UNIVERSIDADE ESTADUAL PAULISTA - UNESP

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS - ZOOLOGIA

Fábio Henrique Carretero Sanches

Distribuição espacial de caranguejos-chama-maré: efeitos do sombreamento, competição interespecífica e seleção sexual

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia), do Instituto de Biociências de Botucatu - Universidade Estadual Paulista, como parte dos requisitos para a obtenção do título de Doutor.

Orientador: Prof. Dr. Rodrigo Egydio Barreto

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Dedico esse trabalho a todos aqueles que destinam

suas vidas para o desenvolvimento da ciência.

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Sumário

Apresentação

Os manguezais são ecossistemas importantes, contribuindo para o apoio das populações de organismos costeiros e comunidades marinhas (Odum & Heald, 1975). Um grupo que possui importante papel ecológico nessas regiões são os caranguejos-chama-maré (veja Shih et al. 2016 para nova classificação filogenética). As espécies de caranguejos-chama-maré são semiterrestres, sociais, e habitam margens de praias abertas, manguezais e marismas, tanto em zonas tropicais como temperadas (Crane 1975). Cada indivíduo concentra suas atividades em torno de uma toca, com as fêmeas possuindo os dois quelípodo de mesmo tamanho, enquanto os machos possuem um dos quelípodo hipertrofiado, usados como armas durante interações agressivas para proteção do território ou durante a corte em um movimento característico de display (Crane 1975). Dessa forma, são excelentes modelos para estudos de competição e seleção sexual. Além disso, a escavação do solo para construção de suas tocas acarreta na maior oxigenação do mesmo, altera as condições de drenagem, distribuição de partículas, disponibilidade de matérias orgânicas e nutrientes (Kristensen 2008; Cannicci et al. 2008; Penha-Lopes et al. 2009; Natálio et al. 2017), sendo assim considerados engenheiros do ecossistema (Jones et al. 1994, 1997; Kristensen 2008). Portanto, alterações nos manguezais que influenciam na distribuição desse grupo de caranguejos podem intensificar potenciais impactos nessas regiões.

Alguns fatores do ambiente podem afetar a distribuição de caranguejos-chama-maré, como por exemplo, o sombreamento devido à cobertura da vegetação (Nobbs 2003). A sombra da cobertura vegetal pode amenizar as condições extremas causadas pelo aumento da temperatura e evaporação (McGuinness 1994; Nobbs 2003). Por outro lado, é possível que algumas espécies evitem essas áreas por usarem sinais visuais mais frequentemente do que espécies que vivem em florestas (Zeil & Hemmi 2006). Assim,

alterações nessas características podem alterar a distribuição dos organismos e, portanto, alterar toda a comunidade. Nesse sentido, ações antrópicas nessas regiões podem ser exemplificadas como fatores que afetam esses organismos, como por exemplo, a construção de estruturas que causam sombreamento em áreas do estuário sem vegetação natural.

Além disso, as espécies que habitam esses ambientes também podem sofrer com efeitos de mudanças climáticas (Godoy & De Lacerda 2015). Animais e plantas nestas áreas são adaptados para viver em um ambiente fisiologicamente estressante, restrita a bandas muito estreitas paralelas à costa às margens dos rios. Esta distribuição em zonas indica que muitos indivíduos vivem a poucos centímetros dos seus limites de tolerância (Lovelock & Ellison 2007). Caso o nível do mar suba devido às mudanças climáticas, as comunidades de manguezais podem migrar para a parte superior do gradiente entre-marés, com as espécies mantendo profundidades, frequências e durações de inundação preferidas (Lovelock & Ellison, 2007). Infelizmente, existem muitas barreiras humanas a essa migração: agricultura, estradas e desenvolvimento urbano. Isso torna a zona entre-marés superior um ecossistema bastante vulnerável (Saintilan & Williams 1999; Gilman et al. 2008). Nesse contexto, com a diminuição da zona entre-marés devido ao aumento do nível do mar, algumas espécies de caranguejos-chama-marés estão sendo comprimidas em áreas de espécies mistas (Backwell, in prep.), o que pode ter implicações para os sistemas sociais e de acasalamento deste grupo. Como as mudanças nas distribuições de espécies também podem alterar a composição da comunidade (Kearney 2006), é importante investigar as interações entre as espécies de caranguejos-chama-maré ao explorar os efeitos de mudanças climáticas (Montoya & Raffaelli 2010).

Baseado nos argumentos anteriores, a tese foi dividida em três capítulos. O estudo do **capítulo 1** foi realizado em manguezais do litoral centro/sul do Estado de São

Paulo, onde investigamos o efeito do sombreamento na distribuição espacial, comportamento e fisiologia de duas espécies de caranguejos-chama-marés: *Leptuca leptodactyla* e *Leptuca urugayensis*. Já o estudo do **capítulo 2** foi concretizado durante período de doutorado sanduíche no exterior, realizado na Austrália. Nele, examinamos o efeito da migração de espécies de áreas adjacentes (*Tubuca elegans* e *Tubuca signata*), relacionados à elevação do nível do mar, sobre o comportamento social e reprodutivo de outra espécie desse grupo de caranguejos, *Austruca mjoebergi*. Ainda durante o período de doutorado sanduíche, realizei o estudo do **capítulo 3** com ênfase em seleção sexual de caranguejos-chama-maré (*Austruca mjoebergi)*, mais relacionado à linha de pesquisa da minha orientadora no exterior, onde examinamos a precisão e o tempo de escolha das fêmeas em relação à velocidade, quantidade e complexidade dos displays dos machos.

Capítulo 1

Shading affects fiddler crabs microhabitat selection and behavior due to physiological limitation

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15   **ABSTRACT**

16   Artificial shading by human-made structures may be prejudicial for many living

17   species. Sunlight shading decreases the solar radiation incidence and heat stress,

18   affecting groups of species in a variety of ways. Here we investigate shading effects on

19   fiddler crabs microhabitat selection, behavior and physiological limitation. Shading

20   influenced their spatial distribution over time; *Leptuca leptodactyla* are more associated

21   to unshaded areas while *Leptuca uruguayensis* to the shaded ones. Furthermore it also

22   altered their behavior, with both species spending less time outside their burrows as the

23   temperature increases, but the second was strongly affected. These effects are related to

24   species physiological limitation (i. e. water loss over time), since they have different

25   responses in thermal tolerance in accordance to their distribution and behavior

26   adjustments. Therefore, artificial shading can negatively affect crabs fitness, since they

27   led to change in the local biodiversity, exposing them to risks when searching for a new

28   territory (e.g. competitors, predators and dehydration) and can influence in primordial

29   activities, such as foraging and mating search.

30   **Keywords:** Dehydration; heat stress; luminous intensity; spatial distribution;

31   thermoregulation

32 **INTRODUCTION**

33   Environmental physical shifts due to anthropogenic activities may be prejudicial

34 for many living species. It is well-known the human pressure that coastal regions have

35 been suffering, where most of the world population lives (Fortes 1988, Nicholls &

36 Small 2003). The direct effects and accompanying losses are easier to detect, such as in

37 mangroves impacts due to land use, which disturbs the whole ecosystem and associated

38 biota (Fortes 1988; Blanco et al. 2012). However, the indirect effects of human-made

39 structures deserve further attention, like the shading by bridges, buildings, or even ports

40 constructions. Sunlight shading decreases the solar radiation incidence and heat stress,

41 affecting species groups in a variety of ways (Quinn et al. 1997, Ruiz & Romero 2001,

42 Nobbs 2003, Kon et al. 2010, Pardal-Souza et al. 2016, Munguia et al. 2017).

43 Understanding their potential harms is essential to comprehend the effects on

44 populations, communities and ecosystems.

45   It was demonstrated by a manipulative approach that artificial shading causes

46 alteration in intertidal benthic communities in different sites and organisms: on a rocky

47 shore benthonic community, shading affected primary producers biomass, primary

48 consumers body size, community structure and recruitment (Pardal-Souza et al. 2016);

49 on mangroves, it reduced microphytobenthos biomass and affected the distribution of

50 benthic epifauna, such as an increased number in shaded areas of gastropods *Pirenella*

51 *cingulata* and *Ovassiminea brevicula*, and the sesarmid crab *Perisesarma indiarum*

52 (Kon et al. 2010); shading also increased the number of sesarmids crabs and fiddler

53 crabs, such as *Tubuca flamula* and *Tubuca signata*, while it decreased the number of

54 *Tubuca elegans* (Nobbs 2003). Therefore, artificial shading can have potential

55 ecological shifts on intertidal organisms from different ecosystems, highlighting the

56 need of understanding and alleviate human pressure over threatened environments.

57   Species behavioral adjustments are important to deal with thermal adverse

58   condition. For example, during the hottest parts of the day, some species of *Sceloporus*

59   lizards needs to retreat into cooler shelters because they can die by overheating. By

60   retreating into refuges, their foraging activities and reproductive behavior are limited,

61   increasing their risk of being extinct (Sinervo et al. 2010). The fiddler crab *Austruca*

62   *mjoebergi* also altered their behavior in this sense, since they remain longer periods

63   foraging and searching for mate in shaded/ cooler microhabitats (Munguia et al. 2017).

64   As thermal tolerance can strongly affect feeding and mating chances, selection should

65   favor individuals that remain longer periods searching mates due to increased chances

66   of reproductive success (Munguia et al. 2017).

67   Changes in species distribution and behavior might be linked to their

68   physiological limitation, especially in intertidal organisms during low tide. Restrictions

69   in thermal tolerances may constrain fiddler crabs to cooler regions for example, such as

70   shaded conditions close to vegetation (Edney 1961, Wilkens & Fingerman 1965, Nobbs

71   2003). On the other hand, it is possible that some species avoid these areas, maybe by

72   using visual signals more frequently than species living in forests (Nobbs 2003).

73   However, it is not well understood how they can deal with sun exposure high

74   temperatures. It is expected that species with lower significant limitations could be less

75   affected.

76   Based on above statements, fiddler crabs are an example of intertidal organisms

77   that potentially are affected by artificial shading. These ecologically significant group

78   plays an essential role due to their bioturbation activities and consequent ecosystem

79   engineering (Jones et al. 1994, Kristensen 2008, Cannicci et al. 2008, Penha-Lopes et al.

80   2009, Kristensen et al. 2012, Citadin et al. 2016, Natálio et al. 2017). During low tide,

81   while courting females on the sediment surface, males faces extreme thermal

conditions, with temperatures frequently exceeding preferred ratios, being lethal in some cases (Allen & Levinton 2014, Darnell et al. 2015). However, temperature does not seem to constrain females' activity (Milner et al. 2010, Darnell et al. 2013). Understanding how artificial shading affects fiddler crabs has broader implications for understanding associated tradeoffs in other intertidal ectotherm organisms.

Given the background, we know of no study that has examined shading effect on the distribution, behavior and physiology of coexisting species. Herein, we used as experimental models fiddler crabs species *Leptuca leptodactyla* and *Leptuca uruguayensis*. We aimed the following questions: (i) How artificial shading affects both species microhabitat selection?; (ii) how temperature affects species behavior?; (iii) do they diverge in physiological limitation?

METHODS

Species and study sites

We use as model the species of fiddler crabs *Leptuca leptodactyla* and *Leptuca uruguayensis* (Note: both species was previously called *Uca leptodactyla* and *Uca uruguayensis*; see Shih et al. 2016 for details). These are small individuals, endemic to the western Atlantic estuaries (Thurman et al. 2013). They are territorial intertidal animals, such as the other species of fiddler crabs. Their territory consists in a central burrow with a small area in the surroundings, where they concentrate their activities (e.g. feeding, fighting with competitors and courting females). The burrow is an important resource, since it can be used as a refuge to protect crabs against predation, dehydration and can be used to mate (Crane 1975). Both species can occur in the same

105   areas, but usually *L. leptodactyla* are associated with open sand banks while *L.*

106   *uruguayensis* remain closer to the vegetation (Masunari 2006).

107   We studied a mixed species population composed mainly by our model species

108   in a non-vegetated muddy-sand bank at Una do Prelado river, in the Ecologic Station

109   Juréia – Itatins (Site A; 24º26'18.11"S, 47º04'20.41"O), south coast of São Paulo State –

110   Brazil. Moreover, we collected *L. leptodactyla* in a non-vegetated muddy-sand bank at

111   Itaguaré river, in the Restinga of Bertioga State Park (Site B; 23°46'42.8"S

112   45°57'46.7"W) and *L. uruguayensis* in a vegetated muddy bank at Jaguareguava river

113   (Site C; 23º48'46.8"S, 46º09'49.3"W), both central coast of São Paulo State – Brazil.

