Universidade estadual paulista “Júlio de Mesquita Filho” Instituto de

Biociências - Campus do Litoral Paulista – CLP

Carolina Guardino Martins

Título: More important than seeing is feeling: The influence of multimodal cospecific signals on the activities of fiddler crab *Leptuca uruguayensis.*

São Vicente - SP

2023

Carolina Guardino Martins

Título: More important than seeing is feeling: the influence of multimodal cospecific signals on the activities of fiddler crab *Leptuca uruguayensis*

Trabalho de Conclusão de Curso apresentado como parte dos requisitos para obtenção do título de bacharel em ciências biológicas, com habilitação em gerenciamento costeiro, pela universidade estadual paulista "júlio de mesquita filho" - unesp, instituto de biociências, campus do litoral paulista.

Orientadora: Prof. Dra. Tânia Marcia Costa

Coorientador: Dr. Fernando Rafael de Grande

São Vicente - SP

2023

M386m

Martins, Carolina Guardino

More important than seeing is feeling: The influence of multimodal cospecific signals on the activities of fiddler crab Leptuca uruguayensis / Carolina Guardino Martins. -- São Vicente, 2023

27 p. : fotos, mapas

Trabalho de conclusão de curso (Bacharelado - Ciências Biológicas) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, São Vicente

Orientadora: Tânia Marcia Costa

Coorientadora: Fernando Rafael De Grande

1. Comportamento animal. 2. Crustáceo. 3. Ecologia. I. Título.

Acknowledgment

This is the moment when I stop to reflect on the events that brought me this far, and i cannot help but remember that to get where I am I learned from many people along this journey. In this way, I want to first thank my family who gave me all the support and who believed in my potential when I didn't even know which career I would decide to follow. To my dear mother, who always supported me in difficult times and always gave me great advice, I am very grateful. there were long years of preparation where I had the opportunity to meet incredible people who helped me a lot during this process towards the public university. I am grateful for the incredible welcome I received at Unesp IB/Clp by my veterans, especially Rodrigo and Beatriz who welcomed me into their apartment during the first days in São Vicente. Special thanks to the Trapiche republic where I was adopted in the first month, especially Luiz Felipe and Radija who named me Leidhy. I want to say a huge thank you to the Xabláu republic, where I lived for 4 years and I was able to meet incredible people that I will never forget, I lived the best moments of my life in that republic and I was able to grow a lot in different aspects. I want to thank professor Tânia Marcia Costa for the opportunity to work with her research team, I am very grateful to be part of this incredible group. In particular, I would like to thank Fernando Rafael de Grande immensely, for accepting to be my co-supervisor in my project and for helping me several times during the TCC execution process, I don't have enough words to express my gratitude for the opportunity to work with such competent researchers. I would like to thank the research funding agencies CAPES and FAPESP for funding my projects with a research grant, which enabled my growth as an academic student. finally, I want to thank the CLP faculty and staff for providing a learning environment.

Abstract

The fiddler crabs are considered key organisms of estuarine environments due to their bioturbation activities in the sediment and contribution on trophic webs. males have a hypertrophied cheliped that is commonly used to attract females through waving and also in combat with other males. the fiddler crabs have been used as a model organism in several experimental studies, mainly those related to sexual selection and ecosystem engineering. in their natural environment, social interaction with other fiddler crabs (conspecific or heterospecific) is recurrent, but many studies involving this animal as model have been carried out with isolated individuals. however, the isolation of an animal can be an interfering factor in behaviors used as experimental variables. many animals can present activities dependent of the social facilitation, i.e. behavior that are stimulated by the presence of other individuals. Thus, the aim of this study was to assess whether social facilitation is a relevant factor for carrying out behaviors related to sexual selection and bioturbation in fiddler crabs *Leptuca uruguayensis*. In the field, we tested whether i) sexual diversity increases the activity of males fiddler crabs. we used as response variables: The number of scoops that the animal performs while feeding, the number of cheliped waves and the time out of the burrow. We found that social facilitation is a mechanism that increased the performance of waves in males of *L. uruguayensis*, but it was not exclusively dependent on visual stimuli. Physical interaction with other conspecific individuals may condition the crab to perform a specific behavior more frequently. Thus, it is important that future approaches with this animal model consider the effect of social facilitation as an interfering variable in its behavior.

