

1 **Seasonal and social factors associated with spacing in a wild** 2 **territorial electric fish**

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26 **Short title**

27 Seasonal factors associated with spacing in territorial fish

28 **Abstract**

29 The expression of territorial behavior in wild species is especially suited to explore how
30 animals integrate individual traits with dynamic environmental and social contexts. In this
31 study, we focused on the seasonal variation of the determinants of territory size in the weakly
32 electric fish *Gymnotus omarorum*. This species is a seasonal breeder that displays year-
33 long territorial aggression, in which female and male dyads exhibit indistinguishable non-
34 breeding territorial agonistic behavior and the only significant predictor of contest outcome
35 is body size. We carried out field surveys across seasons that included the identification of
36 individual location, measurements of water physico-chemical variables, characterization of
37 individual morphometric and physiological traits, and their correlation to spatial distribution.
38 Although *Gymnotus omarorum* tolerates a wide range of dissolved oxygen concentration,
39 territory size correlated with dissolved oxygen in both seasons. In the non-breeding season,
40 we show that territory size is sexually monomorphic and explained only by body size. In the
41 breeding season, while body size no longer correlated with territory size, evidence of sexual
42 differences in territory size determinants emerged. First, the overall spatial arrangement
43 adopted a sexual bias. Second, territory size depended on gonadal hormones in both sexes,
44 which was expected for males, but not previously reported in females. Third, females'
45 territory size correlated with gonadal size and females showed relatively larger territories
46 than males, probably to meet sexually dimorphic energetic requirements. This study
47 provides evidence of seasonal changes in factors correlated with territory size and
48 contributes to the understanding of the mechanisms underlying behavioral plasticity.

49

50 **Keywords**

51 Non-breeding aggression, sexual dimorphism, *Gymnotus omarorum*, gonadal steroids

52

53 **Introduction**

54 The mechanisms underlying behavioral plasticity, by which animals adapt to dynamic
55 environmental and social contexts, are far from being fully understood (1). The study of the
56 modulation of territorial behavior in wild model species is especially suited for this aim, as
57 animals assess the environmental and social clues that determine territory quality and this
58 information is contrasted with individual requirements and fighting abilities, to decide
59 whether to compete over an area or not. Therefore, the distribution of territorial animals in
60 space provide a hint on the integration of individual traits with environmental and social
61 factors. Variation in the ability or motivation to obtain and defend a territory can generate
62 differences in territory size, as traits such as body mass, sex, and reproductive state are
63 known to influence resource holding potential and resource value (2–6). Within a population,
64 body size is associated with territory size across species, as it directly correlates with
65 metabolic requirements. Body size is the universal indicator of physical strength and thus it
66 strongly impacts on contest outcome and territory size (7–10). In species that display
67 territoriality in both sexes, asymmetry in fighting abilities or motivational factors may lead to
68 sex differences in territory size; for example, in red squirrels (*Sciurus vulgaris*), in which
69 males often hold larger territories than females (11) or in the striped plateau lizard
70 (*Sceloporus virgatus*), in which females are more territorial than males (12).

71

72 Many species show territorial behavior only during the breeding season (13). On the other
73 hand, some species across different phylogenetic groups, in spite of being seasonal
74 breeders, show robust territorial aggression all year round, as has been reported in birds
75 (14–17), mammals (18–20), reptiles (21), and fish (22,23). These species offer a valuable

76 opportunity to study the seasonality of environmental features and individual traits, and their
77 relation to territory size in the natural habitat. During the breeding season, male territorial
78 aggression is largely dependent on sexual gonadal steroids across vertebrates (24–26) and,
79 in particular, androgen levels have been related to territory size in the wild (27–30). In
80 contrast, in breeding females, there are few studies on the association between estrogen
81 (E_2) circulating levels and territorial aggression in free-living conditions (31–33), and, to our
82 knowledge, there are no studies reporting the association between circulating E_2 and
83 territory size.

84

85 The weakly electric South American fish, *Gymnotus omarorum* (34), is a seasonal breeder
86 that displays male and female territorial aggression all year-long and thus is an interesting
87 model system to study the seasonal control of territoriality and its sex differences. Previous
88 laboratory results showed that this species presents a remarkably robust non-breeding
89 territorial aggression (initially described in (22)), with well-characterized agonistic behavioral
90 displays including modulations of the electric organ discharge (EOD) to signal submission
91 (35–37) and a dominant phenotype that persists for at least 36 hours (38). Under
92 experimental laboratory conditions, male-male and female-female dyads that display non-
93 breeding territorial behavior have shown no differences in either contest outcome, temporal
94 dynamics of the agonistic encounter, levels of aggression, nor submissive signaling (39).
95 Moreover, the only significant predictor of contest outcome is body size (22), and none of
96 the features of agonistic encounters depends on circulating gonadal hormones (40).

97

98 In this study, we aimed to evaluate the seasonal variation of the ecological, morphometric,
99 and physiological correlates of territory size in the wild in *Gymnotus omarorum*. In the non-
100 breeding season, when gonads are regressed and thus circulating gonadal hormones are

101 low, we stand on previous behavioral results to predict that territory size would be sexually
102 monomorphic and explained mostly by body size, regardless of variations in local
103 environmental characteristics. In the breeding season, when motivational aspects of
104 territoriality may be confounded with the reproductive drive, we expected the emergence of
105 sexual dimorphism in territory size determinants.

106

107 **Materials and Methods**

108

109 Study location and sampling seasons

110 Fieldwork was carried out in the Laguna de los Cisnes, Uruguay (205 ha, 34° 48' S, 55° 18'
111 W) which composes a three-part interconnected shallow system (maximum depth 5 m) and
112 has no inputs of salt or brackish water (Fig. 1A, (41)). The study species, *Gymnotus*
113 *omaronum*, is a weakly electric fish, the only one present in the area of study from the several
114 electric species present in Uruguay. The littoral area of the lake is blanketed by a strip (5-40
115 m with) of dense mats of free-floating aquatic macrophytes that cover the sampling area
116 (Fig. 1B), with *G. omaronum* typically living among the roots of these plants (34).

117

118 **Figure 1:** Study site and sampling method. A. The study site is located in Maldonado, Uruguay, in
119 Laguna de los Cisnes. B. The shores of the lake have water hyacinths creating extensive floating
120 mats that constitute the sampling area. C. Census unit illustrating individual spots. Fish location in
121 individual spots was achieved by carrying out an electric census. Once a fish was located, water
122 dissolved O₂ concentration, temperature, and fish EOD rate were measured in each spot.

123

124 Following Quintana et al. (2004), who identified the breeding season for weakly electric fish
125 species in the neotropical subtropical region from December to February (austral summer),
126 we collected field data during December, corresponding to the early-breeding season before

127 the appearance of offspring (42), and from June to August (austral winter), corresponding
128 to the non-breeding season.

