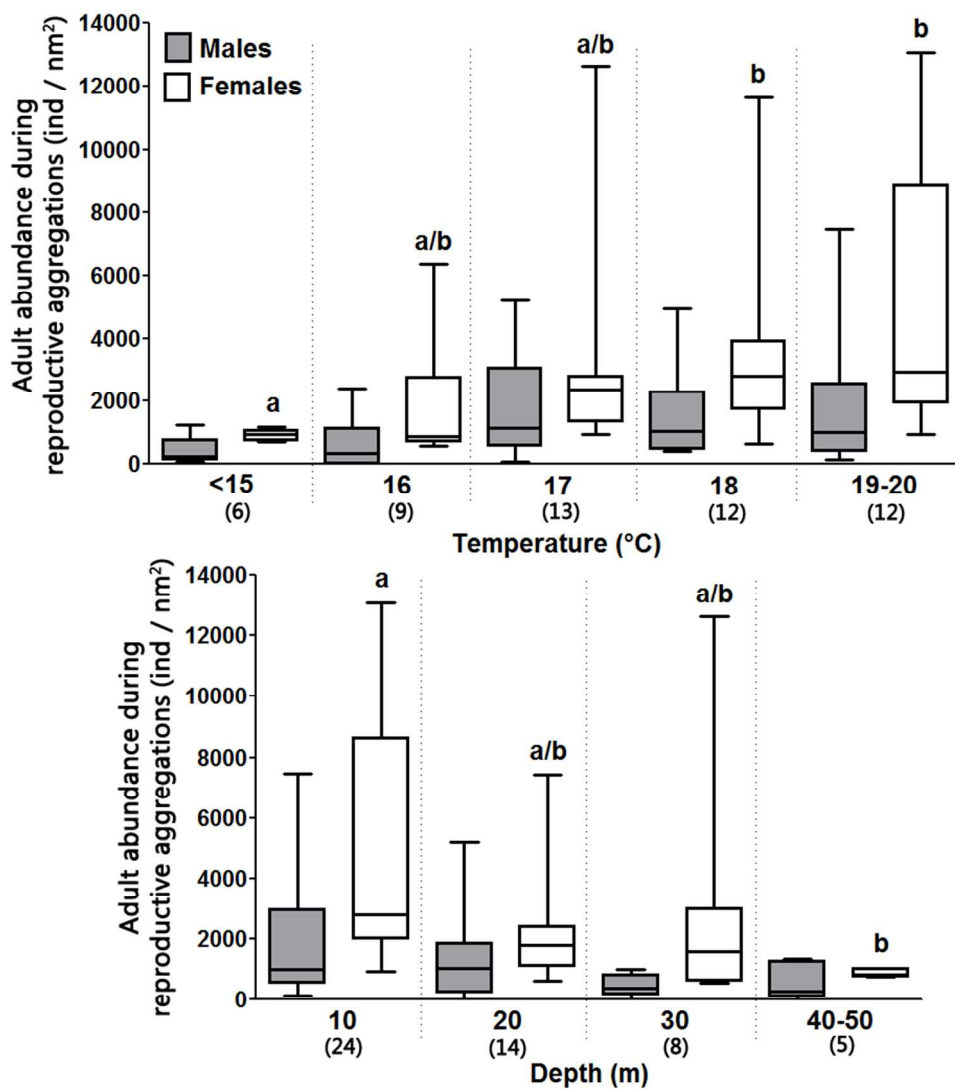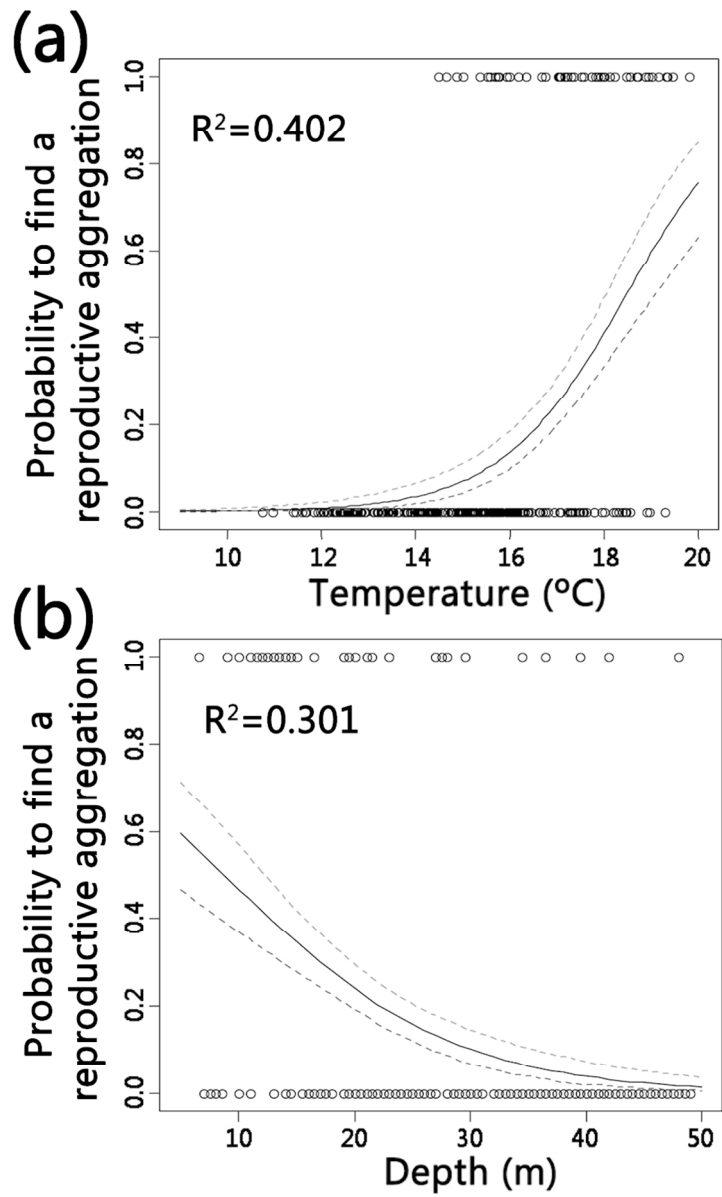| Season	Year	Males (only males)	Pre-reproductive females	Pupping	Pupping-ovulation	Ovulation	Post-mating-ovulation
Winter (NRC)	2004	88.3(50)	50	0	0	0	0
