Title: Fiddler crabs can feel more than we think: The influence of neighbors on the activities of the fiddler crab *Leptuca uruguayensis*

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1718 Abstract

19 Fiddler crabs have been used as model organisms in many laboratory and field studies. In their natural 20 environment, social interaction with other fiddler crabs (conspecific or heterospecific) is recurrent, but manipulative 21 studies involving these crabs as models are often performed with isolated individuals. The isolation of an animal 22 can interfere in the behaviors recorded as response variables. Thus, the aim of this study was to evaluate whether 23 the presence of other individuals affects the performance of behaviors of fiddler crabs Leptuca uruguayensis. We 24 tested two hypotheses in the field: 1) the visual stimulus of the crab assemblage affects the activity of male fiddler 25 crabs; 2) the presence of other conspecific affects the activity of male fiddler crabs depending on the sexes of the 26 individuals present. We found the activities of L. uruguayensis males mediated by social interactions does not 27 depend exclusively on visual stimuli. Physical interaction with other conspecifics of both sexes enables the 28 perception of stimuli which can influence the waving behavior of L. uruguayensis males. We suggest that behavioral 29 studies with this model should consider the presence of other individuals. Understanding the behavioral complexity 30 of a model organism contributes to more robust experiments with greater control of interfering variables.

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32 Keywords: Animal testing, Animal communication, Animal behavior, Mangrove ecosystem, Intertidal zone.

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34 Introduction

Animals have been used in experiments since the nineteenth century (Oparin, 1957). Experiments with animals are associated with the roots of scientific thinking and have allowed for great advances in the understanding of animals themselves and the development of technologies that benefit humanity (Krebs, 1975; Ankeny & Leonelli, 2011; Dietrich et al., 2014). Some well-known animal models are mice, zebrafish, and flies (Hoffmann, 2003; Brown et al., 2015). In addition to being abundant and easy to reproduce, these models have behavioral characteristics that can be used as discrete quantitative variables, which makes their use in experimental approaches viable, as they are behaviors that are easy to detect.

Fiddler crabs are commonly used as animal models in studies of ecology, sexual selection, and animal
behavior (Daleo et al., 2003; Takeshita et al., 2018; Arakaki et al., 2020; De Grande et al., 2021b). These crabs are

44 associated with estuarine environments, inhabiting vegetated areas, such as mangroves and saltmarshes, or non-45 vegetated areas, such as sandbars and mudflats (Thurman et al., 2013; Checon & Costa, 2018). Some key 46 characteristics of fiddler crabs make them good model animals. The main activities of these crabs can be categorized 47 as discrete behaviors, which are often used as response variables in experimental designs (Sanches et al., 2017; De 48 Grande et al., 2018a; Roberts, 2021). They are easily found due their abundance, with some species reaching 49 densities of 70-100 ind/m² (Skov et al., 2002; De Grande et al., 2018a; Arakaki et al., 2020), and their wide 50 distribution around the world as they occur in all tropical and subtropical coastal areas (Crane, 1975; Spivak et al., 51 1991; Thurman et al., 2013). In addition, they are benthic, territorial organisms, which aids with sampling efforts, 52 and sampling can be done with simple tools and methods (e.g., shovels, sampling squares or transects) (Thurman et 53 al., 2013; De Grande et al., 2018a).

54 Many studies that use fiddler crabs as models are on animal communication, especially within the context 55 of sexual competition/selection (Backwell et al., 2000; Nabout et al., 2010; Perez et al., 2016; Sanches et al., 2017; 56 Milner et al., 2010; Takeshita et al., 2018). Males display waves with their hypertrophied claw, which is an important 57 visual signal for females to identify and choose partners (Ryan & Cummings, 2005; Mowles & Ord, 2012; Sanches 58 et al., 2017). Males display aggressive waves against rival males (Perez et al., 2016) and wave for other less obvious 59 functions such as thermoregulation (De Grande et al., 2021b). The displays performed by fiddler crabs are easily 60 perceived by potential receivers such as fertile females and con- or heterospecific neighboring males (Murai & 61 Backwell, 2006; Milner et al., 2012). The receiver, in turn, can emit stimuli in response, which can influence the 62 behavior of the sender when performing their presentation (Murai & Backwell, 2006; Milner et al., 2012). An 63 increase in rhythm or frequency of an animal's behavior due to the presence of other individuals is termed social 64 facilitation (Galef & Laland, 2005; Milner et al., 2012; Herman, 2015). For example, the presence of individuals of 65 the same species stimulates the foraging behavior of crustaceans (Kurta, 1982) and even increased food intake in 66 humans (Karplus et al., 2007; Herman, 2015). Nevertheless, social facilitation may vary with the function or the sex 67 of the individuals. Male fiddler crabs, for example, wave mainly to attract females to mate (Sanches et al., 2017; 68 Takeshita et al., 2018), but the display rate increases with the presence of male rivals (Milner et al., 2012).