129

130 Sampling method

131 Sampling area was homogeneous in depth, distance to shore, and vegetation composition.

132 The sampling area was divided into adjacent transects referred to as census units (Fig. 1B
133 and 1C, defined below), which were each studied in different days, without repeating sites.

134 Sampling was performed during the day, which is the resting phase of animals in this species
135 (43), in two stages: the first one (field survey 1) during the morning from 8 to 12 AM, and the
136 second one (field survey 2) from 1 to 6 PM.

137 All research procedures complied with ASAP/ABS Guidelines for the Use of Animals in
138 Research and were approved by the Institutional Ethical Committee (Comisión de Ética en
139 el Uso de Animales, Instituto Clemente Estable, MEC, 02-2012).

140

141 Field survey 1: Electric census and environmental variables.

142 In order to achieve a first picture of the spatial arrangement of individuals, we carried out an
143 electric census during the resting phase of the animals and measured the distance between
144 each fish and its nearest neighbor (distance to the nearest neighbor, DNN) in the two
145 seasons. To calculate DNN, we considered each focal fish distance to the nearest neighbor,
146 even if the neighbor was not retrieved. The electric census implies the location of individual
147 fish by means of an electronic audio amplifier connected to a pair of electrodes, as described
148 elsewhere (44), and enables to locate individual fish through its EODs when the sound is
149 maximal (detection range is 60 cm). The EOD rate monitoring also allows researchers to
150 confirm that fish remain undisturbed during the measurement of parameters. Gymnotids
151 show a typical behavior called the novelty response (reviewed in (45)), which consists of a

152 transient increase in the EOD rate triggered by changes in nearby impedance, i.e., changes
153 in its surrounding environment (46). To perform the electric census, two experienced
154 researchers waded up to 1.2 m depth in the water, to slowly and carefully access places 1
155 m away from individual fish. Once a fish was located in an individual spot (Fig. 1C), the plant
156 above it was tagged, and conductivity, dissolved oxygen concentration (O_2 , mg/l) and water
157 temperature (T, °C) were measured 30 cm below the surface (using TDSu Testr 3, Cole
158 Parmer for conductivity, and OxyWard, Handy Polaris for O_2 and T). After taking physico-
159 chemical measurements, the EOD of each fish was recorded in situ for 10 seconds by
160 means of two electrodes lowered in the vicinity of the animal, connected (by 10-30 m cables)
161 to an amplifier located on the shore of the lake (World Precision Instruments Inc., Sarasota,
162 FL. DAM-50, AC-coupled). After amplified, signals were recorded on a portable computer,
163 captured by the audio card and stored for further analysis. In order to normalize the potential
164 effect of water temperature on EOD rate, values were corrected to a constant 20°C
165 temperature by using the Q10 value of 1.5 as calculated for electric fish (47,48). Q10 is a
166 unitless quantity calculated as the factor by which the EOD rate increases when the
167 temperature (T) is raised by 10 °C and is calculated as: $Q10 = \frac{\text{EOD rate} \times T}{\text{EOD rate} \times (T$
168 $+ 10)}$.

169 During each sampling day, the measurement of physico-chemical water parameters and
170 EOD rate was repeated for all the fish located within a census unit. A census unit was
171 defined as the area with all fish detected until 12 AM, or the area where we detected a group
172 of fish surrounded by at least 6 meters of water uninhabited by the species (Fig. 1C).

173

174 Field survey 2: Capture and quantification of individual traits.

175 In addition to environment variables, individual traits can influence spatial distribution and
176 be different depending on the season. Therefore, based on field survey 1, we characterized

177 morphometric and physiological traits of retrieved fish in both seasons, and then analyzed
178 its correlation to spatial distribution. Individual spots were revisited in order, and each fish
179 located under the tagged plants was collected using a net.

180 In the breeding season, immediately after netted, fish were anesthetized by immersion in a
181 fast-acting eugenol solution (1.2 mg/l, first dissolved in alcohol 70%) for blood sampling from
182 the caudal vein with a heparinized syringe in less than 3 min, which is the time range usually
183 used to avoid a stress response due to manipulation (49–51). Captured fish were then
184 weighed, measured and their gonads were visually inspected for sex determination. Blood
185 was placed in tubes in ice to be centrifuged and stored at -80 °C in the laboratory (six hours
186 later), gonads dissected in the field were stored in dry ice, and then weighed in the laboratory
187 for gonadosomatic index (GSI) calculation. The index was later calculated for all adult
188 animals as $[\text{Gonad Weight} / \text{Total Tissue Weight}] \times 100$ (52).

189

190 Hormone assays

191 Blood samples were taken in the field as described above, and once in the laboratory plasma
192 was separated by centrifuging the samples at 3000 rpm for 10 min and stored at -80 °C until
193 assayed. 17- β Estradiol (E₂) levels were quantified in breeding females, and 11-
194 Ketotestosterone (11-KT) in breeding males by enzyme-linked immunosorbent assay
195 (ELISA) using commercial kits (IBL International, Hamburg, Germany for E₂ and Cayman
196 Chemical Company, MI, USA for 11-KT). The analyses were carried out according to the
197 manufacturer's instructions and a standard curve was run for each ELISA plate. In all cases,
198 samples were assayed in duplicate and analyses were carried out on samples whose
199 coefficients of variation were below 20% (53). Intra-assay variation was 3.95 % for E₂
200 (detection limit: 25 pg/ml) and 6.2 % for 11-KT (detection limit: 1.56 pg/ml). Pilot assays
201 using three different dilutions of 8 samples (4 samples per sex) were run to establish the

202 appropriate working dilutions, which were 1:2 for breeding female E₂ and 1:30 for breeding
203 male 11-KT. The assays were validated with standards provided in the kit, indicating that
204 each assay effectively detects *G. omarorum* E₂ and 11-KT.

205

206 Data analysis and statistics

207 All data were subjected first to D'Agostino & Pearson omnibus normality test. If data fitted to
208 a gaussian distribution, were analyzed with parametric tests, otherwise non-parametric
209 comparisons were carried out. Environmental variables (O₂ and T) were compared between
210 seasons by Mann-Whitney *U* test. To analyze O₂ and T heterogeneity we calculated the %
211 coefficient of variation (% CV, SD/mean*100) for each census unit and then the mean %CV
212 and SD per season. Fish individual traits (body length and EOD rate), and DNN were
213 compared between sexes within each season by t-tests. Body length, EOD rate and DNN
214 were compared seasonally by t-test for females and males together.