Keywords: Social facilitation, *Leptuca uruguayensis*, Experimental design, Sexual selection, Bioturbation.

Summary

1. Introduction

Experimental approaches using animals have been known since the 19th century (Oparin, 1957; Barré-sinoussi & Montagutelli, 2015; Andersen & Winter, 2019). the importance of using animals in experiments is mainly due to the possibility of understanding specific biological phenomena, which includes behavioral, physiological and genetic processes (Krebs, 1975; Owens, 2006; Ankeny & Leonelli, 2011; Dietrich et al., 2014). Krebs, 1975 cites in his article that “for many problems, there is an animal in which it can be more conveniently studied” that we can understand as the use of simple adaptive models, being a starting point for the understanding of other organisms (Owens, 2006). thus, such findings would be useful and/or applicable to other animals with different levels of complexity (Ankeny & Leonelli, 2011; Dietrich et al., 2014). that is, by starting from a common point, which presents mechanisms shared by many species, we improve the understanding of the functioning of organisms that can present more complex systems than the study model (Ankeny & Leonelli, 2011; Dietrich et al., 2014). some well-known models are mice, a fish called zebra-fish and also the flie such as drosophila (Hoffmann, 2003; Feitsma & Cuppen, 2008; Brown et al., 2015; Steimer, 2022). 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. crustaceans are also widely used as animal models, in some approaches and the present study chose one of its specimens to be your model.

Fiddler crabs are commonly used as animal model in studies of ecology, taxonomy, biogeography, morphology, physiology, animal behavior and sexual selection (Rosenberg, 2002; Daleo et al., 2003; Kristensen, 2008; Thurman et al., 2013; Shih et al., 2016; Masunari et al., 2017; Natálio et al., 2017; Takeshita et al., 2018; Darnell, 2019; Arakaki et al., 2020; De Grande et al. 2021; Silva et al 2022). fiddler crabs representing 106 species in the world, belonging to 11 genera and they are widely known for their marked sexual dimorphism in which males have a hypertrophied claw. (Rosenberg, 2014; Shih et al., 2016). These crabs are part of the fauna associated to the estuarine environment, inhabiting vegetated areas, such as mangroves and saltmarshes, or non-vegetated areas, such as sandbars and mudflats (Thurman et al., 2013; Checon & Costa, 2018). Their distribution can be influenced by biotic factors, such as larval settlement and predation (Daleo et al., 2003; Thurman et al., 2013), and abiotic factors such as salinity, temperature, tidal variation and sediment granulometry (Sanford et al., 2006; Checon & Costa, 2017; de Grande et al., 2021). The fiddler crabs are consumers of deposited matter and use their maxillipeds for mechanical separation of bacteria, microalgae and nematodes from the sediment. (Crane, 1975; Ribeiro et al., 2005; Citadin et al., 2016). they are preyed on birds, mammals and fish, being an important trophic link between the production of the intertidal zone and adjacent environments (Rozas & Lasalle, 1990; Koga et al., 2001; Krumme et al., 2007). Fiddler crabs are considered ecosystem engineers, as their activities in the environment during feeding and burrow digging contribute to the distribution and cycling of nutrients in the system (Skov et al., 2002; Nielsen et al., 2003; Kristensen & Alongi, 2006).