114

115   *Shading effect on species microhabitat selection*

116   The experiment 1 was conducted from August 2014 to September 2015 in Site

117   A, based on Nobbs (2003). It consisted in modulating the luminous intensity in different

118   degrees and to observe its effects in *L. leptodactyla* and *L. uruguayensis* spatial

119   variation over time. For this purpose, we made square structures (60 cm x 60 cm) with

120   pvc pipes (diameter of 3.2 cm) buried in the square vertex, 35 cm depth in the soil, and

121   suspended 25 cm from it. The structures were covered with polyester shade cloth with

122   different mashes size, totalizing 4 treatments: 1- Unshaded; 2- 20% of light absorption;

123   3- 50% of light absorption; 4- 80% of light absorption. Each structure was disposed

124   randomly in the sand bar (~ 2 m distant from each other). There were 7 replicates of

125   each treatment, 28 in total.

126   Crabs were identified and quantified by observation during spring low tide

127   (between 10 to 13 h). We only counted males of our study models, since females from

128   both species are difficult to identify from a certain distance. Each observer had sat

129   without moving for at last 5 min, waiting for the crabs to emerge from their burrows.

130   They were not manipulated in order to avoid interferences in population under the

131   structures through the experiment. In addition, this is a good method for estimating their

132   apparent abundance (Nobbs & McGuinness 1999). Abundance was sampled before (i.e.

133   August 2014), and at 1, 2, 3, 5, 7, 9 and 13 months after the experiment began. As the

134   estuarine zone is very dynamic, some structures have been damaged 5 months after the

135   beginning of the experiment (1 of the 50% of light absorption and 1 of the unshaded

136   treatment) and there was one missing data on the 9th month on 80% of light absorption

137   treatment.

138   Moreover, we measured soil temperature randomly under the structures at 3

139   different days (from October to December 2016) during spring low tide (1-2

140   measurements per day, between 10 to 13 h) with a digital thermometer (0.1°C degree

141   accuracy) with thermosensors wires plugged. We also measured luminous intensity

142   under the structures with a digital luximeter (measuring range 0 to 99999 lux) during

143   the same period (see Table 1 for details of luminous intensity and temperature).

144

145   **Table 1 -** Means ± SD of soil temperature and luminous intensity from the 4 shading structures
146   treatments. Different letter means significant difference between treatments for the same
147   variable (P>0.05; One-Way ANOVA, followed by Fisher LSD test).

Treatment	N	Soil temperature (°C)	Luminous intensity (lux)
0%	33	35.67 ± 3.63 a	93797.58 ± 15880.40 a
20%	25	32.57 ± 3.87 b	77434.80 ± 19040.70 b
50%	29	29.38 ± 2.99 c	41071.17 ± 13005.88 c
80%	35	27.53 ± 2.16 d	16619.27 ± 4395.15 d

148

149   *Behavioral responses*

150   The experiment 2 was conducted between October and December of 2016 in

151   Site A. We observed the behavior of males from both species in relation to the soil

152   surface temperature, comparing the longer distance reached from the burrow and the

153   time spent on soil surface. Therefore, it was 2 treatments: 1- *L. leptodactyla* and 2- *L.*

154   *uruguayensis*. Sample size was 26 for each species, 52 in total.

155   For such, during spring low tide at 3 different days (3-5 measurements per day,

156   between 9 to 14 h), we chose random unshaded areas with both species and placed a

157   tripod with a video camera (Sony DCR SR68) directly above (90^0) to record their

158   behavior for later analysis (we observed 2-3 individuals of each species per video). We

159   measured the temperature by the beginning and the end of each filming (40 min record)

160   with a digital thermometer (0.1°C degree accuracy) with thermosensors wires. Each

161   video had a known scale in order to measure crabs distances. We print screened images

162   from the video at the moment that crabs were on the further position from their burrow,

163   and evaluated the distance using ImageJ (National Institutes of Health, Bethesda, MD,

164   U.S.A.). In relation to the time spent outside the burrows, we randomly observed crabs

165   by the moment they emerged from their burrows until they retreated to it.

166

167   *Species physiological limitation*

168   The experiment 3 was performed in laboratory based on Levinton et al. (2015).

169   It consisted in evaluate and compare the water loss through the time of males from both

170   species from 2 different populations. As major claw length is associated to

171   thermoregulation (Darnell & Munguia 2011), we used it as covariate instead of using

172   initial body mass (covariate used by Levinton et al. 2015), once we did not test females

173   and observed a positively significant relation between those variables for both species

174 (*L. leptodactyla:* β=0.912, $F_{1,37}$=184.059, P=0,001; *L. uruguayensis*: β=0.888 $F_{1, 36}$=

175 135.37, P=0.001). It was 4 treatments in total: 1- *L. leptodactyla* in Site A, 2- *L.*

176 *leptodactyla* in Site B, 3- *L. uruguayensis* in Site A and 4- *L. uruguayensis* in Site C.

177 Sample size was 19 (treatments 1, 3 and 4) and 20 (treatment 2), 77 in total.

178   Crabs were collected manually and kept each species/population in separate

179 buckets (diameter: 35 cm, height: 30 cm) with a 10 cm layer of sediment from

180 respectively site. The laboratory temperature was ~ 28^0C and the photoperiod was 12h

181 of light and 12h of dark. We pick the crabs from each stock population and placed them

182 between 48 to 72 hour in an aquarium (40×24×23cm) with 1 cm deep brackish water, in

183 order to them release their feces (so the loss of weight has no relation to that) and to

184 maintain they hydrated. Later, we cover a new dry aquarium with paper towel where we

185 placed the crabs for one hour to guarantee they were dry. We than weighted the crabs

186 just before the experiment starts with a digital balance (precision of 0.0001 g) and

187 placed them inside an air circulation oven at 30^0C. Crabs were re-weighted at the

188 moment 15, 30, 60, 90 and 120 min after the start of the experiment. Lastly, we

189 measured their carapace width and major claw length (see Table 2 for biometric details).

190

191 **Table 2 -** Means ± SD of carapace width (CW), major claw length (CL) and initial body mass
192 (BM) of males from water loss experiment from respective species and sites. Different letter
193 means significant difference for the same variable between treatments (P>0.05; GLM with Site
194 nested in Species, followed by Fisher LSD test).

Specie	Site	CW (mm)	CL (mm)	BM (g)
L. leptodactyla	Site A	9.24 ± 0.86 b	16.99 ± 2.60 bc	0.33 ± 0.09 b
	Site B	10.21 ± 0.76 a	19.70 ± 2.41 a	0.50 ± 0.11 a
L. uruguayensis	Site A	9.30 ± 0.98 b	15.80 ± 2.37 c	0.33 ± 0.10 b
	Site C	10.53 ± 0.66 a	18.08 ± 1.39 b	0.52 ± 0.09 a

195

Data analysis

197   For the experiment 1, our response variable was the number of *L. leptodactyla*

198 subtracted by the number of *L. uruguayensis*, in order to evaluate the relation between

199 both species over time according to shading gradient. First we tested in each treatment

200 to homocedasticity by Levene test. Once it was confirmed, we used a Repeated Measure

201 Analysis of Variance (ANOVA) comparing the fixed factor Treatment (4 levels:

202 Unshaded, 20% of light absorption; 50% of light absorption and 80% of light

203 absorption) and Time (8 levels: 0, 1, 2, 3, 5, 7, 9 and 13 months from the experiment

204 start). To check the shade effect of the 4 Treatments on soil temperature and luminosity

205 under the structures, we evaluated homocedasticity by Levene or Cochran test and

206 transformed data by log or square root, respectively. Once it was reached, we applied

207 one way ANOVAs comparing the 4 treatments, since the measurements were performed

208 randomly into the structures. We used the post-hoc Fisher LSD test in both analyses.

209   In the case of the experiment 2, we used a Multivariate General Linear model

210 (MGLM) with time spent outside the burrow and longer distance reached from the

211 burrow entrance as dependent variables, soil temperature as covariate and specie as

212 fixed factor. To interpret the multivariate test results, we examined its univariate

213 components. We also ran a linear regression to evaluate the effect of the covariate on

214 each species when it was significant.

215   In the experiment 3, we analyzed data using a General Linear Model (GLM)

216 Repeated Measure. We compared the response variable percentile of water loss between

217 the random factor Site nested in the fixed factor Specie (4 levels: *L. leptodactyla* from

218 Site A and B and *L. uruguayensis* from Site A and C) and Time (5 levels: 15, 30, 60, 90

219 and 120 min), using the post-hoc Fisher LSD test. In addition, we applied a linear

220 regression to evaluate the relation between initial body mas and major claw length. As it

221 was strongly positive related, major claw length was used as a covariate in the model.

222 We ran a linear regression to evaluate the effect of the covariate in each species when

223 significant. In relation to biometric details (carapace width, major claw size and initial

224 body mass) we tested to homocedasticity by Levene or Cochran test. Once it was

225 confirmed, we analyzed data by a GLM with Site nested in Species, followed by the

226 post-hoc Fisher LSD test.

227 Statistical significance was considered when P< 0.05 in all analysis.

228

229 *Ethical note*

230 This study was performed according to Brazilian law and ethics requirements on

231 animal research. We had license to collect animals from Authorization and Information

232 System in Biodiversity (SISBio; protocol number 42907) and to conduct the study into

233 Conservation Unities from Technical Scientific Committee (COTEC; protocol number

234 260108 – 002.036/2014).

235

236 **RESULTS**

237 *Shading effect on species microhabitat selection*

238 A Repeated Measure ANOVA showed a significant interaction between

239 Treatment and Time ($F_{21, 147}=1.736$; P=0.031). There was no difference in crab count

240 between treatments when the experiment started. In, addition, the unshaded treatment

241 had no difference at any period from the beginning to the end of the experiment. The

242 20% and 50% of light absorption had significant higher values in comparison to initial

243 condition only on the 5th month, returning to the same quantities on the following

244   periods, while the 80% of light absorption treatment also had significant higher values

245   than the initial conditions after 5 months and becoming significant lower on the 7th until

246   the end of the experiment (Fig. 1). Comparing between treatments, crab counts were

247   significant greater after 7 months until the end of the experiment for the unshaded

248   treatment, in relation to the 80% of light absorption treatment (Fig. 1). In the same

249   period, the treatments 20% and 50% were the same among themselves, and did not

250   differ from unshaded treatment and 80% of light absorption, except on the 7th month,

251   where the 50% is significant lower than the unshaded treatment (see supplementary

252   material for post-hoc matrices).

253

254   **Figure 1** – Mean number of individuals (*L. leptodactyla* minus *L. uruguayensis*) per

255   experimental plot (0.36 m^2) at each treatment (Unshade; 20% of light absorption; 50% of light

256   absorption; 80% of light absorption) over time. Error bars indicates standard errors (see

257   supplementary material for post-hoc matrices).

258

259   *Behavioral responses*

260   An MGLM showed that species identity affects their behavior ($F_{2, 48}= 12.596$,

261   P=0.001).The effect was driven by differences in the time spent outside the burrows ($F_{1,}$

262    $_{49}= 15.342$, P=0.001) and in the distance travelled ($F_{1, 49}= 16.879$, P=0,001): *L.*

263   *leptodactyla* remained longer outside their burrows and traveled further distances than

264   *L. uruguayensis* (Fig. 2). In addition, temperature affected the time spent outside the

265   burrows, but did not affect the distance traveled ($F_{2, 48}= 21.298$, P= 0.001; time: $F_{1, 49}=$

266   33.989, P= 0.001; distance: $F_{1, 49}= 2.261$, P=0.139). Looking separately at each species,

267   the time spent outside the burrow was negatively significant related to temperature for

268   both species, but stronger to *L. uruguayensis* ($\beta= -0.8$, $F_{1, 24}= 42.803$, P=0.001; Fig. 3)

269   than to *L. leptodactyla* ($\beta= -0.51$, $F_{1, 24}= 8.443$, P=0.008; Fig. 3).