215 To examine the effect of individual traits (see below) on DNN as the dependent variable we
216 used generalized linear models (GLM; (54)), in each season by separate. For the breeding
217 season, we first ran a model with body length, EOD rate and sex as explanatory variables.
218 Because the initial model was non-significant, we ran one model for females with body
219 length, EOD rate, and circulating E₂ as explanatory variables, and a second model for males
220 with body length and EOD rate as explanatory variables. For the non-breeding season, the
221 model included both, females and males together, and explanatory variables were body
222 length, EOD rate and sex. For each season separately, initial models contained all single
223 effects and pairwise interactions of the explanatory variables. To select the most
224 parsimonious GLM, we used the command *bestglm* (55), for a maximum of 3 simultaneous
225 variables, and considering up to second order interactions. Initial models were simplified by
226 the stepwise deletion of the least significant terms in a model and comparing successive

227 steps of model simplification by the Akaike information criterion (AIC), deleting a term
228 whenever there was a difference of more than 2 units between alternative models until
229 arriving to the most parsimonious model that could be fitted. The selection of the best model
230 included the AIC criterion as well as the number and statistical significance of the estimated
231 parameters, discarding models with improvements in the AIC but non-significant
232 parameters. All models were subjected to the customary residual analysis (56).

233 To evaluate if the sex of the nearest neighbor was different from what is expected by a
234 random distribution, we used Binomial tests (57). For the analysis, the focal fish was only
235 considered if the nearest neighbor was retrieved and the sex determined. The proportion of
236 sexes expected for a random distribution was deduced from the empirical sex ratio observed
237 in each season.

238 Parametric, non-parametric statistical analyses, and simple linear regressions were carried
239 out with PAST (58), and GLMs and Binomial tests with software R (59) using RStudio
240 interface.

241

242 **Results**

243

244 Individuals of *Gymnotus omarorum* were located at approximately 30 cm of depth among
245 the dense roots of extensive floating mats of vegetation along the littoral area across
246 seasons (Fig. 1B; i.e., in the breeding season during the austral summer from December to
247 February, and in the non-breeding season during the austral winter from May to August).

248 The water hyacinth *Eichhornia crassipes* dominated both surface and underwater areas
249 accounting for 86% of the total subaquatic biomass. Associated vegetation was composed
250 by the submerged *Egeria densa* and *Miriophyllum aquaticum*, the free-floating *Salvinia*
251 *auriculata*, and the rooted but partly emergent *Ludwigia elegans*, and *Hydrocotyle*

252 *criptocarpa*. This vegetation was present year-long although overall coverage was lower
253 during the non-breeding season.

254 As expected in the subtropical region, water temperature and oxygen content showed
255 significant differences across seasons. Water temperature was higher during the breeding
256 than during the non-breeding season (27.3 ± 0.1 °C, N = 36 vs 11.3 ± 0.5 °C, N = 60; $p <$
257 0.0001 , Mann Whitney U test), whereas O₂ concentration was significantly lower in the
258 breeding compared to the non-breeding season (5.6 ± 0.9 mg/l, N = 36, vs 8.4 ± 0.4 mg/l, N
259 = 65; $p = 0.004$, Mann Whitney U test). In contrast, water conductivity remained consistently
260 below 150 μ S/cm throughout the year.

261

262 **Field survey 1: Fish spatial distribution and environmental variables**

263 Fish (dots in Fig. 1C) were detected by electrical census in individual sites located under the
264 central area of the floating mats, and absent from the edge limiting the open water (Fig. 1C).
265 In both seasons, fish were found in an even distribution, non-aggregated with other
266 conspecifics. The distribution of DNNs was asymmetrical, skewed with a mode at 1.5 m (Fig.
267 2A). The DNN mean value was significantly higher in the breeding season than in the non-
268 breeding season (2.3 ± 0.15 m, N = 47 vs 1.4 ± 0.07 m, N = 73; $p < 1 \text{ exp-}4$, t-test).

269 Water temperature showed low variability among individual spots within each census unit
270 (breeding season mean T CV = 2.0 ± 2.4 %, non-breeding season mean T CV = 6.5 ± 5.5
271 %). In contrast, O₂ concentration showed high variability within each census unit (breeding
272 season mean O₂ CV = 20.4 ± 9.3 %, non-breeding season mean O₂ CV = 25.9 ± 14.6 %),
273 and thus we explored whether O₂ could account for fish spatial patterns. During the breeding
274 season, the distribution of O₂ concentration ranged from 0 to 15 mg/l and was positively
275 skewed (Fig. 2B), with a higher frequency of low values (mode at 1 mg/l). However, during
276 the non-breeding season, O₂ distribution was negatively skewed, showing a single mode at

277 11 mg/l (Fig. 2B). Oxygen concentration and DNN showed a positive and significant
278 association both in the breeding season ($R^2 = 0.44$, $p = 1 \times 10^{-4}$, $N = 31$, Fig. 2C green)
279 and the non-breeding season ($R^2 = 0.21$, $p = 1 \times 10^{-4}$, $N = 50$, Fig. 2C gray).

280

281 **Figure 2:** Fish spatial distribution based on environmental variables. The breeding season is
282 represented in green and the non-breeding season in gray; dark green sections implies overlap of
283 both seasons. A. Frequency distribution versus distances to the nearest neighbor (DNN, in meters).
284 B. Frequency distribution versus oxygen concentration (in mg/l) measured at 30 cm from the surface
285 in each individual spot. C. Linear correlation of distance to the nearest neighbor and oxygen
286 concentration in individual spots Breeding season: $R^2 = 0.44$, $p = 1 \times 10^{-4}$, $N = 31$, non-breeding
287 season: $R^2 = 0.21$, $p = 1 \times 10^{-4}$, $N = 50$.

288

289 **Field survey 2: Fish spatial distribution based on individual traits**

290 There were no sexual differences in body length, EOD rate or DNN in both the breeding and
291 the non-breeding season (Table 1). During breeding, females showed circulating mean E_2
292 levels of 293.7 ± 97.7 pg/ml and males had mean 11-KT circulating levels of 399.1 ± 140.9
293 pg/ml. From a seasonal perspective, breeding fish were significantly larger than non-
294 breeding ones ($p < 1 \times 10^{-4}$, N breeding season = 28, N non-breeding season = 53; t-test).
295 In addition, EOD rate was higher ($p < 1 \times 10^{-4}$, N breeding season = 30, N non-breeding
296 season = 36; t-test) and DNN was larger during the breeding season ($p < 1 \times 10^{-4}$, N
297 breeding season = 31, N non-breeding season = 46, t-test).

298 We evaluated the determinants of DNN separately in the breeding and non-breeding
299 seasons. As the first GLM including breeding females and males, with body length, EOD
300 rate, and sex as explanatory variables was not significant, we separated sexes into two
301 different models and included circulating E_2 levels as an explanatory variable for females.