69 The communication of fiddler crabs is not restricted to displays of hypertrophied chelipeds, it can also take 70 place through tactile and chemical signals, as well as through vibrations carried out in the sediment (Mowles et al., 71 2017; Takeshita et al., 2018; Roberts, 2021). Thus, although visual signaling is recognized as important for fiddler 72 crab communication (Murai & Backwell, 2006; Sanches et al., 2017; Silva et al., 2022), other stimuli may be 73 involved in this process (Crane, 1966; Mowles, 2017; Takeshita et al., 2018). In many studies, the main behavioral 74 activities of fiddler crabs are observed and quantified as response variables: e.g., the total of individuals displaying 75 wave, the number of scoops per minute and/or the time the animal remains outside the burrow (Daleo et al., 2003; 76 Reinsel, 2004; Takeshita, 2018; De Grande et al., 2018b). However, the rates of these behaviors may differ when 77 compared between experiments that prevent contact of the focal animal with other individuals and experiments that 78 do not prevent contact (see, for example, wave rate in De Grande et al., 2021b in comparison to Daleo et al., 2003). 79 One hypothesis that could explain this is that the main behavioral activities of fiddler crabs can be influenced by 80 social facilitation. To further investigate this question, in this study, we evaluated whether the presence of other 81 individuals is an important variable in experiments with fiddler crabs that use the presence/absence of waving 82 behavior, the percentage of time outside the burrow, and number of scoops per minute in the sediment during feeding 83 as response variables. We evaluated whether social facilitation increases the activity of individuals by testing two 84 hypotheses: 1) the visual stimulus of the crab assemblage affects the activity of male fiddler crabs 2) the presence 85 of other conspecific affects the activity of male fiddler crabs depending on the sexes of the individuals present.

87 Materials and methods

88 Study area and model organism

89 The study was carried out in the mangrove forest of the Piaçabuçu Municipal Park, located in Praia Grande, 90 São Paulo State, south-eastern coast of Brazil (23°59'17.4"S – 46°24'23.6"W). The study area is characterized by 91 mangrove forests and semidiurnal tidal systems; the climate is humid subtropical without a dry season (Alvares et 92 al., 2013) and the mean air temperature varies between 17 and 24 °C, with mean annual rainfall between 2,000 and 93 2,500 mm (Municipal City Hall of Praia Grande/SP). We tested whether social facilitation can increase the 94 performance of fiddler crabs' activities. For this, we used the species Leptuca uruguayensis (Nobili, 1901) as an 95 experimental model, which occurs from southern Rio de Janeiro to Mar Chiquita in Argentina (Spivak et al., 1991; 96 Thurman et al., 2013; Colpo & López Greco, 2017). In the studied area, the fiddler crab species L. uruguayensis is 97 observed to inhabit mixed assemblages with other species, including Leptuca leptodactyla (Rathbun, 1898), Leptuca 98 cumulanta (Crane, 1943), Leptuca thaveri (Rathbun, 1900), and Minuca rapax (Smith, 1870) (Checon & Costa, 99 2017; Arakaki et al., 2020). However, it also inhabits monospecific populations, particularly in the southern region 100 of South America, including the state of Rio Grande do Sul in southern Brazil, Uruguay, and Argentina (Truchet et 101 al., 2019). It is an interesting animal model for (1) biogeographical studies as it is the most southerly distributed 102 fiddler crab species in South America (Thurman et al., 2013; Shih & Chan, 2022); (2) physiological studies since 103 this species is sensitive to high temperatures (Vianna et al., 2020; De Andrade et al., 2022); (3) ecological studies 104 since this is the only species in South America that inhabits mangroves and saltmarshes and is the only species that 105 occurs in mixed assemblages or monospecific populations (Thurman et al., 2013; Arakaki et al., 2020; De Grande et 106 al., 2021a, Sanches et al., 2023); and (4) climate change studies as populations further north of the range are expected 107 to become extinct due to increased temperature and populations further south suffer from the expansion of competing 108 or predatory species (Arakaki et al., 2020; De Grande et al., 2021c).