270
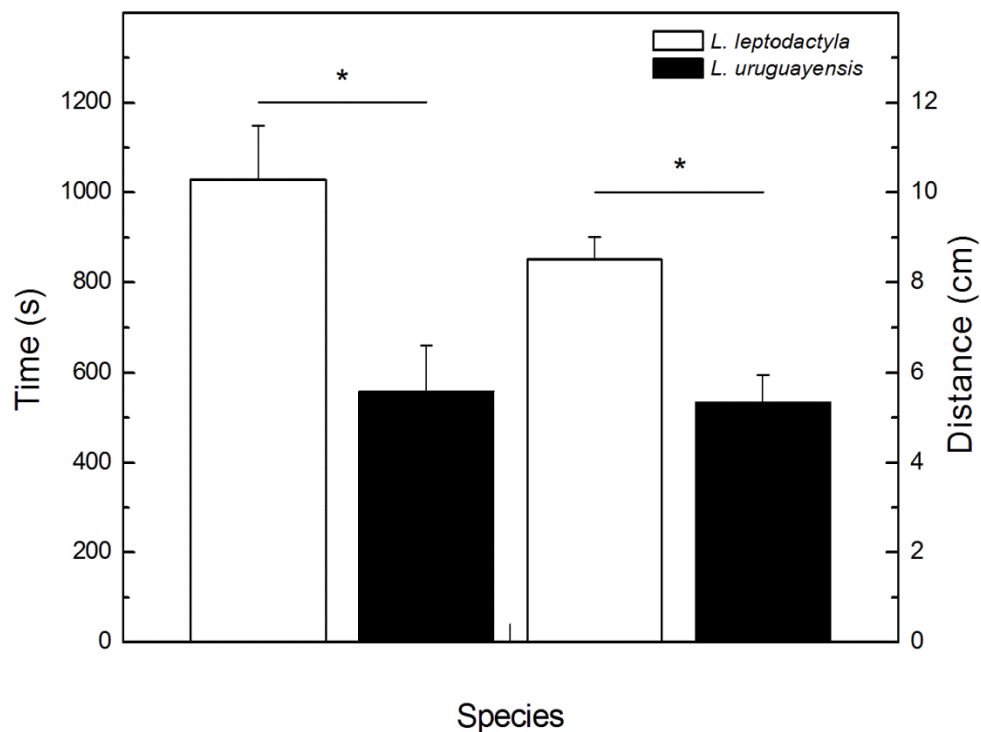
271   **Figure 2** – Mean time spent outside the burrow (left) and distance traveled (right) for *L.*
272   *leptodactyla* (white bars) and *L. uruguayensis* (black bars). Error bars indicates standard errors,
273   while asterisk denotes statistical differences between species (P<0.05).

274
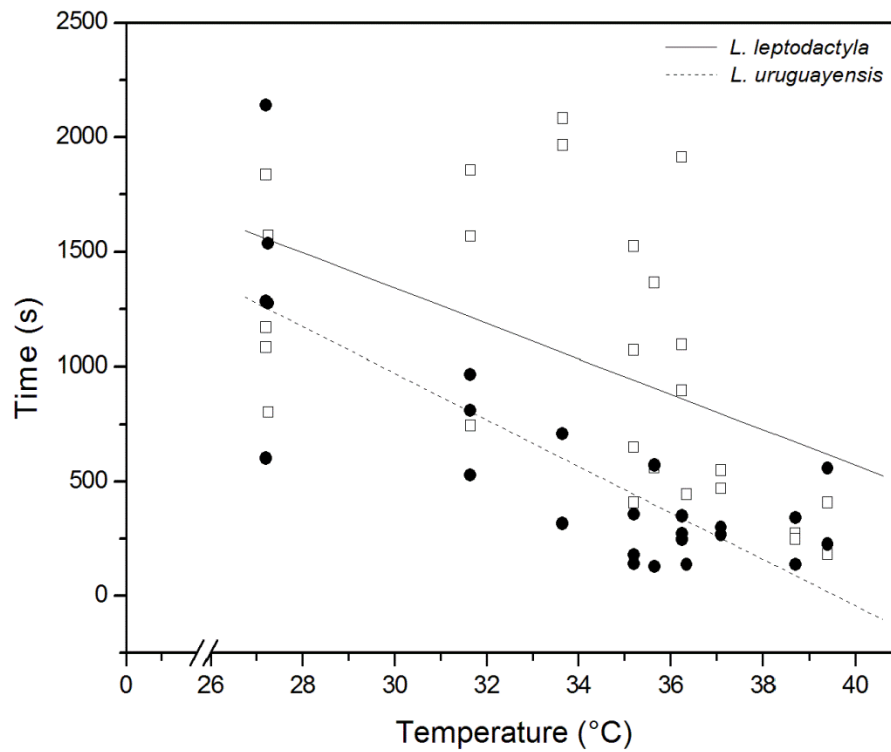
Figure 3 – Time spent outside the burrow of males *L. leptodactyla* (white squares and solid lines) and *L. uruguayensis* (black circles and doted lines) as a function of soil temperature. Lines are least squares fit.

Species physiological limitation

An GLM Repeated Measure showed a significant interaction between Site nested in Specie and Time ($F_{8, 288}$=14.758; P=0.001): *L. leptodactyla* from Site B lost less water on 30 min and the following periods until the end of the experiment, in comparison to *L. uruguayensis* from Site C and both species from Site A, which did not differed from each other at any period (Fig. 4). Furthermore, the major claw length also affected water loss ($F_{1, 72}$=10.374; P=0.002). Looking separately at each species, the claw length was positively significant related to the total water loss (last measure at 120 min) to both species, but stronger to *L. leptodactyla* (β=0.548, $F_{1,37}$=15.875, P=0.001; Fig. 5) than to *L. uruguayensis* (β=0.341, $F_{1, 36}$= 4.754, P=0.036; Fig. 5).

290
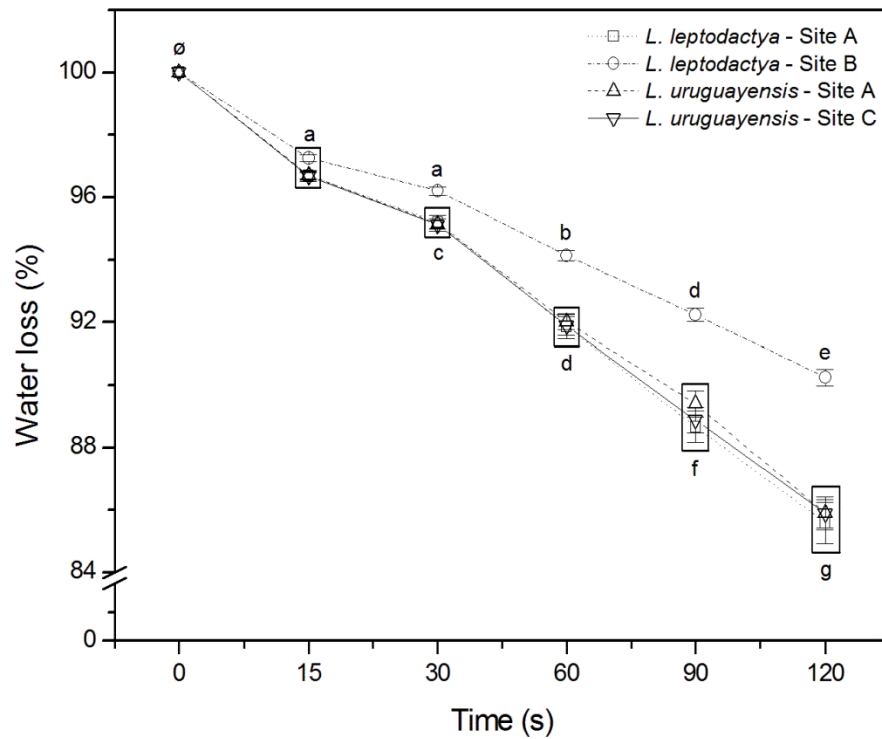
Figure 4 – Pattern of water loss of the two species from different sites over time (*L. leptodactyla* from Sites A and B; *L. uruguayensis* from Sites A and C). Error bars idicates standard error, ø designates that these values were not included in the statistical analyses, boxes indicates similarity between goups, while different letters denotes statistical differences (P<0.05).

296
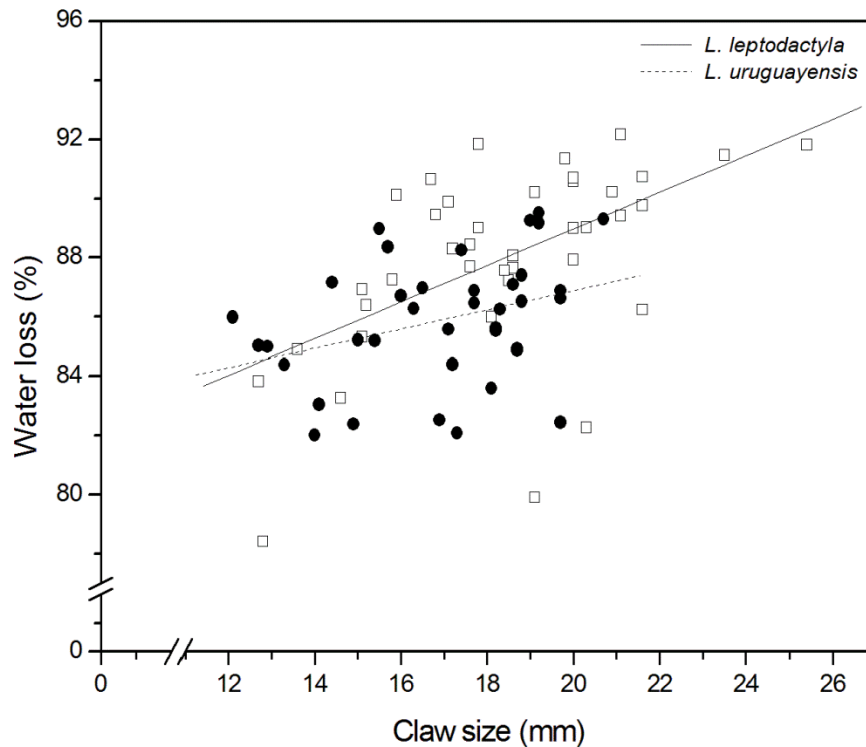
Figure 5 - Water loss after 2 h of drying of males *L. leptodactyla* (white squares and solid lines) and *L. uruguayensis* (black circles and doted lines) as a function of major claw size. Lines are least squares fit.

DISCUSSION

Here, we demonstrate that artificial shading affects microhabitat selection of estuarine intertidal crabs over time. Crabs deal with adverse situation by adjusting their behavior, spending less time outside their burrows as temperature increases. These effects are related to species physiological limitation, since they have different responses in thermal tolerance, in accordance to microhabitat selection and behavior adjustments. Therefore, human-made structures can negatively affect crabs fitness, since they can led to change in the local biodiversity, expose them to risks while searching for a more suitable place and influence in primordial activities, such as foraging and mating search.

312   On the first experiment, our results confirm that there is, in fact, an effect of

313 shading on species microhabitat selection. We used the number of *L. leptodactyla* minus

314 *L. uruguayensis* in each structure as variable response in order to see the relation

315 between both species in the environment. Positive values show that most of the crabs

316 are from the first specie, and as it decreases, increases the number of the second one (so

317 negative values reveals higher quantities *L. uruguayensis*). In this sense, *L. leptodactyla*

318 are more related to unshaded areas while *L. uruguayensis* with shaded ones. This effect

319 occurs only for the shade that absorbs greater amount of light, without marked influence

320 on the treatments of 50% and 20% light absorption. Our findings is in accordance to

321 distribution descriptive approaches for this species, since *L. leptodactyla* are associated

322 to non-vegetated areas, while *L. uruguayensis* to vegetated ones (Masunari 2006,

323 Thurman et al. 2013), but none of them test it by a manipulative approach. Furthermore,

324 it was suggested to other fiddler crabs species (Nobbs 2003) that this effect are indeed

325 related to vegetation shade (as observed in our study) instead of vegetation physical

326 structures (i.e. stems and roots). It is known that mobile species can exploit habitat

327 heterogeneity to find suitable places, reducing exposure to thermal extremes (Munguia

328 et al. 2017). Therefore, this change in spatial distribution can negatively affect mobile

329 species (as fiddler crabs) since they might abandon their own burrows searching for a

330 more appropriate area, spending energy fighting for territories, time that they could be

331 investing in reproduction and becoming more exposed to risks (e.g. predators and

332 dehydration).