The expressive use of the fiddler crabs in several approaches in the literature may explain by the fact that they are animals with characteristics that favor experimental studies (Nabout et al., 2010). They are easily found due their extensive abundance, with some species reaching a density of 70-100 ind/m² (Skov et al., 2002; De Grande et al., 2018; Arakaki et al., 2020), and due their wide distribution around the world occurring in all tropical and subtropical coastal areas. (Crane, 1975; Spivak et al., 1991; Thurman et al., 2013). They live in intertidal zone, where they are easily accessible by humans without the need for specific equipment to access them (e.g., diving gear). Their sampling is also facilitated by the fact that they are benthic and territorial organisms, and it can be done with simple tools and methods (e.g., shovels, sampling square or transects) (Costa & Negreiros, 2002; Thurman et al., 2013; De Grande et al., 2018a). In addition, they are animals that tolerate human presence, quickly getting used to observers (Arakaki et al., 2020; De Grande et al., 2021; Silva et al 2022). Its maintenance in laboratory is quite simple, requiring only aquariums with a reduced amount of water, since they are crabs that have bimodal breathing (Paoli et al., 2015). Their feeding in captivity also does not demand much complexity, since they are animals with low-cost generalist food, and can be fed with ornamental fish feed (Allen et al., 2012; Darnell et al., 2015; de Grande et al 2021). The main activities of these crabs can be categorized as discrete behaviors witch often used as response variables in experimental designs (Sanches, et al. 2017; De Grande et al. 2018a; Roberts, 2021). Some authors have suggested that these animals are good models for understanding contemporary global issues, such as monitoring climate change (Colpo et al, 2017; De Grande et al., 2021b). In addition, these animals can be indicators of physiological stress, since they inhabit areas that already present drastic variations in temperature, salinity and ph (Reinsel et al, 2004; Marochi et al, 2022).

Fiddler crabs have been even categorize as “climate sentinels”, which could be a key species to understand how organisms will react to climatic stressors over the decades (Capparelli et al., 2018, 2022; Truchet et al., 2022). Fiddler crabs are used as animal models in studies that assess animal communication, especially within the context of sexual selection (Backwell et al., 2000; Nabout et al., 2010; Perez et al., 2016; Sanches et al., 2017; Milner et al., 2010; Takeshita et al., 2018). Their marked sexual dimorphism fits the classical pattern of sexual selection described by Darwin. The wave pattern and speed can vary among species, but for all them it is important visual signals for females to identify and choose a partner (Ryan & Cummings, 2005; Mowles & Ord, 2012; Sanches, et al. 2017). Male waves are also performed aggressively in combat with other rival males and for other functions less obvious, such as thermoregulation (Perez et al., 2016; De Grande et al., 2021b). The communication of fiddler crabs is not restricted to displays of hypertrophied chelipeds, it can also take place in a tactile, chemical, coloring and also through vibrations carried out in the sediment where these animals usually forage (Hill, 2001; Popper et al. al., 2001; Detto et al., 2006; Hill, 2009; Mowles et al., 2017; Takeshita et al., 2018; Roberts & Laidre, 2019; Roberts, 2021). Fiddler crabs can even communicate through extracorporeal structures built at the entrance to their burrows, such as pillars, domes, chimneys and trenches, which are used in visual communication to attract females (Saher et al., 2017; Kim et al., 2017; Pardo et al., 2020).

Fiddler crabs can live in highly dense mono-or heterospecific populations (De Grande et al., 2018b; Arakaki et al., 2020), which can increase the pressure for intraspecific competition, but also favor reproductive success (Chuang & Peterson, 2016; Sanches et al., 2018). Fiddler crabs, in an attempt to safeguard their own territory, may team up with a neighbor to expel an invading crab from its burrow (Detto et al., 2010; Sanches et al., 2018; Fogo et al., 2019). The complex social context in which fiddler crabs live favors a well-developed ability to communicate. the displays perform by the fiddler crabs are easily perceived by a potential receptor, as fertile females or monospecific or heterospecific neighboring males (Murai & Backwell, 2006; Milner et al., 2012). The receivers, in turn, can emit stimuli in response, which can influence the behavior of the sender when performing their presentation (Murai & Backwell; Milner et., 2012). When the presence of conspecifics is able to increase a pattern of behavior, both in rhythm and frequency, we say that there has been social facilitation (Galef & Laland, 2005; Milner et al., 2012; Herman, 2015). For example, the presence of individuals of the same species stimulates the foraging behavior of crustaceans (Kurta, 1982), as well as an increase in food intake in humans (Herman, 2015). Depending on density and sexual diversity, it is possible to observe variations in the effects that social facilitation can condition. in the violinist crab, for example, males use the waving mainly when they want to attract females to mate (Sanches et al, 2017; Takeshita et al., 2018). Thus, social facilitation can further stimulate this behavior linked to sexual selection in the presence of conspecifics. as an example, one study found through experimentation that male fiddler crabs adjust the wave rate according to the presence of other males (Milner et al. 2012).