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110 Experimental design and procedures

111 Experiment 1: Presence of conspecifics and heterospecifics in the crab assemblages

112 In the first experiment, we tested the hypothesis that the visual stimuli emitted by the presence of other crabs 113 (conspecifics and heterospecific) of the assemblage in an intertidal zone can affected the activities of the fiddler crab 114 L. uruguayensis males. Initially, we used 40 cm diameter arenas to isolate a part of the fiddler crab assemblage. 115 Then, within the larger arena area, we isolated the area around the burrow of an individual male L. uruguayensis 116 using small circular arenas with a diameter of 12 cm (113 cm²). These small arenas were divided into two treatments 117 (fixed factor, with two levels): 1. Opaque arenas, which prevented the male from seeing the other individuals from 118 the crab assemblage (n = 15; Fig. 1a); 2. Transparent arenas, which allowed the male to see the other individuals 119 from the crab assemblage (n = 14; Fig. 1b). The experimental units (the set of both arenas) were kept at least one 120 meter apart from each other. After the arenas were installed, we waited 5 min for the animals to acclimate to the 121 experimental condition and return to their normal activities outside their burrows. The focal animal was observed 122 for 10 minutes by an observer, at a distance of 50 cm. In both treatments, we recorded, as response variables for 123 each individual, the presence/absence of waving, the percentage of time spent out of the burrow, and the number of 124 sediment scoops per minute during feeding behavior. Males of some species of fiddler crabs exhibit different types 125 of waves, which may be employed during the courtship of females or in territorial defense against other males (How 126 et al., 2007; Muramatsu, 2011). However, the functions of the different types of waves varies between species and 127 this issue has not yet been investigated for L. uruguayensis. Since we were unable to recognize the different types of waves emitted by *L. uruguayensis*, we quantified all instances of males displaying wave behavior during our observations. The average abundance of crabs that made up the assemblages of treatments with opaque arenas was 9 ± 5 individuals (mean \pm standard deviation), whereas in treatments with transparent arenas it was 7 ± 3 individuals. To ensure standardization of the assembly's individual count and thereby eliminate it as a confounding variable in our experimental design, we conducted a Student's t-test and found no statistically significant difference between the treatments (t test, $t_{29} = 1.0726$, p = 0.29). At the end of the experiments, the burrows were excavated, and all crabs were captured and identified.

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136 Experiment 2: The activity of male L. uruguayensis as a function of the presence of conspecifics of different sexes 137 We tested whether activity of male L. uruguayensis differed as a function of the presence of other conspecific 138 males, females, and both sexes simultaneously. For this, we used an opaque arena of 12 cm (113 cm²), which was 139 sufficient to cover the territories of three individuals, based on the maximum density of the L. uruguayensis 140 population in Praia Grande (according to De Grande et al., 2018a). The arenas were adjusted around the burrows of 141 focal males and their neighbors according to the following treatments (fixed factor, with 4 levels): control, a burrow 142 of a focal male without the presence of neighboring crab burrows (n = 18); a focal male burrow with two neighboring 143 females burrows (n = 22); a focal male burrow with two neighboring males burrows (n = 17); a focal male burrow 144 with neighbor burrows of both sexes (i.e., one male and one female; n = 17; Fig. 2). After the arenas were installed, 145 we waited 5 min for the animals to acclimate to the experimental condition and return to their activities outside the 146 burrow. Then, the focal animal was observed for 10 min by an observer, at a distance of 50 cm, and, similar to the 147 previous experiment, the following variables were recorded: the presence/absence of waving, the percentage of time 148 outside the burrow, and number of scoops per minute in the sediment during feeding. At the end of the experiment 149 the burrows were excavated, and all crabs were identified.