333   Behavioral patterns are also affected by shading. As the temperature is a key

334 factor in this sense, our behavioral observation in relation to the temperature instead of

335 shading is valid, once the shade had a strong effect on soil surface temperature (see

336 Table 1). We observed that *L. leptodactyla* stays longer periods outside their burrows

337   and reach further distances than *L. uruguayensis*. Furthermore, for the last species,

338   temperature has stronger negative relationship with time outside the burrows, revealing

339   that *L. uruguayensis* are more sensitive to high temperature condition, what explain in

340   part their association to shaded and cooler areas, while *L. leptodactyla* can tolerate

341   hotter temperatures and habit unshaded places. However, even with higher thermal

342   tolerance of the last specie, the increase in temperature also decreases their activity

343   time. Our finding is in accordance to what described for *A. mjoebergi*, for example

344   (Munguia et al. 2017). This species inhabits high intertidal zone where the mudflat are

345   open unshaded areas interspersed with areas shaded by vegetation. The soil temperature

346   can reach the extreme of about 43^{0}C in unshaded areas, while in shaded temperature are

347   around 35^{0}C by the same time of the day. Crabs are more active in shaded/ cooler

348   conditions, do not needing to retreat to their burrow frequently, increasing their activity

349   time in soil surface. Therefore, the role of thermal tolerance and associated

350   microhabitats can influence opportunities of foraging and searching for a mate, affecting

351   crab fitness and reproductive success.

352   These distributional and behavioral changes found in our study can be explained

353   by species physiological limitation. We observed that the population of *L. leptodactyla*

354   with bigger claws retained greater percentage of water than the same species and both *L.*

355   *uruguayensis* populations. As fiddler crab thermoregulation is related to major claw size

356   (Darnell & Munguia 2011), this factor partially explain our findings, since the

357   population with bigger major claw length lost less water than the other ones. However,

358   not only the claw was important in this sense, but also the specie identity, since the two

359   populations of *L. uruguayensis* lost the same amount of water, irrespective of having

360   different claw length. In addition, both bigger population of *L. leptodactyla* and *L.*

361   *uruguayensis* was similar sized, but the first species have bigger claw regardless of

362   having same carapace size and body mass. Reinforce this findings the fact that *L.*

363   *leptodactyla* has stronger correlation of claw length with water loss than *L.*

364   *uruguayensis*. The natural distribution and water loss pattern of our species corroborates

365   to what described for other fiddler crabs species, since the high-dry-sandy *Leptuca*

366   *pugilator* loses water more slowly than the low-wet-muddy *Minuca pugnax* (Levinton et

367   al. 2015). However, those authors observed water loss rate negatively related to initial

368   mass, without correlating it with males major claw length. We observed that claw length

369   has strong correlation with initial mass to our both model species, what might also be

370   the true to males of *L. pugilator* and *M. pugnax*, reinforcing the major claw length

371   function in thermoregulation.

372   Additionally, others fiddler crabs sexually characters (like the major claw) might

373   be selected by thermoregulation, since it allows males to remain on soil surface for

374   longer periods and thus increasing reproductive success. For example, *L. leptodactyla*

375   constructs biogenic sand structures (hoods) during hottest months of the year (their

376   reproductive period; Masunari 2012), that affects burrows superficial layer temperature

377   (up to 2 cm; Fogo, in prep.), probably permitting males to remain courting longer

378   without often retreating deep into their burrows. Also during the reproductive cycle,

379   their color pattern changes from pale to white (de O. Rodrigues et al. 2016), what might

380   beneficiate them in thermoregulation, as it does for *Leptuca panacea* and *L. pugilator*

381   (Silbiger & Munguia 2008, Munguia et al. 2013, Kronstadt et al. 2013). It is important

382   to notice that sexual ornamentation associated to thermoregulation (like hoods or

383   carapace color change) had not been describe to *L. uruguayensis* in our study sites. A

384   final point in this question is the difference in both species latitudinal distribution

385   pattern (Thurman et al. 2013), since *L. leptodactyla* habits neo-tropical shores of the

386   western Atlantic, while *L. uruguayensis* habits subtropical and temperate along South

387 American coast, corroborating therefore to their water loss capacity and sexually

388 selected ornaments, explaining the behavioral patter in relation to the temperature and

389 the observed change by manipulative approach in species distribution to shaded/ cooler

390 areas.

391   In summary, artificial shading by human-made structures plays an essential role

392 in intertidal species microhabitat selection and behavior. As shading alters substrate

393 temperature, a possible explanation is due to species physiological limitation. Change in

394 distribution and behavior negatively affects species, since they led to change in the local

395 biodiversity, exposing them to risks when searching for a new territory (e.g.

396 competitors, predators and dehydration) and can influence in primordial activities, such

397 as foraging and mating search. Moreover, these structures can impact not only fiddler

398 crabs, but also intertidal organisms in a general manner from others ecosystems. Lastly,

399 our study highlights the importance of behavioral and physiological approach to

400 understand and mitigate ecological aspects related to human pressure over threatened

401 environments.

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510 **Supplementary material**

Fisher LSD post-hoc matrices

	Group	Time	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)	(30)	(31)	(32)
1	80%	0		0,13	0,19	0,78	**0,02**	**0,00**	**0,00**	**0,00**	0,47	1,00	0,73	0,39	**0,00**	0,09	0,23	0,25	0,44	0,50	0,58	0,96	**0,01**	0,15	0,33	0,42	1,00	0,94	0,47	0,40	0,81	0,42	0,42	0,65
2	80%	1	0,13		0,83	0,21	**0,00**	0,10	0,12	0,16	0,54	0,18	0,09	**0,03**	**0,00**	0,73	0,89	0,85	0,53	0,47	0,05	0,15	**0,00**	0,97	0,68	0,56	0,18	0,21	**0,04**	**0,03**	0,11	0,59	**0,03**	0,07
3	80%	2	0,19	0,83		0,30	**0,00**	0,06	0,08	0,10	0,67	0,25	0,13	**0,04**	**0,00**	0,59	0,96	1,00	0,66	0,59	0,08	0,21	**0,00**	0,81	0,83	0,70	0,25	0,28	0,06	0,05	0,16	0,73	0,05	0,11
4	80%	3	0,78	0,21	0,30		**0,01**	**0,00**	**0,01**	**0,01**	0,63	0,81	0,55	0,27	**0,00**	0,15	0,33	0,36	0,61	0,67	0,42	0,76	**0,01**	0,24	0,46	0,57	0,81	0,87	0,33	0,28	0,63	0,57	0,29	0,48
5	80%	5	**0,02**	**0,00**	**0,00**	**0,01**		**0,00**	**0,00**	**0,01**	**0,01**	**0,04**	0,09	**0,00**	**0,00**	**0,00**	0,11	0,10	**0,00**	**0,01**	**0,00**	**0,04**	0,72	**0,00**	0,06	**0,00**	**0,04**	**0,03**	**0,00**	0,22	0,07	**0,00**	0,21	0,11
6	80%	7	**0,00**	0,10	0,06	**0,00**	**0,00**		0,93		**0,04**	**0,01**	**0,00**	**0,00**	**0,00**	0,27	0,11	0,10	**0,03**	**0,03**	**0,00**	**0,00**	**0,00**	0,14	0,06	**0,04**	**0,01**	**0,01**	**0,00**	**0,00**	**0,00**	0,05	**0,00**	**0,00**
7	80%	9	**0,00**	0,12	0,08	**0,01**	**0,00**	0,93		0,88	0,05	**0,00**	**0,00**	**0,00**	**0,00**	0,31	0,13	0,12	**0,04**	**0,03**	**0,00**	**0,00**	**0,00**	0,17	0,07	**0,05**	**0,00**	**0,01**	**0,00**	**0,00**	**0,00**	0,06	**0,00**	**0,00**
8	80%	13	**0,00**	0,16	0,10	**0,01**	**0,01**		0,88		0,06	**0,01**	**0,00**	**0,00**	**0,00**	0,37	0,17	0,15	0,06	0,05	**0,00**	**0,01**	**0,00**	0,21	0,09	0,06	**0,01**	**0,01**	**0,00**	**0,00**	**0,01**	0,08	**0,00**	**0,00**
9	50%	0	0,47	0,54	0,67	0,63	**0,01**	**0,04**	0,05	0,06		0,41	0,22	0,07	**0,00**	0,27	0,05	0,62	0,99	0,93	0,19	0,42	**0,00**	0,49	0,82	0,95	0,47	0,52	0,15	0,12	0,33	0,94	0,13	0,24
10	50%	1	1,00	0,18	0,25	0,81	**0,04**	**0,01**	**0,00**	**0,01**	0,41		0,69	0,33	**0,00**	0,05	0,17	0,19	0,44	0,50	0,39	0,96	0,15	0,15	0,33	0,42	1,00	0,94	0,47	0,40	0,69	0,87	0,13	0,25
11	50%	2	0,73	0,09	0,13	0,55	0,09	**0,00**	**0,00**	**0,00**	0,22	0,69		0,56	**0,00**	**0,02**	0,08	0,09	0,26	0,30	0,85	0,75	**0,03**	0,86	0,78	0,66	0,23	0,05	0,05	**0,04**	0,15	0,25	0,65	0,91
12	50%	3	0,39	**0,03**	**0,04**	0,27	**0,00**	**0,00**	**0,00**	**0,00**	0,07	0,33	0,56		**0,02**	**0,00**	**0,02**	**0,02**	0,10	0,12	0,73	0,40	0,11	0,06	0,83	0,09	0,35	0,06	0,89	0,98	0,54	0,10	0,96	0,69
13	50%	5	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,02**		**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,02**	**0,00**	0,65	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,03**	**0,04**	**0,01**	**0,00**	**0,04**	**0,02**
14	50%	7	0,09	0,73	0,59	0,15	**0,00**	0,27	0,31	0,37	0,27	0,05	**0,02**	**0,00**	**0,00**		0,58	0,54	0,32	0,28	0,02	0,07	**0,00**	0,75	0,44	0,34	0,09	0,11	**0,02**	**0,01**	0,05	0,37	**0,01**	**0,03**
15	50%	9	0,23	0,89	0,96	0,33	0,11	0,11	0,13	0,17	0,05	0,17	0,08	**0,02**	**0,00**	0,58		0,95	0,62	0,56	0,07	0,19	**0,00**	0,86	0,78	0,66	0,50	0,56	0,15	0,13	0,15	0,69	**0,04**	0,10
16	50%	13	0,25	0,85	1,00	0,36	0,10	0,10	0,12	0,15	0,62	0,19	0,09	**0,02**	**0,00**	0,54	0,95		0,66	0,59	0,08	0,21	**0,00**	0,81	0,83	0,70	0,05	0,28	0,06	**0,05**	0,16	0,73	0,05	0,11
17	20%	0	0,44	0,53	0,66	0,61	**0,00**	**0,03**	**0,04**	0,06	0,99	0,44	0,26	0,10	**0,00**	0,32	0,62	0,66		0,91	0,07	0,33	**0,00**	0,62	0,94	0,95	0,44	0,49	0,13	0,10	0,31	0,94	0,11	0,21
18	20%	1	0,50	0,47	0,59	0,67	**0,01**	**0,03**	**0,03**	0,05	0,93	0,50	0,30	0,12	**0,00**	0,28	0,56	0,59	0,91		0,15	0,39	**0,00**	0,36	0,71	0,86	0,50	0,56	0,15	0,13	0,36	0,87	0,13	0,25
19	20%	2	0,58	0,05	0,08	0,42	**0,00**	**0,00**	**0,00**	**0,00**	0,19	0,58	0,85	0,73	**0,02**	**0,02**	0,07	0,08	0,12	0,15		0,55	**0,02**	**0,02**	0,07	0,11	0,58	0,53	0,84	0,75	0,76	0,17	0,77	0,94
20	20%	3	0,96	0,15	0,21	0,76	**0,04**	**0,00**	**0,00**	**0,01**	0,42	0,96	0,75	0,40	**0,00**	0,07	0,19	0,21	0,33	0,39	0,55		**0,00**	0,08	0,22	0,31	0,96	0,89	0,48	0,41	0,84	0,38	0,43	0,67
21	20%	5	**0,01**	**0,00**	**0,00**	**0,01**	0,72	**0,00**	**0,00**	**0,00**	**0,00**	0,15	**0,03**	0,11	0,65	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,02**	**0,00**		**0,00**	0,07	**0,00**	**0,01**	**0,01**	0,08	0,10	**0,02**	**0,00**	0,09	**0,04**
22	20%	7	0,15	0,97	0,81	0,24	**0,00**	0,14	0,17	0,21	0,49	0,15	0,07	**0,02**	**0,00**	0,75	0,86	0,81	0,43	0,36	**0,02**	0,08	**0,00**		0,59	0,46	0,15	0,18	**0,03**	**0,02**	0,09	0,55	**0,02**	0,06
23	20%	9	0,33	0,68	0,83	0,46	**0,00**	0,06	0,07	0,09	0,82	0,33	0,18	0,06	**0,00**	0,44	0,78	0,83	0,80	0,71	0,07	0,22	**0,00**	0,59		0,84	0,33	0,37	0,08	0,07	0,22	0,89	0,07	0,15
24	20%	13	0,42	0,56	0,70	0,57	**0,00**	**0,04**	0,05	0,06	0,95	0,42	0,24	0,09	**0,00**	0,34	0,66	0,70	0,95	0,86	0,11	0,31	**0,00**	0,46	0,84		0,42	0,46	0,12	0,09	0,29	0,98	0,10	0,20
25	0%	0	1,00	0,18	0,25	0,81	**0,04**	**0,01**	**0,01**	**0,01**	0,47	1,00	0,73	0,39	**0,00**	0,09	0,23	0,25	0,44	0,50	0,58	0,96	**0,01**	0,15	0,33	0,42		0,93	0,41	0,34	0,78	0,36	0,36	0,60
26	0%	1	0,94	0,21	0,28	0,87	**0,03**	**0,01**	**0,01**	**0,01**	0,52	0,94	0,67	0,35	**0,00**	0,11	0,26	0,28	0,49	0,56	0,53	0,89	**0,01**	0,18	0,37	0,46	0,93		0,36	0,30	0,71	0,41	0,31	0,54
27	0%	2	0,47	**0,04**	0,06	0,33	0,18	**0,00**	**0,00**	**0,00**	0,15	0,47	0,71	0,89	**0,03**	**0,02**	0,05	0,06	0,13	0,15	0,84	0,48	0,08	**0,03**	0,08	0,12	0,41	0,36		0,90	0,58	0,08	0,93	0,76
28	0%	3	0,40	**0,03**	0,05	0,28	0,22	**0,00**	**0,00**	**0,00**	0,12	0,40	0,63	0,98	**0,04**	**0,01**	**0,04**	**0,05**	0,10	0,13	0,75	0,41	0,10	**0,02**	0,07	0,09	0,34	0,30	0,90		0,50	0,06	0,98	0,67
29	0%	5	0,81	0,11	0,16	0,63	0,07	**0,00**	**0,00**	**0,01**	0,33	0,81	0,91	0,54	**0,01**	0,05	0,15	0,16	0,31	0,36	0,76	0,84	**0,02**	0,09	0,22	0,29	0,78	0,71	0,58	0,50		0,23	0,52	0,81
30	0%	7	0,42	0,59	0,73	0,57	**0,00**	0,05	0,06	0,08	0,94	0,42	0,25	0,10	**0,00**	0,37	0,69	0,73	0,94	0,87	0,17	0,38	**0,00**	0,55	0,89	0,98	0,36	0,41	0,08	0,06	0,23		0,07	0,15
31	0%	9	0,42	**0,03**	0,05	0,29	0,21	**0,00**	**0,00**	**0,00**	0,13	0,42	0,65	0,96	**0,04**	**0,01**	**0,04**	0,05	0,11	0,13	0,77	0,43	0,09	**0,02**	0,07	0,10	0,36	0,31	0,93	0,98	0,52	0,07		0,69
32	0%	13	0,65	0,07	0,11	0,48	0,11	**0,00**	**0,00**	**0,00**	0,24	0,65	0,91	0,69	**0,02**	**0,03**	0,10	0,11	0,21	0,25	0,94	0,67	**0,04**	0,06	0,15	0,20	0,60	0,54	0,76	0,67	0,81	0,15	0,69	