302 We obtained two significant models in females, both equivalent according to the AIC criterion
 303 (Table 2). In the model with the best adjustment (model 1), DNN showed a positive
 304 correlation with circulating E₂, and a negative correlation with EOD rate. In model 2, EOD
 305 rate was not a significant explanatory variable for DNN and only E₂ had a significant positive
 306 correlation. For males, we were unable to find a correlation between the independent
 307 variables tested and DNN. Although androgen levels were not included in the model (due to
 308 a low number of valid samples), it is worth mentioning that circulating 11-KT levels showed
 309 a positive trend with DNN in a simple linear regression (p = 0.049; R² = 0.77; N = 5). During
 310 the non-breeding season, as all traits quantified were represented in both sexes, we were
 311 able to run females and males together when testing the influence of individual traits on
 312 DNN. We explored if individual sex, body size, and EOD rate correlated with DNN, and found
 313 that body size, but not sex nor EOD rate, correlated positively with DNN (Table 3).

314 **Table 1:** Sexual comparison of individual traits and distance to the nearest neighbor in the breeding
 315 season and in the non-breeding season. Individual traits: Body length and electric organ discharge
 316 (EOD) rate corrected by water temperature. Distance to the nearest neighbor (DNN). Values are
 317 expressed as mean ± standard error of the mean (SEM), and statistical comparisons carried out by
 318 t-test.

319

	Body size (cm)		EOD rate _{temp}		DNN (m)	
	♀	♂	♀	♂	♀	♂
A- Breeding season	22.4 ± 0.5 n = 13	24.5 ± 0.9 n = 15	41.0 ± 1.1 n = 13	39.3 ± 0.9 n = 17	2.2 ± 0.2 n = 13	2.3 ± 0.3 n = 18
	♀ vs ♂ p = 0.2		♀ vs ♂ p = 0.3		♀ vs ♂ p = 0.8	

B- Non-breeding season	16.9 ± 0.9 n = 26	17.4 ± 0.8 n = 27	20.0 ± 1.2 n = 18	19.3 ± 0.95 n = 18	1.13 ± 0.1 n = 23	1.2 ± 0.1 n = 23
	♀ vs ♂ p = 0.7		♀ vs ♂ p = 0.6		♀ vs ♂ p = 0.5	

320

321 **Table 2:** GLM models that presented the best adjustment to explain the distance to the nearest
 322 neighbor in females during the breeding season. Model intercept and explanatory variables are
 323 expressed as Mean (SD). For each parameter the value is shown in bold if statistically significant
 324 (<0.05), in italic if marginal (<0.1), and expressed as NS if non-significant. Model 1 and Model 2 did
 325 not present significant differences by the Akaike information criterion (AIC).

Model	Intercept	Circulating E ₂	EOD rate	N	P model	Adjusted R ²	AIC
M1	4.52 (1.41)	1.4e-3 (0.03)	<i>-0.051</i> (0.027)	13	0.022	0.44	20.24
M2	1.88 (0.18)	1e-3 (4e-4)	NS	13	0.028	0.31	22.21

326

327

328 **Table 3:** GLM model that presented the best adjustment to explain the distance to the nearest
 329 neighbor in both sexes during the non-breeding season. Model intercept and explanatory variable are
 330 expressed as Mean (SD). For each parameter the value is shown in bold if statistically significant
 331 (<0.05).

Intercept	Body size	N	P model	Adjusted R ²	AIC
0.364 (0.34)	0.052 (0.02)	48	0.014	0.11	79.75

332

333 Although DNN was larger in the breeding season in both sexes, this seasonal difference
 334 disappeared in males when DNN was normalized by fish body size (0.08 ± 0.007, N = 17 vs
 335 0.1 ± 0.01, N = 22; p = 0.16, t-test, Fig 3A). Interestingly, in females, DNN normalized by
 336 body size was significantly higher during the breeding season than in the non-breeding
 337 season (0.1 ± 0.008, N = 13 vs 0.07 ± 0.006, N = 23; p = 0.01, t-test, Fig 3A).

338

339

340 **Figure 3:** Emergence of sex dimorphism during the breeding season.

341 The plots show values for females (left panels, represented as squares) and males (right panels,
342 represented as circles); colors indicate breeding (green) and non-breeding (grey) seasons.

343 **A-** Distance to the nearest neighbor (DNN in m) corrected by body length (cm). **B-** Gonadosomatic
344 index (GSI). Dots represent individual values, and in A and B horizontal line represent mean values,
345 and error bars represent SEM. For each sex, breeding values are shown in the left (green) and non-
346 breeding values in the right (grey). * indicate statistically significance ($p < 0.05$) t-test. **C-** Linear
347 regression between body size (cm) and DNN (m) in the breeding season. Females: $p = 0.8$, $R^2 =$
348 0.007 , $N = 13$; males $p = 0.7$, $R^2 = 0.01$, $N = 15$. **D-** Linear regression between GSI and DNN (m) in
349 the breeding season. Females: $p = 0.001$, $R^2 = 0.8$, $N = 9$; males: $p = 0.14$, $R^2 = 0.25$, $N = 10$.

350

351 Animals sampled in both seasons differed in their gonadosomatic index depending on the
352 sex (Fig. 3B). In females, GSI was significantly higher in the breeding season than in the
353 non-breeding season ($1.1 \pm 0.22\%$ $N = 9$ vs $0.6 \pm 0.09\%$ $N = 11$; $p = 0.02$, t-test), whereas
354 males did not show seasonal differences ($0.24 \pm 0.03\%$ $N = 10$ vs $0.23 \pm 0.03\%$ $N = 16$; $p =$
355 0.87 , t-test). Body size did not correlate with DNN in the breeding season (females, $p = 0.8$,
356 $R^2 = 0.007$, $N = 13$; males $p = 0.7$, $R^2 = 0.01$, $N = 15$; Fig. 3C). In the breeding season, GSI
357 strongly correlated with DNN in females ($p = 0.001$, $R^2 = 0.8$, $N = 9$; Fig. 3D), whereas the
358 correlation was not significant in males ($p = 0.14$, $R^2 = 0.25$, $N = 10$, Fig. 3D).

359

360 In the breeding season, the spatial arrangement of sexes showed a specific configuration in
361 which the percentage of females with a female as the nearest neighbor was significantly
362 lower than the random distribution ($p = 0.03$, $N = 11$; Binomial exact test, Fig. 4A). In the same
363 sense, the percentage of males with a female as the nearest neighbor was significantly
364 higher than the random distribution ($p = 0.04$, $N = 16$; Binomial exact test, Fig. 4A). In contrast,
365 the spatial configuration of the population in the non-breeding season showed a random

366 arrangement of both males and females. The probability of having a female as the nearest
367 neighbor was not significantly different from the random distribution for both females
368 ($p=0.82$, $N = 20$; Binomial exact test, Fig. 4B) and males ($p=0.12$, $N = 15$; Binomial exact
369 test, Fig. 4B).