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151 Statistical analyses

152 We analyzed whether the presence of other fiddler crabs affected the activity of L. uruguayensis males. In 153 the first experiment, we employed the predictor variable of whether it was possible (transparent arenas) or not 154 possible (opaque arenas) to visualize the members of the assembly. In the second experiment, the predictor variable 155 encompassed the presence of two conspecific males, two conspecific females, both conspecific sexes 156 simultaneously, and the absence of conspecifics. The response variables used were the presence/absence of waving, 157 the percentage of time outside the burrow and number of scoops per minute in both experiments. Initially, we 158 assessed the normality and homoscedasticity assumptions of the data using the Shapiro-Wilk and Bartlett tests, 159 respectively. The analyses were conducted using R version 4.3.2 (R Core Team, 2023). For a given response 160 variable, and considering data dispersion, the same distribution family analysis was applied in both experiments. 161 Throughout our observations, wave behavior was extremely variable between individuals (including individuals 162 within the same treatment). The total number of waves per individual during the observation time ranged from 0 to 163 145 in the first experiment, and from 0 to 75 in the second experiment. Given substantial variability and limited 164 statistical power for comparing average wave behavior (e.g., average waves per minute, as in previous studies, e.g., 165 Daleo et al., 2003; De Grande et al., 2021b), we instead compared the incidence of waving behavior among 166 treatments, treating it as a binary response to categorize individuals based on its presence or absence. We analyzed 167 the number of focal males that waved using a Generalized Linear Models with binomial error distributions and logit 168 link functions, as these are suitable for binary data. The percentage of time out of the burrow met the assumptions 169 of normality and homoscedasticity, and the models were fitted using General Linear Models assuming a Gaussian

- 170 distribution. Since scoops per minute were an over dispersed count variable, we performed other Generalized Linear
- 171 Models with negative binomial distribution to compare it between treatments. A significance level of $\alpha = 0.05$ was
- 172 adopted, Response variables are represented as mean values and standard error in the figures. Results are presented
- 173 as outcome tables of global significance tests for Generalized Linear Models (GLM), assessing the overall impact
- 174 of experimental treatments on male activity in *L. uruguayensis*.
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176 **Results**

177 Experiment 1: Presence of conspecifics and heterospecifics in the crab assemblages

178 The total number of individuals exhibiting waving behavior in the treatment with visual access to the 179 assemblages was not significantly different from the number of animals that waved in the treatment without visual 180 access to the assemblages. (Table 1; Fig. 3a). In treatments with transparent arenas, 35.7% of the individuals 181 displayed wave behavior, whereas in treatments with opaque arenas, 33.3% of individuals exhibited waving. 182 Likewise, the percentage of time out of the burrow for the treatment where they could see other crabs in the 183 assemblages was not significantly different from the time out of the burrow for the treatment where they could not 184 see them (Table 1; Fig. 3b). The mean time (± standard error) that the crab was out of the burrow in treatments with 185 opaque arenas was $25\% \pm 7.8$, and in transparent arena was $43\% \pm 9.1$ of the total observation time. The mean 186 number of scoops (\pm standard error) in the treatment in which the crabs could see the assemblages also was not 187 significantly different from the number of scoops in the treatment in which the crabs could see the assemblage (Table 1; Fig. 3c). The mean number of scoops (\pm standard error) in treatments with opaque arenas was 2.9 \pm 1.1, and in 188 189 transparent arena was 7.4 ± 3.3 , times per minute while feeding.