511

1   # Capítulo 2

2   **The cost of living in mixed species populations: a fiddler crab example**

3

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6

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16

17 **ABSTRACT**

18   Rising sea level is reducing the size of the inter-tidal zone in many Australian

19 mangrove forests. This breaks down the normal species distribution patterns of fiddler

20 crabs and there is an increasing number of herospecifics moving from adjacent zones

21 into an area normally occupied by a single species of fiddler crab. Here we examine the

22 interspecific social and sexual interactions that have resulted in this context. We show

23 that male *Austruca mjoebergi* are just as likely to help their small conspecific neighbor

24 fight off an intruder when the intruder is a conspecific or heterospecific male. It appears

25 that keeping a known neighbor is preferable to having *any* new neighbor (even a

26 heterospecific neighbor that would not compete for receptive females) since the costs of

27 renegotiating territory boundaries would be the same whatever the species of the new

28 neighbour. We also show that males court females of their own species just as

29 vigorously as those of two heterospecific species. Courtship is costly, so the time and

30 energy spent courting heterospecific females is wasted: a potentially high cost of living

31 among heterospecifics.

32 **Keywords:** Climate change; coalition; competition; mate choice; sea level rise

33

34 **INTRODUCTION**

35   Climate change is already rapidly and irreversibly altering ecosystems (Hoegh-

36 Guldberg and Bruno, 2010). Inter-tidal communities are especially vulnerable because

37 the plants and animals that live there are adapted to a physiologically stressful

38 environment. Marine zonation, with species restricted to very narrow bands, graphically

39 illustrates that many individuals live within a few centimeters of their tolerance limits

40 (Lovelock and Ellison, 2007). As sea levels rise, mangrove communities migrate

41 landward up the inter-tidal gradient, with species maintaining their preferred depths,

42 frequencies and durations of tidal inundation (Lovelock and Ellison, 2007). Mangroves

43 have historically moved landward in response to rising sea level (Krauss et al., 2008;

44 Lovelock and Ellison, 2007). Unfortunately, there are now many human-induced

45 barriers to migration: agriculture, roads, urban development and steeply sloped levees.

46 This makes the upper inter-tidal zone one of the most vulnerable ecosystems (Gilman et

47 al., 2008; Saintilan and Williams, 1999). In Australia, for example, four well-studied

48 salt flats have already been reduced by rising sea level to 75%, 51%, 33% and 20%

49 respectively of their original size (Gilman et al., 2007).

50   Within mangrove ecosystems, fiddler crabs are arguably among the most

51 ecologically important fauna, playing an essential role due to their bioturbation

52 activities, resulting in ecosystem engineering (Cannicci et al., 2008; Citadin et al., 2016;

53 Jones et al., 1994; Kristensen, 2008; Kristensen et al., 2012; Natálio et al., 2017; Penha-

54 Lopes et al., 2009). Most species live in single-species populations due to specific

55 habitat requirements (Booksmythe et al., 2011). They are vulnerable to climate change

56 effects, particularly those species that live in the upper inter-tidal zones. In this sense,

57 rising sea levels and the concurrent landward migration is already eating away the

58 mangroves and mudflats in Darwin Harbour. Over the past six years, however, two

59 fiddler crab species moving from adjacent zones have gradually but steadily been

60 increasing in number within a previously monospecific population of *Austruca*

61 *mjoebergi* (Backwell, in prep.). *Tubuca elegans* and, to a lesser extent *Tubuca signata*,

62 are now commonly found within the *A. mjoebergi* population. This breakdown in the

63 normal mosaic distribution pattern has potentially far reaching implications for the

64 social and mating systems of these species. Species interactions, especially interference,

65 can have profound effects on the timing and form of sexual signalling, even leading to

66 evolutionary divergence in sexual traits (Martin et al., 1996).

67   *Austruca mjoebergi* is a small fiddler crab that lives in large, high density,

68 mixed-sex populations on inter-tidal mudflats in Northern Australia. Individuals of both

69 sexes aggressively defend their territories from intruders(Booksmythe et al., 2010). The

70 territory consists of a small area of mudflat (approximately 20 cm diameter) with a

71 central burrow. The burrow is used as a water source, heat sink, an escape from

72 predators and tidal inundation, as well as a mating and incubation site. The surface area

73 around the burrow is used as a feeding site and as a courtship site for males attempting

74 to attract females to their burrows for mating (Crane, 1975). Owning a territory is

75 important for all individuals, but particularly so for males since they are unable to mate

76 if they do not have a burrow (although it is possible for them to surface mate, the level

77 of paternity is <6%, Reaney et al. 2012).

78   Male *A. mjoebergi* have been shown to form coalitions in which neighbours help

79 each other to defend their territories from intruders (Backwell and Jennions, 2004): a

80 male will assist his smaller neighbour to fight of an intruder if the intruder is larger than

81 the resident (so the resident is likely to lose the fight) and if the helper is larger than the

82 intruder (so the helper is likely to win the fight). Coalitions are likely to be due to by-

83 product mutualism: the helper pays to retain an established neighbor and the neighbor

84   keeps his territory (Backwell and Jennions, 2004). Coalition formation also occurs in *T.*

85   *elegans*: males helped their conspecific neighbours in 50% of the attacks; they were

86   significantly less likely to help a heterospecific (*A. mjoebergi*) neighbor, doing so in

87   only 15% of attacks (Booksmythe et al., 2010).

88   In *A. mjoebergi*, mating occurs over a five day period every two weeks (a semi-

89   lunar cycle). Receptive females leave their territories and wander through the population

90   of courting males, visiting several males before selecting a mate (see Reaney &

91   Backwell 2007). Courting males wave their enlarged claw in a species-specific wave

92   pattern to attract the females (Crane, 1975). Females select their mates based on

93   numerous criteria including claw size, wave rate, the production of temporally leading

94   waves, male size and, ultimately, burrow quality (see Reaney & Backwell 2007; Kahn

95   et al. 2014 and refs therein). When a female selects a male, she remains in his burrow,

96   the male enters the burrow and seals the pair in. Mating takes place in the burrow within

97   a few hours and the male then guards the female for 1-5 days, until she extrudes her

98   eggs onto her pleopods (after which she can no longer re-mate). The males leaves and

99   fights for a new territory; the female remains underground for approximately 18 days,

100   before releasing her larvae during a nocturnal spring tide.

101   Waving is energetically expensive (Matsumasa and Murai, 2005) and

102   indiscriminate courtship would impose substantial costs for *A. mjoebergi* males

103   (Booksmythe et al., 2011). Time spent courting heterospecifics would reduce a male's

104   opportunity to attract conspecific females. Under natural conditions, males encounter

105   females sequentially (Reading and Backwell, 2007). Earlier work has shown that, when

106   a conspecific or a heterospecific (*T. elegans*) female is released in the population (one at

107   a time), nearly every male they passed waved at them (Booksmythe et al., 2011). When

108   presented with a conspecific and heterospecific (*T. elegans*) female simultaneously,

109　males gave significantly more waves at conspecific females (Booksmythe et al., 2011).

110　We do not know, however, whether males adjust wave rates at conspecific or

111　heterospecific females or take greater risks by moving further away from their burrows

112　when courting them.

113　　　　This study aims to examine the effect of mixed-species populations on the

114　behavior and mating success of *A. mjoebergi*. We specifically want to understand (i)

115　whether a male *A. mjoebergi* is as likely to assist his neighbour when it is attacked by a

116　conspecific or a heterospecific (either *T. elegans* or *T. signata*) male; and (ii) whether a

117　male *A. mjoebergi* alters waves rate or travel greater distances from their burrows when

118　courting a conspecific or heterospecific (either *T. elegans* or *T. signata*) female.

119

120　**METHODS**

121　　　　The study was conducted at East Point Reserve, Darwin, Australia

122　(12°24'31.89"S 130°49'49.12"E) from September to December 2015. Fiddler crabs

123　(Note: *Austruca mjoebergi* was previously called *Uca mjoebergi*; *Tubuca elegans* was

124　previously *Uca elegans; Tubuca signata* was previously *Uca signata*; see Shih et al.

125　2016 for details) were captured randomly in the population with the use of shovels. All

126　individuals that were hold until the use on experiments were housed individually in a

127　cup containing 0.2 cm deep sea water in a shaded area to prevent them from

128　overheating.

129

130　*Cooperation*

131　　　　We captured males of the three species (*A. mjoebergi, T. elegans and T. signata*)

132　from a distant part of the population and tethered them (a 3cm length of cotton thread

133　superglued to their carapace and tied to a 3cm long nail). We selected a pair of naturally

134 occurring *A. mjoebergi* males where one male was considerably larger than the other

135 and measured the distance between their burrows. We placed a tethered male

136 (randomizing the species of intruder between trials) 3cm away from the smaller

137 resident, on the opposite side of the burrow to the larger resident. The tethered male was

138 always intermediate in size between the two resident *A. mjoebergi* males. The tethered

139 male could approach the burrow entrance but not enter the burrow of the smaller

140 resident. The placement of the 'intruder' on the opposite side of the burrow to the larger

141 resident ensured that the larger *A. mjoebergi* male was not fighting the 'intruder' in

142 order to defend his own territory (see Backwell & Jennions 2004). We scored helping

143 behavior when the larger resident fought (physical contact between the claws of the

144 'helper' and 'intruder') the tethered intruder within five minutes of all males being

145 surface active. After each trial, we captured and measured the claw length of all three

146 males. Each female and male was used in only one trial. We did not use males with

147 regenerated claws. Males intruders sample sizes were: N=14 to *A. mjoebergi*; N=19 to

148 *T. elegans*; and N=18 to *T. signata*.