Spring	2003	78.5 (50)	7.1	0	2.4	38.1	2.4
	2005	80.6 (41.7)	0	0	22.2	30.6	5.6
	2008	75 (25)	66.7	0	8.3	0	0
	2011	58.8 (29.4)	11.8	17.6	35.3	5.9	0
	2012	87 (65.2)	0	0	13	17.4	4.3
	Total	77.7 (45.4)	10	2.3	14.6	24.6	3
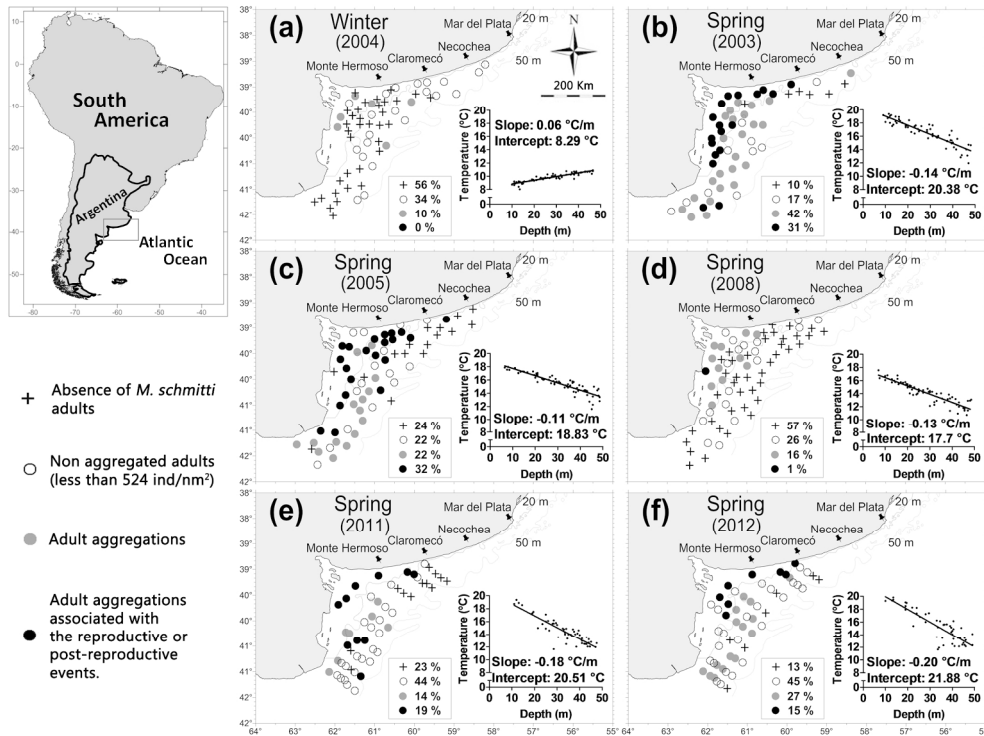