370

371 **Figure 4:** Seasonality in the spatial distribution of sexes. Sex of the nearest neighbor (expressed in
372 percentage) when the focal fish is a female or a male, both in the breeding season (A, top), and in
373 the non-breeding season (B, bottom). Dashed line represents the expected percentage to have a
374 female as nearest neighbor for a random distribution, according to the empirical sex ratio (41% in the
375 breeding season, and 50.9% in the non-breeding season). * indicate statistical significance ($p<0.05$)
376 according to the binomial exact test.

377

378 Discussion

379 This study contributes to the understanding of the mechanisms underlying behavioral
380 plasticity by reporting for the first time seasonal and sexual differences in the determinants
381 of territory size in a teleost species. In the first place, we found that *Gymnotus omaroum*
382 presents a spatial arrangement in the natural habitat consistent with territoriality across
383 seasons. In addition, we confirmed our predictions: a) In the non-breeding season, territory
384 size was sexually monomorphic and partially determined by individual body size; and b) in
385 the breeding season, sexual differences emerged given that the spatial arrangement
386 adopted a sexual bias and that females hold gonadal-dependent larger territories.

387

388 Body mass has strong relationships with a variety of physiological and ecological attributes,
389 such as home range and metabolic rates and is key to understanding how animals use the
390 environment (60–63). The distribution of differently sized animals can be shaped by
391 behavioral interactions such as the defense of foraging resources (64). These ideas, put

392 forth initially to analyze interspecific interactions, can be also applied to interpret distribution
393 of individuals of the same species, as in this study (65). We found that body size predicted
394 DNN in the non-breeding season but not in the breeding season (Tables 2 and 3). This
395 suggests that during reproduction other physiological, behavioral, and motivational aspects
396 may be overriding body size as a predicting trait on DNN, a proxy for territory size.

397 Oxygen is a limiting physico-chemical variable in aquatic ecosystems, and low
398 concentrations of dissolved O₂ has been related to fish mortality (66). Interestingly,
399 *Gymnotus* has been shown to be tolerant to a wide range of dissolved O₂ concentration and
400 can survive in places with hypoxia (67), as it is capable of breathing from air in addition to
401 metabolic adaptations which compensate for any potential damages hypoxia may cause
402 (68). Although different individual spots showed variable O₂ concentration, and O₂ correlated
403 with territory size in both seasons, it remains to be understood whether high O₂
404 concentration may be a valuable attribute of territories in this species. In this regard, despite
405 being tolerant to hypoxia, higher concentration of available O₂ could most probably offer a
406 less demanding context for fish.

407

408 Non-breeding spacing: body size dependent and sex independent

409 In teleosts, year-round territoriality has been mostly approached in populations of coral reef
410 communities (for example *Stegastes fuscus*, (69)). Here, we evaluated the seasonal spatial
411 organization in a subtropical freshwater fish in the field and found that spatial distribution
412 during winter was consistent with territoriality. Why would *Gymnotus* defend non-breeding
413 territories? The acquisition and maintenance of territories are known to be mediated by
414 agonistic behavior (70). In particular, previous reports of laboratory experiments
415 demonstrated that *Gymnotus omarorum* exhibits non-breeding agonistic behavior that
416 mediates territory access (38) and that body size is the main determinant of the outcome of

417 fights (22). Electric fish present high basal metabolic requirements associated to
418 electrogeneration (71), which impose an additional constant need to forage. In line with this,
419 we have found that larger fish hold larger territories (Table 3). Body size is sexually
420 monomorphic in this species and behavioral experiments have shown that agonistic
421 behavior is non-sex-biased (22,39). In this study, we confirmed that non-breeding males and
422 females hold sexually monomorphic territory sizes in the wild, probably to cope with
423 energetic requirements that are not expected to be sexually different during winter.

424 Electric fish use electric communication signals as behavioral displays. As EOD encodes
425 information about body size and physiological state (72–74), territory boundaries could be
426 maintained in the wild by remotely assessing the EOD of neighbors. Status dependent EOD
427 rate-rank occurs only when fish are kept in close quarters after conflict resolution, but not
428 when allowed to distance themselves (38). Thus, we did not expect to find correlations
429 between EOD rate and territory size in the field, as was the case (Table 3). EOD rate rank
430 may be a behavior needed to reinforce submission when a subordinate individual is unable
431 to escape from the dominant as in confined laboratory conditions but not in the wild under
432 the population densities observed in this study.

433

434 Breeding spacing: the emergence of sexual dimorphism in territory determinants

435 While competition for reproductive opportunities is usually sexually dimorphic, competition
436 over non-sexual resources can be expected to be balanced between males and females
437 (75,76). Moreover, species of different phylogenetic groups with male and female
438 territoriality have been found to display sexual monomorphism in body size and signal traits
439 (77–79). Consistently, we found that during the breeding season absolute territory sizes
440 were sexually monomorphic, which is consistent with the lack of sex dimorphism in body
441 size (Table 1). However, during breeding, body size did not correlate with territory size (Fig.

442 3A and B), suggesting there are other factors overcoming body size influence on habitat
443 resource acquisition and maintenance.

444 Interestingly, a closer analysis of our data showed that territory size relative to body size
445 was sexually dimorphic. Females seemed to need larger territories in the breeding season
446 compared to the non-breeding season (Fig. 3A), but males showed no seasonal difference
447 (Fig. 3B). This result can be interpreted in the context of the energetic cost imposed by
448 ovarian maturation, as GSI showed an excellent predicting power on female territory size
449 (Fig. 3 A). Steroid hormones are crucial as mediators of behavioral plasticity (1). Sex steroids
450 orchestrate integrated responses in the organism, and also respond depending on the social
451 environment (80,81). Not surprisingly, circulating E_2 positively correlated with territory size
452 in females (Table 2). The fact that both GSI and E_2 correlated with female territory size, in
453 addition with reports in which E_2 promotes female aggression (82–84), suggests that ovarian
454 E_2 modulates territorial behavior in *G. omarorum*. We hypothesize that E_2 is integrating
455 female metabolic requirements with the social environment, through the expression of
456 territorial behavior. On the other hand, males showed a trend between circulating 11-KT and
457 territory size, which was expected given the well documented relationship between
458 androgens and male territoriality (24–26).