149

150 *Courtship*

151 We collected females of the three species and tethered them (a 3cm length of

152 cotton thread superglued to their carapace and tied to a 3cm long nail). We placed the

153 nail 20 cm in front of an *A. mjoebergi* male burrow (this is the distance at which

154 females make their choice: Callander et al. 2011). After the male emerged from his

155 burrow, we counted the number of waved directed at the female and evaluate the

156 distance the male moved towards the female while courting her within five minutes the

157 male was surface active or until he touched the female with his legs. So we evaluate

158 wave rate as the number of waving per time of courting. We randomized the order of

159   presentation of females between trials. After each trial, we captured and measured the

160   male and female. Each female and male was used in only one trial. We did not use

161   males with regenerated claws. Presented females sample sizes were: N= 20 to *A.*

162   *mjoebergi*; N=10 to *T. elegans*; and N= 14 to *T. signata*.

163

164   *Data analyses*

165   We used one way ANOVAs to examine the differences between the three

166   species in the cooperation trials (distance between male burrows; claw lengths of helper,

167   intruder and resident males; size difference between helper and intruder and between

168   intruder and resident males) and the courtship trials (wave rate; distance travelled

169   towards the female; female carapace widths; male claw lengths). In addition, we used a

170   Likelihood ratio test to determine whether there was a difference in the number of times

171   a male helped his neighbour when the intruder was an *A. mjoebergi*, a *T. elegans* or a *T.*

172   *signata* male and to evaluate if there was difference between the 3 species in the number

173   of females were touched by males. All these variables were homoscedastic (Levene

174   test). The analyses were performed in SPSS version 23.0 (SPSS Inc., Armonk, NY,

175   U.S.A.). The significance level was set at $\alpha < 0.05$.

176

177   *Ethical note*

178   No ethics permit was required for this study. We limited the handling and the

179   amount of time each crab was used as much as possible. No crab was injured during the

180   research, and they all continued their regular activities after release. The tethered crabs

181   were released after the cotton thread had been cut as short as possible (such that the only

182   thread remaining was fully glued to the carapace and would be lost at the next moult).

183   The work was conducted under a research permit from the Darwin City Council (permit

184   no. 2322876).

185

186   **RESULTS**

187   *Cooperation*

188   The size of the intruder, helper and resident did not differ between the three

189   trials type (when the intruder was an *A. mjoebergi* male, a *T. elegans* male, or a *T.*

190   *signata* male). Neither the size difference between the helper and intruder, nor the size

191   difference between the intruder and the resident male differed between the three trial

192   types. The distance between the burrows of the helper and resident males also did not

193   differ between the three trial types (see Table 1). The three types of trials are therefore

194   directly comparable.

195   When the intruding male was a conspecific *A. mjoebergi* male, the neighbor

196   helped a resident in 8 of the 14 trials (57%). When the intruder was a heterospecific *T.*

197   *elegans* male, the neighbor helped in 5 of the 19 trials (26%). When the intruder was a

198   heterospecific *T. signata* male, the neighbor helped in 6 of the 18 trials (33%). A

199   likelihood ratio test showed that there was no difference in the likelihood of help

200   between the three trial types (LR χ^2 = 3.41, df = 2, P = 0.18).

201

202 **Table 3 -** Distance between burrows (cm), male sizes (mm) and size differences for the three
203 species of intruder (mm). Data is presented as sample size (N), mean ($\bar{x}$), standard deviation of
204 the mean (sd), degrees of freedom (df), F statistic from a one-way ANOVA (F), and probability
205 (P).

	Intruder	N	$\bar{x}$	Sd	Df	F	P
Distance	*A. mjoebergi*	14	15.36	2.81	2,48	0.93	0.4
between	*T. elegans*	19	16.71	2.73			
burrows	*T. signata*	18	16.04	2.96			
Intruder	*A. mjoebergi*	14	15.56	1.83	2,48	2.25	0.12
claw	*T. elegans*	19	14.88	1.93			
length	*T. signata*	18	14.14	1.9			
Helper	*A. mjoebergi*	14	18.94	1.83	2,48	2.33	0.11
claw	*T. elegans*	19	19.77	1.91			
length	*T. signata*	18	18.47	1.83			
Resident	*A. mjoebergi*	14	12.5	2.48	2,48	0.69	0.5
claw	*T. elegans*	19	12.91	2.21			
length	*T. signata*	18	12.09	1.61			
Size diff	*A. mjoebergi*	14	3.37	2.06	2,48	2.04	1.41
helper-	*T. elegans*	19	4.89	2.23			
intruder	*T. signata*	18	4.33	2.12			
Size diff	*A. mjoebergi*	14	3.06	1.73	2,48	2.71	0.08
intruder-	*T. elegans*	19	1.97	1.3			
resident	*T. signata*	18	2.04	1.37			

206

207

208   *Courtship*

209   Neither the size of the waving male nor the tethered female differed between the

210   three types of trials (when the specie of presented female was *A. mjoebergi*, *T. elegans*,

211   or *T. signata*, see Table 2). The three types of trials are therefore directly comparable.

212   The wave rate did not differ between the three trial types and males moved an

213   equivalent distance towards the female in all three trial types (Table 2).

214   When the presented female was a conspecific *A. mjoebergi*, the male touched

215   her with his legs in 13 of the 20 trials (65%). When the female was a heterospecific *T.*

216   *elegans*, the male touched her in 4 of the 10 trials (40%). When the female was a

217   heterospecific *T. signata*, the male touched her in 7 of the 14 trials (50%). A likelihood

218   ratio test showed that there was no difference in the likelihood of touching females

219   between the three trial types (LR $\chi^2 = 0.56$, df = 2, P = 0.76).

220

Table 4 - The wave rate (waves/ second), distance moved towards female (cm), male claw length (mm) and female carapace width (mm) for trials with the three species of intruder. Data is presented as sample size (N), mean ($\bar{x}$), standard deviation of the mean (sd), degrees of freedom (df), F statistic from a one-way ANOVA (F), and probability (P).

	Intruder	N	$\bar{x}$	Sd	df	F	P
Wave rate	*A. mjoebergi*	20	0.22	0.07	2,41	0.04	0.97
	T. elegans	10	0.21	0.07			
	T. signata	14	0.21	0.06			
Distance moved towards female	*A. mjoebergi*	20	16.25	4.48	2,41	1.26	0.29
	T. elegans	10	14.50	5.38			
	T. signata	14	16.25	4.04			
Male claw length	*A. mjoebergi*	20	17.28	3.23	2,41	1.36	0.27
	T. elegans	10	18.90	2.13			
	T. signata	14	18.40	2.42			
Female carapace width	*A. mjoebergi*	20	9.59	0.83	2,41	2.98	0.06
	T. elegans	10	9.96	1.36			
	T. signata	14	8.96	1.04			

DISCUSSION

Our study indicated that can be costly to fiddler crabs living in mixed species areas, with implications for the social and mating systems of this group. The identity of intruder specie did not affect male coalition, probably because keeping a known neighbor is preferable than renegotiating territory boundaries with a new one, irrespective of the specie. Furthermore, the identity of female specie did not affect

232   males courting behaviors, making them waste time and energy being exposed to risks

233   courting females that they will not mate.

234   Males of *A. mjoebergi* are just as likely to help their small conspecific

235   neighbours fight off intruders when the intruders are conspecific or heterospecific

236   males. When the intruder is intermediate in size between the small resident he is

237   attacking and it's larger neighbor (see Backwell & Jennions 2004), then it is irrelevant

238   whether the intruder is a conspecific or a heterospecific male. However, *A. mjoebergi* is

239   smaller than these two heterospecific species: the average size of *A. mjoebergi* males is

240   17.8 ± 0.12 mm claw length (Morrell et al., 2005); *T. elegans* have a mean claw length

241   of 21.07 ± 0.31 mm (Booksmythe et al., 2010); and *T. signata* have claws that are 21.0

242   ± 0.31 mm long (Booksmythe et al., 2008). This difference in size means that there

243   would be fewer occasions when a heterospecific intruder had a size intermediate

244   between a small and a large *A. mjoebergi,* so fewer occasions when helping behaviour

245   would occur (in comparison to intrusions by conspecific males). However, when the

246   required size patterns holds, it appears that *A. mjoebergi* males are prepared to pay a

247   cost for keeping their small conspecific neighbor. The opposite would be expected once

248   it is possible that a heterospecific neighbor would be preferable to a conspecific since

249   they would not compete for the attention of mate-searching females. Alternatively, it is

250   probable that keeping a known neighbor is preferable to having *any* new neighbor since

251   the costs of renegotiating territory boundaries would be the same whatever the species

252   of the new neighbor.

253   *Austruca mjoebergi* males are also non-discriminatory in their courtship of

254   females: they wave at the same rate and move an equivalent distance away from their

255   burrows when courting conspecific and heterospecific females. They also are just as

256   likely to touch with the legs conspecific or heterospecific females. Why do males not

discriminate between females of different species? The most obvious answer is that they are unable to recognize conspecific females. We know, however, that this is not true: when presented with a conspecific and heterospecific female simultaneously (an event that is unlikely to occur naturally), males waved faster and for longer periods of time to the conspecific female (Booksmythe et al., 2011). From this, we know that they can comparatively differentiate between conspecific and heterospecific females. We suggest that males court indiscriminately because the operational sex ratio is very male biased (45:1; Reading & Backwell 2007). The low probability of encountering a receptive female may favor indiscriminate courtship since the lost time and energy of courting a heterospecific female may be outweighed by the chance of not courting a conspecific female (see Booksmythe et al. 2011).

Changes in species behavior can potentially affect their distributions and thus alter community composition (Kearney, 2006). It is important to understand the behavioural interactions between species that are forced to coexist due to habitat loss, particularly due to the effects of sea level rise (Montoya and Raffaelli, 2010). Understanding the effect of sea-lever rise and the consequent overlap of species can give us a greater understanding of the potential outcomes from unabated climate change. We concluded that fiddler crabs pays a high cost by environmental shifts associated with habitat loss due to rising sea level, and this has far reaching implications for the social and mating systems of this group. More behavioral studies in the field with hypothetically affected species are needed in order to better comprehend this question.

287

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379

1 **Capítulo 3**

2 **Faster male displays and less complex choice are more attractive to female fiddler**

3 **crabs as they reduce search costs**

4

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17

18 **ABSTRACT**

19 Females that quickly and accurately locate and assess males can reduce their risks of

20 predation, dehydration and heat stress while mate searching. Here we measured the

21 accuracy and time it took female fiddler crabs, *Uca mjoebergi,* to approach robotic

22 claws that simulated males' courtship signals. We ran six experiments: three one-choice

23 experiments varying in waving display rate (fast, medium and slow) and three three-

24 choice experiments with increased number of displays (all with fast wave rate) and

25 complexity (each one at the three different rates; and the three different rates presented

26 at different distances, with the fast wave rate further from the female and the slow wave

27 rate closer to the female). Females approached all waving robots with an accuracy of 9–

28 18°. They approached faster-waving claws more quickly even when they were

29 presented in sets of three claws, but it took females longer to approach a claw in the

30 more complex situation, with claws waving at different rates and distances. Females

31 may approach waving claws more rapidly simply because they present a more

32 continuous and less ambiguous stimulus. The results suggest that high signalling rates

33 may attract females because they reduce female search costs, and they may or may not

34 additionally signal male quality.

35

36 *Keywords*: accuracy; mate choice; robotic crab; sexual selection; waving display

37

INTRODUCTION

Mate choice is expensive for females. Any increase in searching time will further increase her costs (energy, predation risk, dehydration, overheating). If a male's signal is difficult to localize, a female would take a more circuitous path to the signaller and spend more time exposed to risks. It is not surprising, therefore, that many animals are able to localize signals with great precision. Females usually follow a zigzag path towards displaying males, and the average error of each movement from the target axis is used to estimate the precision of approach. Most female anurans have an approach error angle of 16–23°, but one species has an accuracy of 1° (Rheinlaender et al., 1979; Shen et al., 2008; Ursprung et al., 2009); crickets have an accuracy of 10−14° (Schöneich & Hedwig, 2010); a fly was shown to have an accuracy of 1−2° (Mason et al., 2001).