Fig. 1
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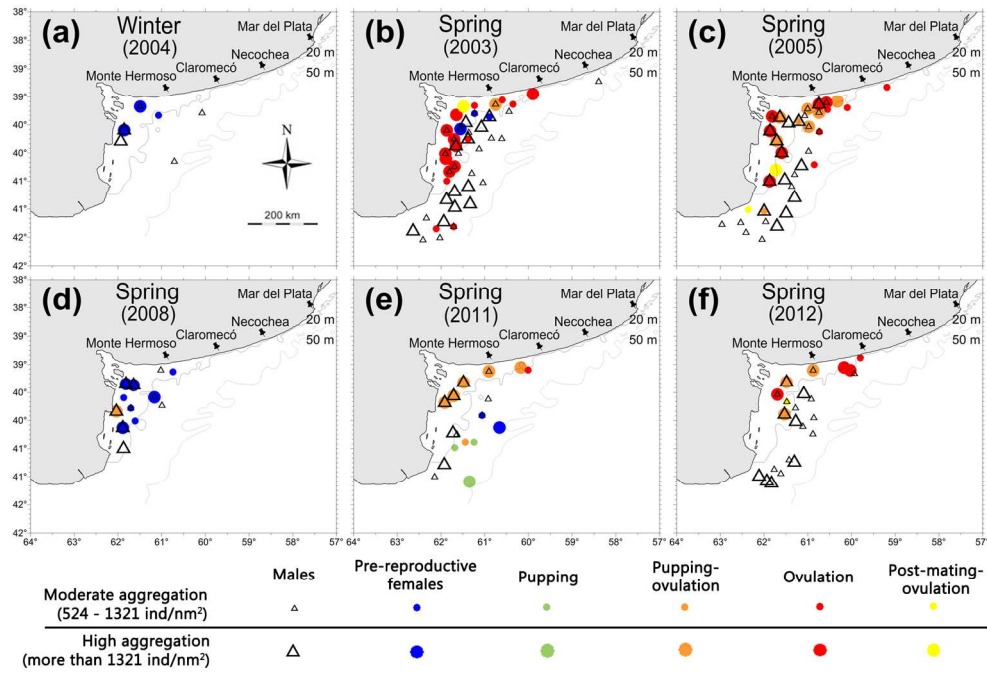
Fig. 2

Fig. 3

Fig. 4

Fig. 5
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Fig. 6
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