The most commons behaviors of the fiddler crab are used as response variables, such as the number of waves, the number of scooping and the time the animal remains out of the burrow (Botto & Iribarne, 2000; Reinsel, 2004; Takeshita, 2018; De Grande et al., 2018b). However, these behaviors observed in the natural environment may differ when compared to the same experiments performed in the laboratory (see, for example, Botto & Iribarne, 2000 in comparison to De Grande et al., 2018b). One hypothesis that could explain this is that in the laboratory, in most studies (Posey, 1987; Botto & Iribarne, 2000; Reinsel, 2004; De Grande et al., 2018b), animals are isolated without the opportunity of interaction with conspecifics. In order to investigate this issue, in this study we evaluated whether the presence of other individuals is an important variable in experiments with fiddler crabs that use as response variables: waves, time spent outside the burrow and the number of scoops in the sediment during feeding. We evaluated whether the social facilitation increase in the activity of individuals by testing the hypothese: i) the physical stimulus caused by the presence of other individuals increases the activity of fiddler crabs males, depending on the sex of the individuals present.

2. Materials and methods

2.1 Study area and model organism

The study was carried out in the mangrove of the Piaçabuçu municipal park, located in Praia Grande, São Paulo State, Southeastern Coast of Brazil (23°59'17.4"s - 46°24'23.6"w). the study area is characterized by mangrove forests and semidiurnal tidal systems; the climate is humid subtropical without a dry season (Alvares et al., 2014) and the average air temperature varies between 17° and 24° c, with average annual rainfall between 2000-2500 mm (Municipal City hall of Praia Grande/Sp). We tested whether social facilitation can influence the performance of fiddler crabs' activities. for this, we used as experimental model the species *Leptuca uruguayensis*, which occurs from the south of Rio de Janeiro to the mar Chiquita in Argentina (Spivak et al., 1991; Thurman et al., 2013; Colpo & López Greco, 2017). This species of fiddler crab inhabits mixed banks with another species (*Leptuca leptodactyla, Leptuca cumulanta, Minuca rapax, Leptuca thayeri*) (Checon & costa, 2017;

Arakaki et al., 2020, but can also inhabit monospecific banks (Checon & Costa, 2017, 2018).

Figure 1. (a) Experimental area in the mangrove located in Praia Grande, sp. the marker in red indicates the area where the experiment was carried out. **(b)** Exemplary male of *Leptuca uruguayensis.*

2.2 Experimental design and procedures

2.2.1 The activity of males of *l. uruguayensis* as a function of the presence of conspecifics of different sexes

We tested the increased activity of males of *l. uruguayensis* as a function of the presence of other conspecifics males, females and both sexes simultaneously. For this, the territory of a focal male of *l. uruguayensis* was isolated, which presented in its borders the territory of two other neighboring conspecifics (males or females) through an opaque arena, 12 cm of diameter, which presents an area enough for the territory of up to three neighboring crabs (De Grande et al., 2018). The treatments (fixed factor, with 4 levels) were divided into: control, where a focal male was isolated without the presence of other neighboring crabs (n = 18); a focal male with the presence of two other neighboring males (n = 22); a focal male with the presence of two females (n = 17); a focal male with the presence of neighbors of both sexes (i.e. one male and one female; n= 17; figure 2). After the arenas were installed, we waited 5 minutes for the animals to acclimate to the experimental condition and return to their activities outside the burrow. the focal animal was observed for 10 minutes by an observer, at a distance of 50 cm, and the following was recorded: the time the crab was out of the burrow;

the number of consecutive scoops on the sediment during feeding; the number of waves performed. at the end of the experiment the burrows were excavated, all crabs were captured and measured (maximum carapace width in mm).