By making his signal stand out, a male can make himself more detectable and more locatable, and this can attract more females (Mowles & Ord, 2012; Ryan & Cummings, 2005; Wilson & Mennill, 2011). In many species, the same male traits that increase the conspicuousness or locatability of a signaller may also signal his quality or act as a handicap (Mowles & Ord, 2012; Ryan & Cummings, 2005). High signalling rate is one example: it is expensive for males to signal at a high rate (time, energy and predation risk), so display rate is often considered to signal male quality or act as a handicap (Mowles & Ord, 2012; Ryan & Cummings, 2005). In field crickets, males that signal more rapidly accumulate greater energetic costs, and it was suggested that females select mates based on their ability to bear these costs (Mowles, 2014). In chickadees, a slow display rate prevented females from locating the stimulus, and a high display rate caused females to approach the speakers more quickly (Wilson & Mennill, 2011). By increasing the display rate when a female is detected, a male may make

himself more visible and more locatable. In a fiddler crab, for example, males increase their wave rate when they detect wandering females (or when they detect the increased wave rate of other males that have seen a female); this increases their conspicuousness and consequently elevates their likelihood of being approached by the female (Milner et al., 2010). If the signal functions entirely to facilitate male localization, it would still be energetically expensive and males would still succumb to the energetic costs, but females would approach the more rapidly waving males simply because they are more locatable. There may or may not be additional benefits (e.g. females that mate with more easily detected males may produce sons that are also more easily detected), meaning that the locatability of a signal and its possible role in mate assessment may be closely linked.

Enhanced locatability of complex over simple calls has been suggested as a potential reason why females strongly prefer complex calls in the túngara frog, *Physalaemus pustulosis* (Bonachea & Ryan, 2011). It was shown that females chose more quickly when presented with complex calls than when listening to simple calls, but the accuracy of approach was no different between simple and complex calls (Bonachea & Ryan, 2011). Female tree frogs were also found to approach complex three-component and simpler one-component calls with equal accuracy (Rheinlaender et al., 1979). In the leaf-folding frog, *Afrixalus delicatus,* neither the approach accuracy nor the time to reach the signal were affected by call complexity or number of males present (Backwell & Passmore, 1991).

All the above studies were on acoustically communicating species. This probably reflects the ease with which sound signals can be manipulated and phonotaxis experiments conducted. Visual signals are more difficult since they often require the use of robotic models or video presentations of courtship displays. We know of no study

88   that has examined the accuracy of mate attraction to visual/movement-based signals that

89   differ in signalling rate, signal complexity or the number of signallers present. Here we

90   use robotics to examine the accuracy and speed of female approaches to signals in the

91   movement-based courtship of a fiddler crab. We test the effect of display rate and

92   choice complexity on the accuracy and speed of female approach. We specifically ask

93   whether the accuracy or duration of female approaches are affected by (1) wave rate, (2)

94   the number of waving claws or (3) the complexity of the choice context (variation in

95   signals and distances).

96

97   **METHODS**

98   We studied a population of the fiddler crab *Uca mjoebergi* from September to

99   December 2015 at East Point Reserve, Darwin, Australia (12°24'31.89"S,

100   130°49'49.12"E). *Uca mjoebergi* is a small fiddler crab (mean ± SD carapace width

101   =10.16 ± 1.43 mm; $N = 200$) that occurs on the northern coast of Australia. Both males

102   and females defend territories within a large, mixed-sex population. A territory consists

103   of a small area of sediment surface with a central burrow. Males court females from the

104   surface around their burrow by waving their enlarged claw. When a female is ready to

105   mate, she will leave her territory and move through the population of waving males.

106   Males form small clusters (2–6) around the female and, as she moves, males join in or

107   drop out of the cluster. The female visits one of the males in the cluster by walking

108   directly towards him and briefly entering his burrow. She then either leaves the male to

109   continue searching, or she accepts the male and remains underground in his burrow. The

110   chosen male enters the burrow and plugs its entrance with sand; mating occurs within 1

111   h. The male remains underground with the female, guarding her until she extrudes her

112   eggs onto her pleopods 1–5 days later. The female is then unable to remate, and the

113   male leaves, resealing her in the burrow.

114   Female preferences were tested using custom-built robotic crabs consisting of a

115   twin-cam motor that moved a small metal arm in a motion exactly mimicking the

116   courtship wave of the species. The motor is remotely controlled to regulate the exact

117   timing of each wave using custom-designed software (for further details of the robotic

118   crabs, see Booksmythe et al., 2008; Holman et al., 2014; Reaney et al., 2008). The

119   motor was buried under the testing arena with only the metal arm protruding through

120   the arena floor. The arm had a plaster replica of *U. mjoebergi* claw attached to it. For all

121   trials, we used replicas of the same claw, each measuring 24 mm and painted a yellow

122   that matched the natural claw colour of this species (for details of the claw and paint

123   colour, see Detto et al., 2006). The choice arena was a cleared area of mudflat that was

124   levelled to provide a uniform surface. We placed a video camera (Sony DCR-SR65E)

125   directly above the centre of the arena so that we could film an area of 45×45 cm of the

126   choice arena.

127   Mate-searching females were captured as they wandered through the population

128   of courting males. We housed them individually in shaded cups containing 0.2 cm deep

129   sea water until we used them in the choice trials. For each trial, the female was placed at

130   the release point on one end of the test arena, in a small translucent cup that was

131   remotely lifted once the female had seen three waves of the robotic crabs (for more

132   details, see Booksmythe et al., 2008; Reaney, 2009). A positive response was scored

133   when the female touched (or approached to within 5 cm) a robotic crab arm. Trials were

134   discarded if the female darted, ran to the edge of the area, or remained stationary for >3

135   min. Each female was retested up to a maximum of three times (each in a different

136   experiment and in a random order), but females were never tested in the same trial more

137   than once. Females were released after they were tested so they could continue mate

138 searching. Females naturally visit numerous males so it is not unreasonable to test them

139 in multiple trials.

140 We tested 20 females in each of six experiments, and filmed each trial. We

141 analysed the videos using ImageJ (National Institutes of Health, Bethesda, MD,

142 U.S.A.). Trial durations were measured as the time from female release (lifting the

143 translucent cup) until the female reached the robotic claw. We calculated the error

144 angle of each trial by dividing the area between the release point and the robotic crabs

145 into four sections, each 5 cm long. Then, we marked the female's position as she

146 crossed each line and calculated the angle as the difference between (1) the line joining

147 the female with the robotic claw (target axis) and (2) the line joining the female at

148 position n with her position at $n + 1$ (the jump axis between successive lines; Fig. 1).

149 This resulted in three error angles (Fig. 1). To make the readings comparable to

150 published work (Murphey & Zaretsky, 1972; Rheinlaender et al., 1979), we summed the

151 three error angles and divided the total by four (since the final error angle is necessarily

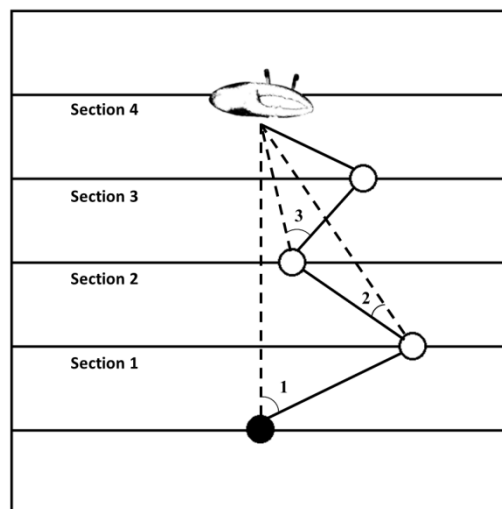152 zero). This gave the 'accuracy' score for each female.

153

154 **Figure 6.** Experimental design with robotic crab (male claw) and female positions (the black

155 circle is the release point and the white circles are the positions when she crossed the lines that

156 divided the arena into four sections of 5 cm). Error angles (1, 2 and 3) were calculated from the

157 angle between (1) a straight line from the female starting position to the robotic crab (dotted

158   connecting lines) and (2) the line between the female starting position and the female position at

159   the next line (solid connecting lines).

160

161   *Wave Rate Experiments*

162   We ran three one-choice experiments to determine the effect of wave rate on the

163   duration and accuracy of female approaches. In all three, the female was released 20 cm

164   away from the robotic crab. For the fast wave rate experiment (F), the claw waved at

165   16.8 waves/min. For the medium wave rate experiment (M), the claw waved at 8.4

166   waves/min, and for the slow wave rate experiment (S), the claw waved at 4.2

167   waves/min. We tested 20 females in each experiment.

168

169   *Choice Complexity Experiments*

170   We ran three three-choice experiments with increasing complexity to determine

171   their effect on the duration and accuracy of female approaches. In the least complex

172   experiment (FFF), we presented the female with three robotic claws in an arc 20 cm in

173   front of and directly facing her. All three waved at the fast rate (16.8 waves/min). In the

174   mid-level complexity experiment (FMS), we presented the female with three robotic

175   claws in an arc 20 cm in front of and directly facing her. One claw waved at the fast rate

176   (16.8 waves/min), one at the medium rate (8. 4 waves/min) and one at the slow rate (4.2

177   waves/min). The most complex trial (FMSdd) was the same as the previous trial but

178   with the stimuli presented at different distances. The fast-waving claw was placed 22

179   cm away from the female release point; the medium-waving claw was placed at 20 cm;

180   and the slow-waving claw was placed at 18 cm. We tested 20 females in each

181   experiment.

182   In the three-choice trials, we measured the duration and accuracy of the female

183 approach to her chosen robotic male. In most cases, the female approached the fastest

184 waving male, but in 7/60 trials the female approached either the medium or slow

185 waving robot.

186

187 *Statistical Analysis*

188   We analysed the first three (one-choice) trials using a multivariate general linear

189 model (MGLM) with trial duration and accuracy as the dependent variables, female

190 carapace width as a covariate and experiment type (F, M or S) as a fixed factor. The

191 standardized residuals were normally distributed and the scatterplot of predicted values

192 against residuals had a shotgun pattern. To interpret the multivariate test results, we

193 examined its univariate components (duration and error angle).

194   We examined the effect of 'number of waving claws' by comparing the trial

195 duration and accuracy between the fast-wave-rate one-choice trial and the fast-wave-

196 rate three-choice trial (F versus FFF). We used an MGLM as above.

197   We analysed the last three experiments (three-choice trials) in the same way.

198 Since the females did not always select the fast waving male, we calculated the

199 difference in duration and accuracy in the three-choice trial with the associated mean

200 duration and accuracy for the one-choice trials. If the female approached the slow

201 waving robot in the three-choice trial, we subtracted the mean duration and accuracy

202 score of the one-choice 'slow' trials from her duration and accuracy scores in the three-

203 choice trial. If the female approached the fast waving robot in the three-choice trial, we

204 subtracted the mean duration and accuracy score of the one-choice 'fast' trials from her

205 duration and accuracy scores in the three-choice trial. If the female approached the

206 medium waving robot in the three-choice trial, we subtracted the mean duration and

207 accuracy score of the one-choice 'medium' trials from her duration and accuracy scores

208 in the three-choice trial. This gave us a measure of the change in duration and accuracy

209 between the simple one-choice trial and the more complex three-choice trial. In the

210 trials where claws were presented at different distances (FMSdd), all females selected

211 the fast-waving claw and it was presented at 22 cm away from the female (2 cm further

212 than in all other trials).

213 We analysed 'difference in duration and accuracy' for the three complex trials

214 using a multivariate general linear model (MGLM) with duration difference and

215 accuracy difference as the dependent variables, female carapace width as a covariate

216 and experiment type (FFF, FMS or FMSdd) as a fixed factor. The standardized residuals

217 were normally distributed and the scatterplot of predicted values against residuals had a

218 shotgun pattern. To interpret the multivariate test results, we examined its univariate

219 components (duration and accuracy).

220 Statistical analyses were conducted in SPSS version 23.0 (SPSS Inc., Armonk,

221 NY, U.S.A.) and the alpha level was set at $P = 0.05$.

222

223 *Ethical Note*

224 No ethics permit was required for this study. We limited the handling and the

225 amount of time each crab was used as much as possible. No crab was injured during the

226 research, and they all continued their regular activities after release. The work was

227 conducted under a research permit from the Darwin City Council (permit no. 2322876).