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191 Experiment 2: Activity of male *L. uruguayensis* as a function of the presence and sex of conspecifics

192 The total number of individuals exhibiting waving behavior differed statistically between the treatments 193 (Table 2). However, it's important to note that when we conducted post hoc comparisons using Tukey test, we did 194 not find statistically significant differences between the specific treatments (p > 0.05). In the control treatment, none 195 of the 18 observed male crabs exhibited waving behavior during the experiment (Fig. 4a). Conversely, in the other 196 treatments, 13% of males waved in the presence of two conspecific males, 23% waved in the presence of two 197 conspecific females, and 27% of males exhibited waving behavior in the presence of both sexes. The time out of the 198 burrow did not exhibit a statistically significant difference across all treatments, regardless of the presence or sex of 199 other individuals. Nevertheless, a visual difference is discernible in the graph representations. (Table 3; Fig. 4b). 200 The mean time (\pm standard error) that the crab was out of the burrow in control treatment was 50% \pm 7,3, in the 201 presence of two conspecific males was $70\% \pm 5.0$, with two conspecific females was $69\% \pm 6.6$, and with presence 202 of both sexes was $70\% \pm 6.9$ of the total observation time. The number of scoops in the treatments with conspecifics 203 was similar to the control treatment, regardless of the sex of the individuals present in the arenas (Table 3; Fig. 4c). 204 The mean number of scoops (\pm standard error) in control treatment was 9,4 \pm 3,0, in the presence of two conspecific 205 males was 15.7 ± 1.6 , with two conspecific females was 16.5 ± 3.0 , and with presence of both sexes was 16.8 ± 2.8 206 times per minute while feeding.

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208 Discussion

209 Our results indicate that social facilitation may stimulate the waves of male *L. uruguayensis* when physical 210 interaction with other conspecific individuals is possible. However, social facilitation did not produce any 211 perceptible effect on other evaluated behaviors, i.e., time spent outside the burrow and the number of sediment scoops during feeding. Contrary to what was expected, none of the three behaviors tested in *L. uruguayensis* males was affected exclusively by visual stimuli emitted by conspecific or heterospecific individuals of the fiddler crab assemblage. This finding led us to hypothesize that social facilitation in male *L. uruguayensis* may not be exclusively dependent on visual stimuli but may involve other stimuli resulting from physical interactions between neighboring crabs. Consequently, the social facilitation resulting from the interaction between individuals should be considered as an interfering variable in the behavior of *L. uruguayensis* when they are used as animal models in future designs.

218 As demonstrated in the present study with L. uruguayensis, male Afruca tangeri (Eydoux, 219 1835) fiddler crabs isolated from visual stimulus and contact with other individuals also wave less than non-isolated 220 males (Oliveira et al., 1998). In a field experiment, it was shown that male Leptuca pugilator (Bosc, 1801) wave 221 less when isolated than when in the presence of females (Pope, 2000a). Waving is an energetically costly process 222 for fiddler crabs, implying a continuous oxygen debt and increasing lactic acid accumulation (Murai & Backwell, 223 2006; Mowles, 2017). Prolonged waving can reduce individuals' running speed performance, which consequently 224 makes them vulnerable to predation (Mowles, 2017). When the cost of waving outweighs the potential benefits, 225 some species may reduce wave rates depending on the level of competition and distance from reproductive females 226 (Tina, 2020).

227 Despite the importance of visual stimuli, communication between some fiddler crab species is sensitive to 228 multimodal stimuli (Mowles, 2017; Takeshita et al., 2018). This may explain why in our first experiment just 229 viewing the crabs in the assemblage was not enough to influence the wave performance of L. uruguayensis males, 230 while in our second experiment contact among individuals stimulated the animals to wave. Although we did not 231 systematically quantify or record the stimuli emitted by conspecifics of L. uruguavensis in our study, in other species, 232 for example Austruca lactea (De Haan, 1835), and Austruca mjobergi (Rathbun, 1924) the courtship of females on 233 the sediment surface involves multimodal signals, including visual signals emitted by agitating chelipeds, 234 constructing sedimentary structures at the burrow entrance and sound signals emitted by the drumming of male 235 chelipeds (Mowles, 2017; Takeshita et al., 2018). Tactile signals produced during direct contact between individuals, 236 using both chelipeds and/or ambulatory legs, may also be important for fiddler crab communication (Crane, 1966). 237 At night, when visual communication is limited, male L. pugilator's attract females by drumming their chelipeds, 238 but when they are touched by them, they increase the frequency of this sound display (Salmon & Atsaides, 1968). 239 Thus, the use of multimodal signals may represent an adaptive strategy that allows L. uruguayensis males to not 240 spend energy waving unnecessarily.