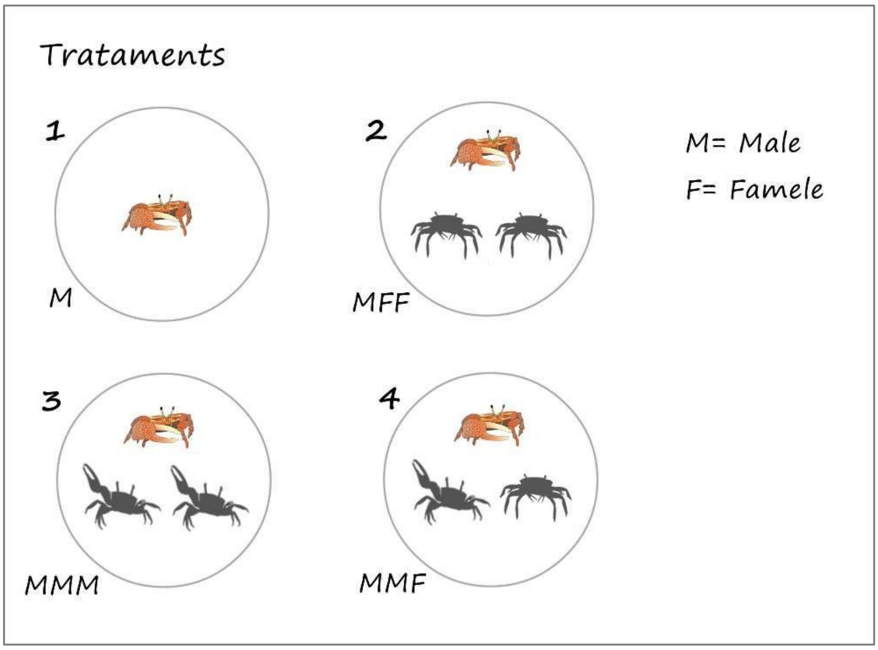

Figure 2. 1- Control treatment with a focal male of *leptuca uruguayensis* in isolation. 2- Treatment with a focal male *l. uruguayensis* and two conspecific females. 3- Treatment of a focal male with two conspecific males. 4- Treatment of a focal male with a conspecific male and female.

3. Statistical analysis

We used the generalized linear model (glm) to compare whether the activities of the fiddler crab *Leptuca uruguayensis* differed between the treatment with opaque arena, without possibility of visualization and transparent with possibility of visualization of other individuals. the response variables were compared between treatments of sexual diversity. we used the generalized linear model (glm) to compare the activities of treatments (fixed factor, with 4 levels) where each focal crab was exposed to different groups of neighboring crabs. when necessary, the data were transformed and in both cases we adopted a minimum significance level of 0.05.

4. Results

4.1 Activity of males of *l. uruguayensis* as a function of the presence and sex of conspecifics

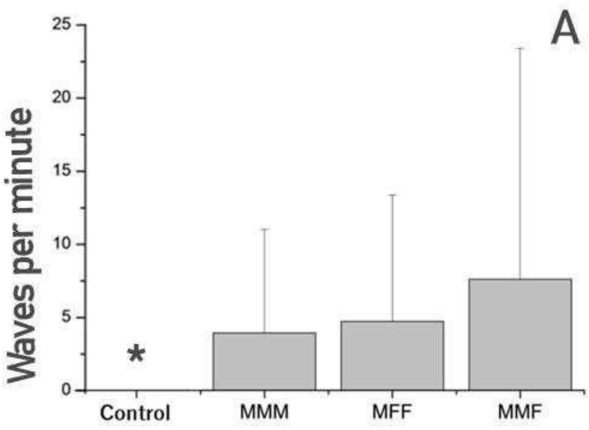

Figure 3. (**a**) mean (± standard deviation) of the number of waves per minute, performed by *l.uruguayensis* males in treatments with three males (mmm), one male and two females (mff), two males and one female (mmf) and control with a focal male, **p** < 0.05.