228

229 **RESULTS**

230 *Effect of Wave Rate on Accuracy and Duration*

231 An MGLM showed that female size did not affect the accuracy or duration of

232   approach (Hotelling's trace: $F_{2,55} = 0.31$, $P = 0.73$; see Table 1 for female sizes). Wave

233   rate did, however, have an effect (Hotelling's trace: $F_{4,108} = 7.20$, $P < 0.001$; see Table 1

234   for descriptive statistics). The effect was driven by differences in the approach durations

235   (univariate effects: duration: $F_{2,56} = 14.14$, $P < 0.001$; accuracy: $F_{2,56} = 0.56$, $P = 0.58$).

236   Females took longer to approach when the wave rate was slower, but their approach was

237   equally accurate at all wave rates (Fig. 2).

238

239   **Table 5.** Descriptive statistics (means ± SD) for female size (carapace width), trial duration and

240   accuracy for the six experiments.

Experiment	Female size (mm)	Duration (s)	Accuracy (degrees)
F	8.82 ±0.83	19.25±13.44	9.94±7.14
M	8.97±0.95	64.25±42.20	12.16±9.22
S	9.15±0.94	109.45±82.43	9.73±7.51
FFF	8.95±0.90	23.50±16.22	11.28±5.81
FMS	8.96±0.91	32.40±28.90	17.80±11.88
FMSdd	8.95±0.86	44.05±32.46	15.35±8.83

241   F = one-choice test with fast wave rate; M = one-choice test with medium wave rate; S = one-

242   choice test with slow wave rate; FFF = three-choice test all with fast wave rate; FMS = three-

243   choice test with one fast, one medium and one slow wave rate; FMSdd = three-choice test with

244   one fast, one medium and one slow wave rate, presented at different distances (fast = 22 cm;

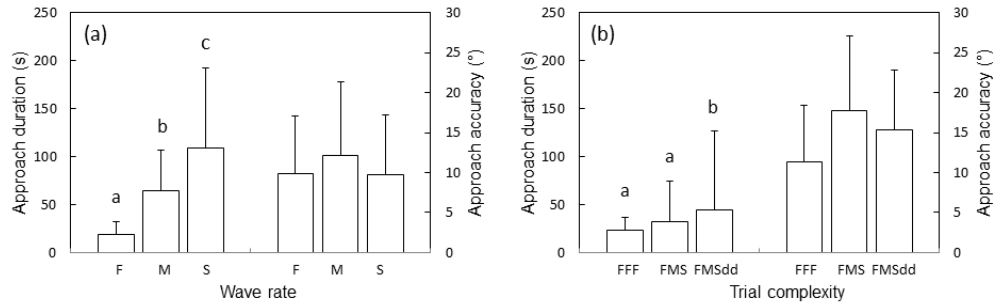245   medium = 20 cm; slow = 18 cm).

246

247

248 **Figure 7.** Approach duration and accuracy for (a) one-choice trials that differed in wave rate
249 and (b) three-choice trials that differed in complexity for the six experiments: F = one-choice
250 test with fast wave rate; M = one-choice test with medium wave rate; S = one-choice test with
251 slow wave rate; FFF = three-choice test all with fast wave rate; FMS = three-choice test with
252 one fast, one medium and one slow wave rate; FMSdd = three-choice test with one fast, one
253 medium and one slow wave rate, presented at different distances (fast = 22 cm; medium = 20
254 cm; slow = 18 cm). Different letters above bars denote statistical differences ($P < 0.05$).

255

256 *Effect of Number of Waving Claws on Accuracy and Duration*

257   An MGLM showed that female size did not affect the accuracy or duration of
258 approach (Hotelling's trace: $F_{2,36} = 0.85$, $P = 0.43$). The number of robotic waving
259 claws (one or three robots) did not affect the approach accuracy or trial duration
260 (Hotelling's trace: $F_{2,36} = 0.71$, $P = 0.50$; univariate effects: duration: $F_{1,37} = 0.92$, $P =$
261 0.35; accuracy: $F_{1,37} = 0.32$, $P = 0.57$). The female approach was equally accurate and
262 took the same amount of time when there was a single waving claw and when there
263 were three waving claws.

264

265 *Effect of Choice Complexity on Accuracy and Duration*

266   An MGLM showed that female size did not affect the accuracy or duration of
267 approach (Hotelling's trace: $F_{2,55} = 0.86$, $P = 0.43$; see Table 1 for female sizes). The
268 complexity of the choice arena did, however, have an effect (Hotelling's trace: $F_{4,108} =$
269 3.04, $P = 0.02$; see Table 1 for descriptive statistics). The effect was driven by

270 differences in the approach durations rather than approach accuracy (univariate effects:

271 duration: $F_{2,56} = 3.91$, $P = 0.03$; accuracy: $F_{2,56} = 2.34$, $P = 0.11$). Females took longer

272 to approach when the choices were more complex, but their approach was equally

273 accurate irrespective of the wave rate (Fig. 2).

274

275 **DISCUSSION**

276 *Accuracy of Localization*

277   Female *U. mjoebergi* approached the waving claw of the robotic male crabs with

278 an accuracy of 9–18°. To our knowledge, this is the first documentation of approach

279 accuracy to a movement-based visual signal, so it is interesting that the level of error

280 was equivalent to most frogs (±20°; Rheinlaender et al., 1979; Shen et al., 2008) and

281 crickets (±12°; Schöneich & Hedwig, 2010). However, the approach accuracy measured

282 here may be considerably less than the accuracy with which females approach males in

283 the field: males of many species (including the study species) move towards a female

284 and 'lead' her back to their burrow by waving at an elevated rate and walking backward

285 to allow her to follow (Crane, 1975; How et al., 2008). This 'leading' behaviour may

286 increase female approach accuracy under natural conditions.

287   The accuracy of approach was not affected by the wave rate: females approached

288 a slow-waving claw as accurately as a fast-waving claw. The complexity of the choice

289 scenario also did not affect approach accuracy: females approached a single stimulus as

290 accurately as they approached one presented alongside two others that waved at

291 different rates and were at different distances from the female. This result has also been

292 found in other species: the repetition rate of a dendrobatid frog did not affect the

293 accuracy of approach (Ursprung et al., 2009); neither did the call complexity affect

294 accuracy in three frog species (Backwell & Passmore, 1991; Bonachea & Ryan, 2011;

295   Rheinlaender et al., 1979). Although simultaneous calling by neighbouring frogs was

296   predicted to reduce their locatability (Awbrey, 1978), female approach accuracy in the

297   painted reed frog, *Hyperolius marmoratus,* was unaffected by call overlap (Passmore &

298   Telford, 1981). The accuracy with which a female approached a calling male in a pond,

299   under natural conditions, was no different to the accuracy in controlled, single-stimulus

300   laboratory trials (leaf-folding frog: Backwell & Passmore, 1991; dendrobatid frog:

301   Gerhardt, 1980). Even in a non-mate-searching context, the accuracy of approach by a

302   parasitoid fly to its hosts' (cricket) calls was only slightly less accurate when the

303   repetition rate was halved (Muller & Robert, 2002).

304   It does not appear that repetition rate or the complexity of the signal or choice

305   environment influences the accuracy with which a female can locate a stimulus. This is

306   surprising and it suggests that the accuracy of localization is constrained by the female's

307   sensory system (see Bonachea & Ryan, 2011; Ursprung et al., 2009).

308

309   *Time to locate*

310   In contrast, the time it took for female *U. mjoebergi* to approach the waving claw

311   was significantly affected by wave rate: it took females ±110 s to approach a slow-

312   waving claw; ±64 s to approach a claw waved at a medium rate; and only ±19 s to

313   approach a fast-waving claw. The number of stimuli did not affect the approach time:

314   females took just as long to approach a fast-waving claw when it was presented alone or

315   with two additional fast-waving claws. However, when the context of choice was more

316   complex, females took longer to approach the claw: it took 24 s for the female to

317   approach when three identical, fast-waving stimuli were presented; it took an additional

318   10 s when the stimuli had three different wave rates; and a further 12 s more when the

319   stimuli were at different distances from the female. Although the approach distance in

320 the FMSdd trials was 2 cm further than in other trials, this 10% increase in distance

321 cannot explain the 36% increase in the time taken.

322   It is not surprising that it took females longer to locate a signal with a low

323 repetition rate since there are fewer waves per unit time to guide her approach and allow

324 her to make corrections to her approach path rapidly. In a dendrobatid frog, females also

325 took less time to approach a rapidly repeated call than a slower call, but this was

326 because they only jumped towards the sound source during signal production and not

327 during the intercall intervals (Ursprung et al., 2009). An increase in signal repetition rate

328 does not always decrease the time it takes to reach a signal source: repetition rate had no

329 effect on approach time in the leaf-folding frog (Backwell & Passmore, 1991) or in the

330 approach of a parasitoid fly to the calls of its host (Muller & Robert, 2002).

331   The effect of choice complexity on the approach time is similarly inconsistent in

332 other species. Female túngara frogs approached complex calls more quickly than simple

333 calls (Bonachea & Ryan, 2011); but in the leaf-folding frog, females were able to locate

334 a naturally calling male in a pond just as quickly as a single stimulus presented under

335 highly controlled experimental conditions (Backwell & Passmore, 1991).

336

337 *Conclusions*

338   Finding a mate is an expensive process, and females need to quickly and

339 accurately detect a male, locate him and then (possibly) assess his suitability as a mate.

340 Any increase in the time a female must spend to achieve this will increase her exposure

341 to risks (predation, dehydration, overheating, etc.). We suggest that, while a female's

342 ability to increase the accuracy of localization would be constrained by her sensory

343 system, she is able to decrease her approach time by selecting signals with high

344 repetition rates and less complex choice environments.

345   Males could increase their chances of mating by signalling as fast as possible.

346   When females are unpredictable in space and time, vigorous and constant display may

347   be costly (Ryan & Cummings, 2005). High signalling rates may attract females because

348   they reduce the female search costs; and they may or may not additionally signal male

349   quality. In *U. mjoebergi*, males with fast wave rates are preferentially approached by

350   mate-searching females (Callander et al., 2012), possibly because wave rate signals

351   male quality, but probably also (or even entirely) because faster wave rates can be

352   located more quickly.

353

360

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Considerações finais

Por fim, chegamos às seguintes conclusões em cada um de nossos capítulos:

Capítulo 1

O sombreamento artificial desempenha um papel importante na distribuição e comportamento dos caranguejos-chama-marés. Como o sombreamento altera a temperatura do substrato, uma possível explicação deve-se à limitação fisiológica das espécies. A mudança na distribuição espacial e no comportamento dos animais possui um enfeito negativo nos mesmos, pois os expõe a riscos durante a busca por um novo território (por exemplo, competidores, predadores e desidratação) e podem influenciar nas atividades primordiais como forrageamento e na busca por parceiros sexuais. Além disso, essas estruturas podem afetar não apenas os caranguejos, mas também os organismos das regiões entre-marés de maneira geral, sendo necessários mais estudos nesse sentido.

Capítulo 2

Concluímos que os caranguejos-chama-maré pagam um alto custo por mudanças ambientais associadas à perda de habitat devido ao aumento do nível do mar, e isso tem implicações para os sistemas sociais e de acasalamento deste grupo. A identidade de espécie invasora não afeta a coalizão dos machos, provavelmente porque é preferível manter um vizinho conhecido que renegociar limites de território com um desconhecido, independentemente da espécie. Além disso, a identidade da espécie de fêmea apresentada aos machos, não afeta os comportamentos de corte dos mesmos, fazendo-os gastar tempo, energia e sendo expostos a riscos (predadores, desidratação,

etc.) enquanto cortejam fêmeas que eles não vão acasalar.

Capítulo 3

Encontrar um companheiro é um processo custoso, então as fêmeas necessitam detectar um macho de forma rápida e precisa. Qualquer aumento no tempo de localização e escolha de um parceiro aumentará sua exposição a riscos (predação, desidratação, superaquecimento, etc.). Sugerimos que, embora a habilidade das fêmeas de aumentar a precisão da localização seja limitada pelo seu sistema sensorial, ela é capaz de diminuir seu tempo de aproximação ao selecionar sinais com altas taxas de repetição e ambientes de escolha menos complexos. Por outro lado, os machos podem aumentar suas chances de acasalamento, sinalizando o mais rápido possível. Taxas de sinalização elevadas podem atrair fêmeas devido à redução dos custos da escolha e podem sinalizar adicionalmente a qualidade do macho. Assim, os machos com taxas de displays mais rápidos são preferencialmente abordados por fêmeas, possivelmente não apenas devido à taxa sinalizar a qualidade dos mesmos, mas também por ser mais fácil de ser localizado.

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