459

460 In the breeding season, sexually dimorphic individual traits may influence motivation towards
461 territory defense, and thus be reflected in the spatial pattern (11,12). The results presented
462 in this study suggest that *G. omarorum* can assess territory features and use this information
463 to relocate at different times of the year. In the non-breeding season individual fish had a
464 closest neighbor which was randomly either of the same or opposite sex, whereas in the
465 breeding season, it was more likely to have an opposite-sex closest neighbor (Fig. 4). This
466 evidence supports the idea that the sex of the nearest neighbor becomes a relevant factor

467 for territory value, but only during breeding. This can be considered a good example of
468 behavioral plasticity by which individuals respond differently to the same social stimulus
469 (e.g., sex of the nearest neighbor), depending on variations in their internal state (sexually
470 dimorphic hormones).

471

472 Concluding remarks

473 *Gymnotus omarorum* is a species which offers the opportunity to analyze seasonal changes
474 in year-round territoriality. Although we found no differences in absolute territory size
475 between sexes across the year, territory size was truly sexually monomorphic only in the
476 non-breeding season, in which body size was the only significant determinant of territory
477 size. In contrast, in the breeding season sex became relevant for the territorial behavior of
478 this species: a) the sex of neighbors matters; b) territory size depended on gonadal
479 hormones in both sexes, which was expected for males, but not previously reported in
480 females; and c) females needed relatively larger territories than males which may reflect
481 particular sex-biased reproductive requirements consistent with metabolic demands of
482 anisogamy. This study contributes to bridge the gap between behavioral plasticity of natural
483 territorial behavior and its underlying mechanisms.

484

485 **Author's contributions**

486 L.Z., L.Q., and A.S. conceived and designed the overall study.

487 M.M. and F.T.M. contributed to the design of field sampling.

488 L.Z., L.Q., F.T.M., M.M., and A.S. collected the data.

489 L.Z., R.H.M., and R.G.M. processed and interpreted hormone data.

490 L.Z. and D.H. analyzed all data.

491 L.Z., L.Q., D.H., F.T.M., M.M., and A.S. interpreted results.

492 L.Z., L.Q., and A.S. led the writing of the manuscript.

493 L.Q. and A.S. funding acquisition.

494 All authors contributed critically to the drafts and gave final approval for publication.

495

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503

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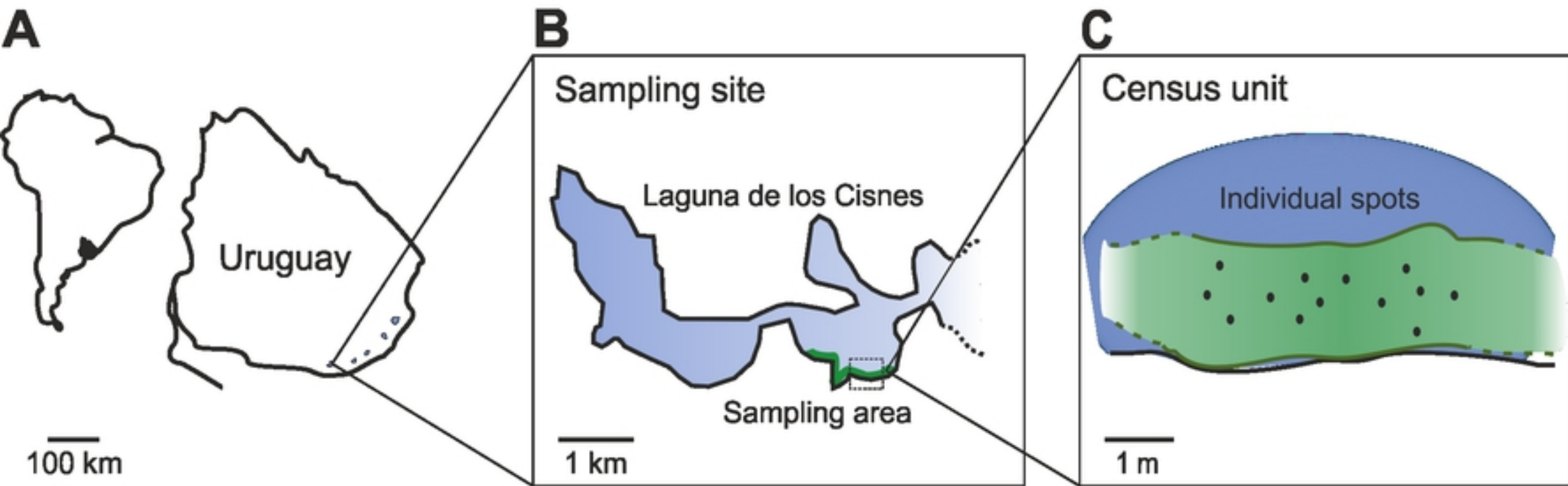