241 The presence of other individuals did not affect the time that L. uruguayensis spent outside the burrow. Other 242 factors may be more important for determining the time L. uruguayensis spends outside the burrow. Burrows are 243 used by fiddler crabs as shelter during high tide and as a form of refuge from high temperatures and predators (Crane, 244 1975; Rossi & Chapman, 2003). For example, the time L. uruguayensis spends outside the burrow may decrease 245 according to the increase in ambient temperature on the sediment surface (De Grande et al., 2021a). Male L. 246 uruguayensis also decrease the time spent outside the burrow according to the risk of predation by the predatory 247 crab Neohelice granulata (Dana, 1851) (Daleo, 2003). Some abiotic variables, such as physiological stressors, can 248 modulate the activities that crabs perform when they are out of the burrow independent of sympatric interactions 249 (Nobbs & Blamires, 2017).

In contrast to the *L. uruguayensis* in the present study, *L. pugilator's* feeding activity in a laboratory study was affected by the presence of other individuals, both through physical stimuli (i.e., physical interactions between conspecifics) and visual stimuli (i.e., isolated individuals with the ability to see other conspecifics) (Horst, 1995). Perhaps this difference between species can be explained by the difference in their eating habits. Some species of fiddler crabs, such as *L. pugilator*, leave their burrows in the supratidal region, which is poor in food, to foraging in seagrass to the downshore region, which is rich in food (Ens et al., 1993). Thus, for this species, the visual stimulus caused by individuals feeding could help form foraging flocks and indicate feeding patches (Viscido & Wethey, 2002). On the other hand, *L. uruguayensis* is a sedentary species, which forages in a radius of a few centimeters around its burrow from where it extracts all its food (De Grande et al., 2018b; Arakaki et al., 2020). The decision of when to feed in *L. uruguayensis* probably involves other mechanisms, such as the chemical perception of substances in the sediment that indicate the presence of food.

261 Our findings bring practical consequences for the elaboration of studies that use L. uruguayensis as an 262 experimental model. Since the presence of neighboring individuals can affect the activity of these animals, it is 263 necessary to consider this as a source of variation to be controlled in experimental designs that use this organism as 264 a model. In this sense, studies that aim to test the relationship of a certain predictor variable on a behavioral response 265 of fiddler crabs should consider whether the behavioral variable is influenced or not by the presence of other 266 individuals. For example, De Grande et al. (2021b) tested whether temperature is associated with the wave rate of 267 L. uruguayensis males in a field experiment by isolating L. uruguayensis males in arenas that prevented physical 268 and visual contact with neighboring crabs. According to the present work, it was a proper approach to control the 269 interference of the presence of other crabs on the tested variable in that study. In other situations, visual isolation of 270 animals would not be as necessary. For example, studies that used the number of scoops in the sediment as a response 271 variable as a function of abiotic predictor variables (i.e., sediment organic matter content, temperature and humidity) 272 isolated focal animals from visual contact with other individuals, including studies with L. uruguayensis (e.g., De 273 Grande et al., 2018a,b; Dyson et al., 2020). However, since we demonstrated that L. uruguayensis does not change 274 its feeding activity due to the presence of other individuals, we suggest that isolation of the focal animal in future 275 studies using the scoops number is not necessary, which can save time and resources during research.

276 Nevertheless, in some situations, exposure to a visual stimulus alone may not be enough to operationalize the 277 response variable. For example, in experiments on communication of fiddler crabs, it is common to provide only 278 visual stimuli to the focal animal, isolating it behind a transparent fence or presenting it with video footage (Pope, 279 200b; Murai et al., 2022). However, visual communication alone may be insufficient for communication between 280 fiddler crabs and information mediated by physical contact may be necessary variables, as demonstrated in the 281 present study. In these cases, it is preferable that the focal animal can interact with the other individuals (for example, 282 see Arakaki et al., 2020). Despite these considerations regarding experimentation, the responses of fiddler crabs to 283 the presence of other individuals, conditioning their main activities, may be species-specific. Expanding the 284 knowledge about which stimuli and mechanisms are involved in the behavioral responses of different fiddler crab 285 species is crucial for the use of these crustaceans as experimental models.