Figure 1

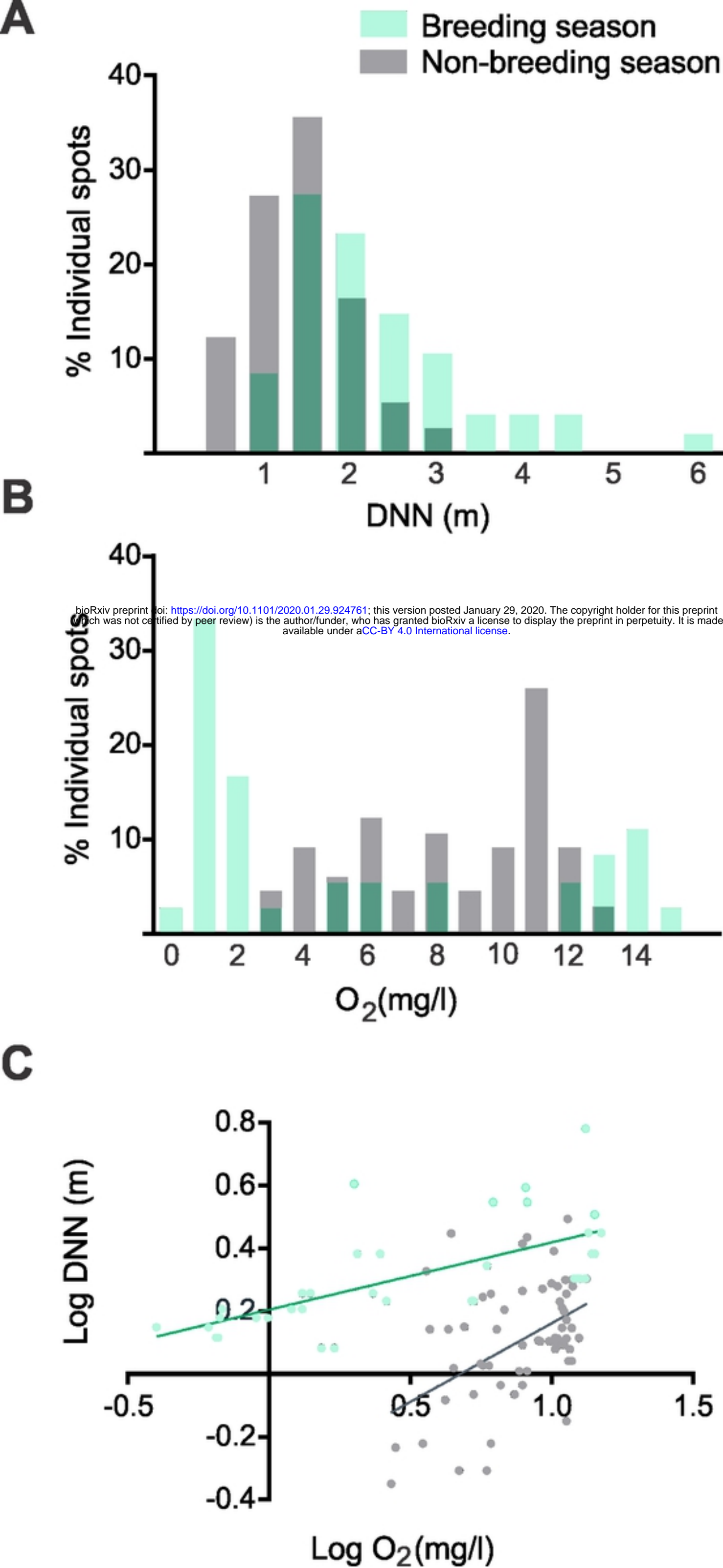


Figure 2

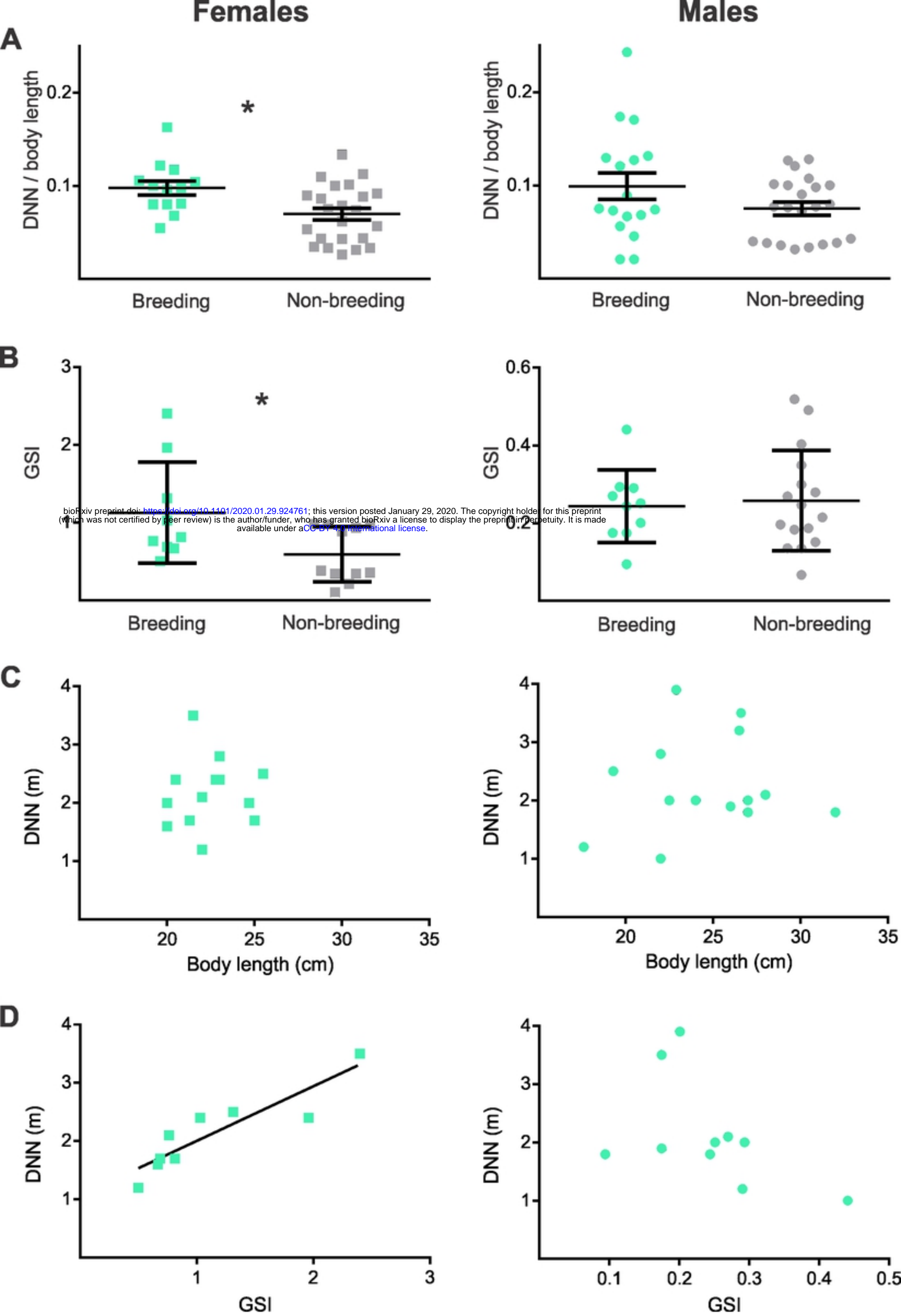
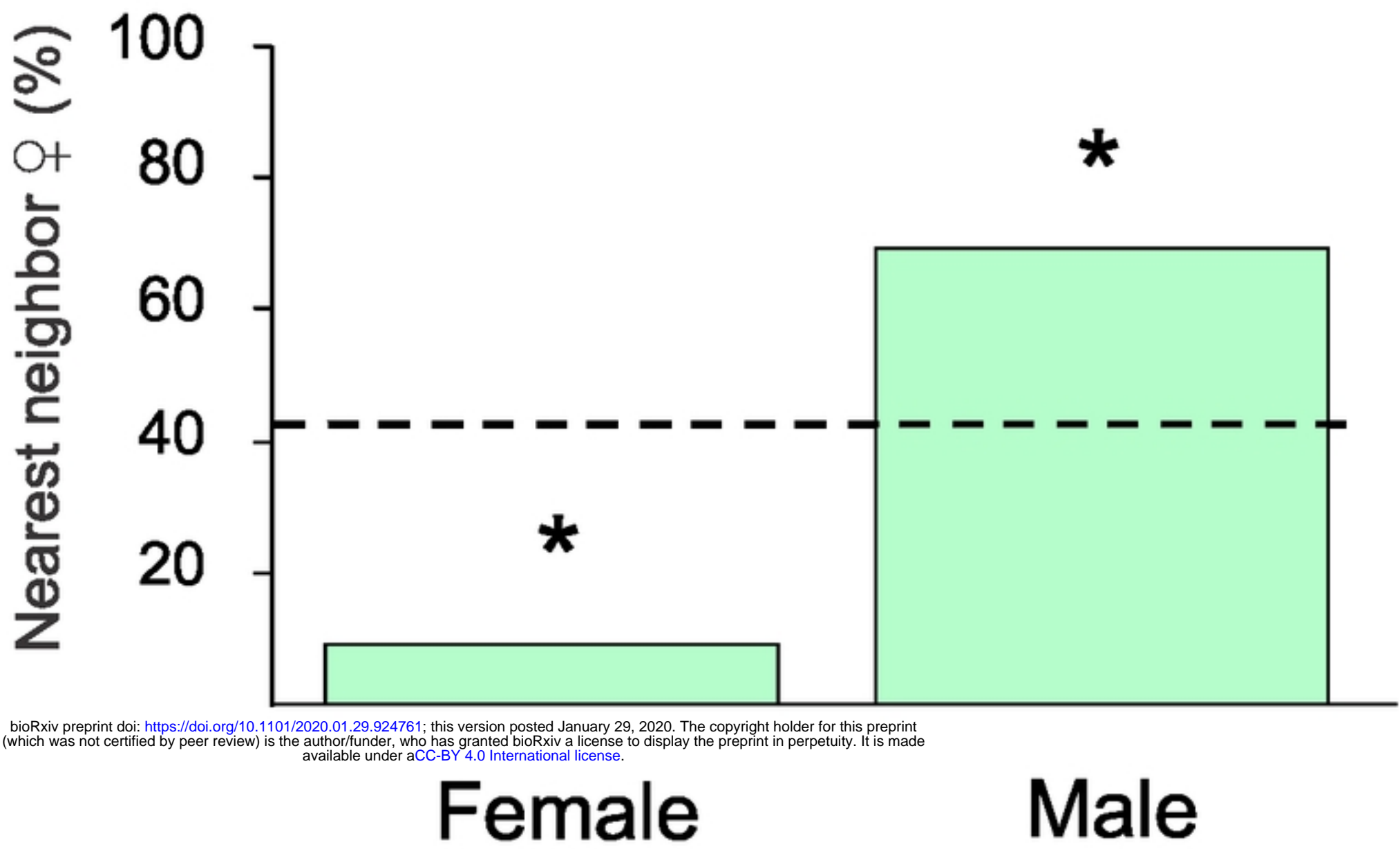


Figure 3

A

Breeding season

**B**

Non-breeding season

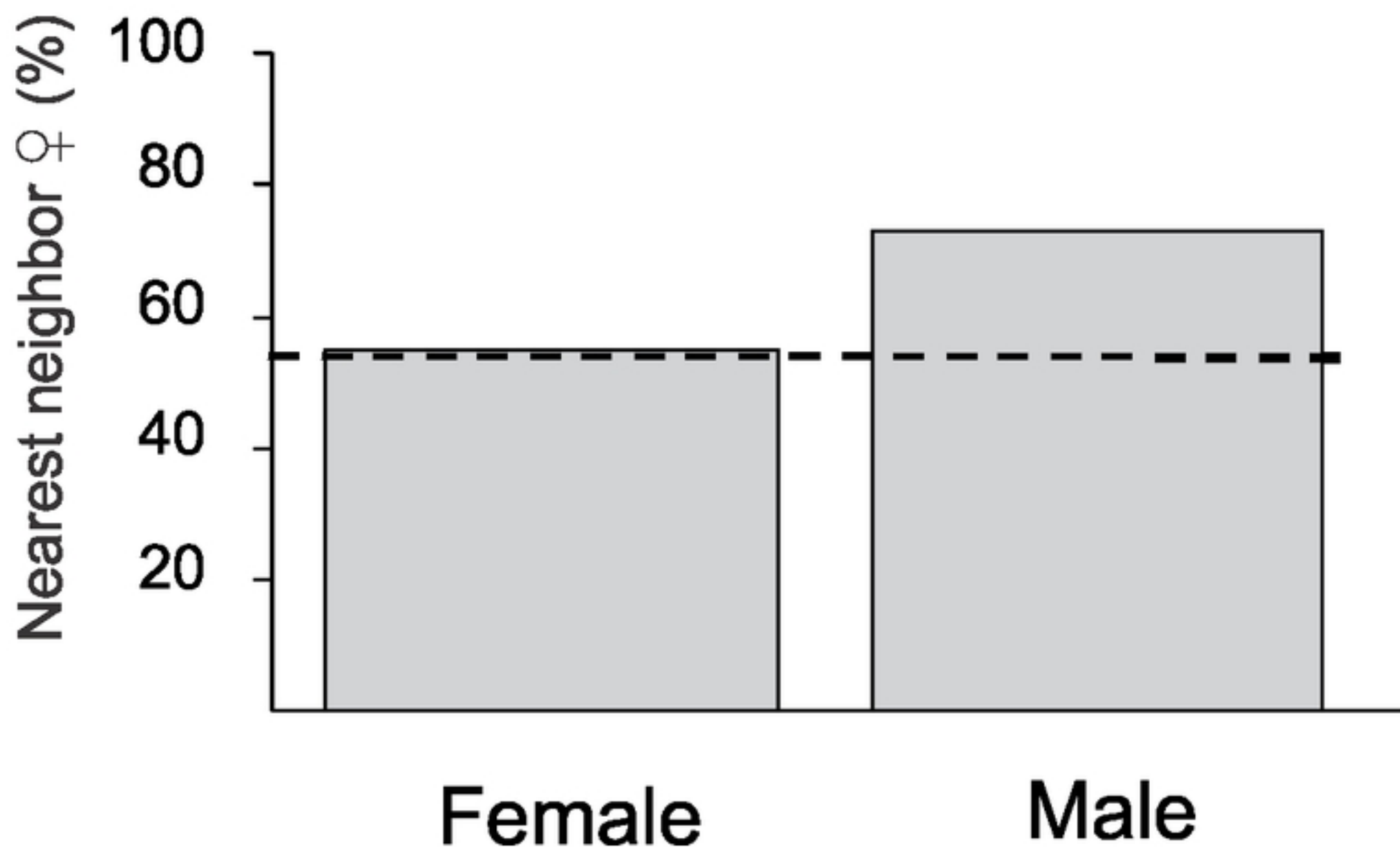


Figure 4