286 Conclusion

287 Understanding how the activities of organisms can be modulated by abiotic and biotic factors contributes to 288 the advancement of animal experimentation, such as clarifying whether the organism is capable of carrying out its 289 activities even when isolated from other animals, or whether it can adapt well to a controlled laboratory environment 290 or an experimental approach carried out in the field. We concluded that the fiddler crab L. uruguavensis should be 291 used in a non-isolated way in experimental approaches that use behavioral predictor variables, especially in wave 292 communication studies. Thus, using multimodal stimuli in experiments, such as the interaction of conspecifics, will 293 avoid problems during the performance and quantification of the activities of the animal model. In the case of studies 294 involving non-behavioral variables, it would be desirable to avoid the presence of conspecifics, as the mechanism 295 of social facilitation can act as a confounding factor in the behavior of the animal model.

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297 Compliance with ethical standards

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301 Conflicts of Interest

302 The authors do not have any conflicts of interest.

303 Ethical approval

304 All applicable international, national, and/or institutional guidelines for the care and use of animals were

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307 Data availability

308 The datasets generated during and/or analyzed during the current study are available from the corresponding author 309 on request.

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Figures



Fig. 1 Overview of the experimental apparatus used to test whether the behaviors of *Leptuca uruguayensis* males are influenced by visual signals emitted by other neighboring fiddler crabs. A large circular arena was used to isolate part of the fiddler crab assemblage in the intertidal zone. Within this large arena, another smaller arena was used to isolate just the territory around the burrow of a focal male *L. uruguayensis*. a) Small opaque arenas prevented focal males from viewing other crabs in the assemblages. b) Small transparent arenas allowed focal males to view other crabs in the assemblages.



Fig. 2 Experimental design of experiment 2: a) without contact with other neighboring crabs (control), b) the presence of two conspecific females, c) the presence of two conspecific males and, d) the presence of a conspecific male and a conspecific female (both sexes).



Fig. 3 Behaviors of *Leptuca uruguayensis* males observed in treatments using opaque and transparent arenas, a) Total number of individuals displaying wave behavior during observations, b) Mean ± EB (Error Bars) of the percentage of time that males were out of the burrow, c) Mean ± EB (Error Bars) of the number of scoops per minute performed by males during observations.



Fig. 4 Behaviors of *Leptuca uruguayensis* males observed in treatments with the presence of other male or female crabs. The treatments represented on the X axes refer to the behavior of male *L. uruguayensis* in an arena without contact with other neighboring crabs (control), in the presence of two conspecific males (males), in the presence of two conspecific females (females) and in the presence of a conspecific male and a conspecific female (both sexes), (a) Total number of displaying wave behavior during observations, (b) Mean \pm SD of the percentage that males of were out of their burrows according to treatment, (c) Mean \pm SD of the number of scoops per minute by males according to treatment. The asterisk and horizontal bar in the figure 4a indicate statistical significance.

514Table 1. Global Significance Test for Generalized Linear Models (GLM) comparisons of male activity of516Leptuca uruguayensis within opaque and transparent arenas in the crab assemblage. Significance Level: α 517= 0.05.

Response variables	df	Deviance	Residual df	Residual deviance	р
Presence/aubsence of wave	1	0.3592	28	39.336	0.5489
Time out of the burrow	1	8.0493	27	95.474	0.1121
Scoops per minute	1	287.94	27	3145.7	0.1555

Table 2. Global Significance Test for Generalized Linear Models (GLM) assessing male activity of Leptu

ca uruguayensis across treatment groups. Significance Level: $\alpha = 0.05$.

Response variables	df	Deviance	Residual df	Residual deviance	р
Presence/aubsence of wave	3	86.044	71	57.346	0.0350*
Time out of the burrow	3	54.603	71	548.9	0.0699
Scoops per minute	3	491.15	71	6771.2	0.1537

#