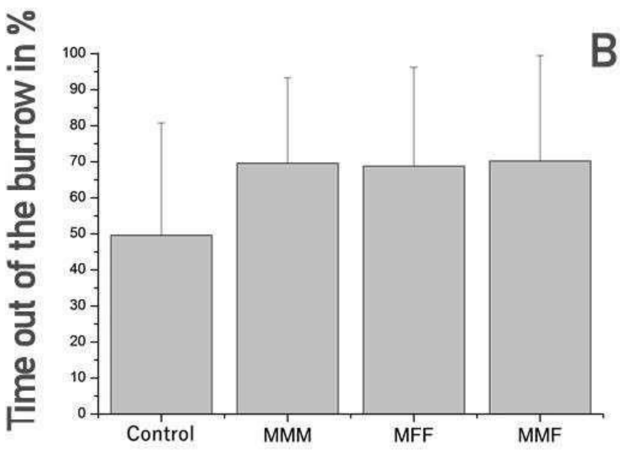

Figure 4. (**b**) mean (± standard deviation) of the percentage that males of *l. uruguayensis* were out of the burrow in treatments with three males (mmm), one male and two females (mff), two males and one female (mmf) and control with a focal male.

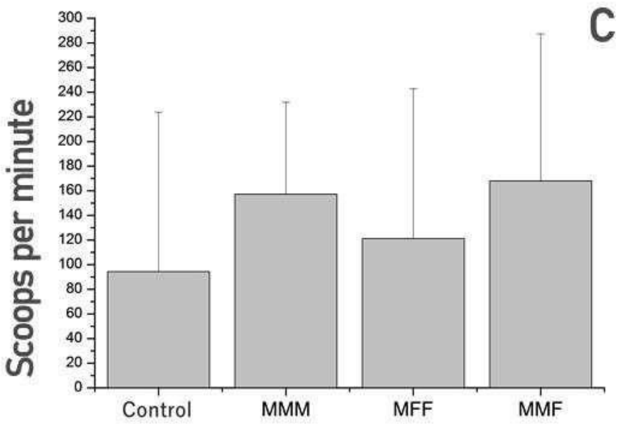

Figure 5. (**c**) mean (± standard deviation) of the number of scoops per minute, performed by *l. uruguayensis* males in the treatments: control (one male), with three males (mmm), one male and two females (mff), two males and one female (mmf).

the number of waves showed a significant difference between treatments (glm, pr (>chi) = 0.03) (figure 4 - a). in treatments with the presence/sex of other individuals, the crabs waved an average of 2.7 ± 10.9 (± sd) times per minute while feeding. in the control treatment, none of the crabs waved while doing their activities. time out of the burrow was similar in all treatments, regardless of the presence or sex of other individuals (anova, f1.71 = 2.3, ms = 1114, p = 0.70) (figure 5). the average percentage that crabs were out of the burrow in the four treatments was 64.8% ± 28.6% (± sd) of the total observation time. the number of scoops in the treatments that presented conspecifics were similar to the control treatment, regardless of the sex of the individuals present in the arenas (glm, pr(>chi) = 0.3), (figure 6). crabs scooped the sediment on average (±sd) 146.5 ± 113.1 times per minute while feeding independently of treatment.

5. Discussion

Our results indicate that social facilitation is a mechanism that may stimuli the performance of waves in males of *L. uruguayensis* when physical interaction with other conspecific individuals is possible. however, the social facilitation did not produce any perceptible effect on other behaviors evaluated, i.e., time spent outside the burrow and the number of sediment scoops during feeding. This finding makes us hypothesize that social facilitation in males of *L. uruguayensis* may not be exclusively dependent on visual stimuli, but may involve other stimuli resulting from physical interaction between neighboring crabs, such as touch between individuals and cues propagated by sediment vibration. consequently, the use of *L. uruguayensis* as an experimental model must consider the social facilitation resulting from the interaction between individuals as an interfering variable in the behavior of the animal model in future designs.

As demonstrated in the present study with *L. uruguayenisis*, males of *Uca tangeri* isolated from the visual stimulus and from contact with other individuals also wave less than non-isolated males, but increase the time they spend feeding (Oliveira et al., 1998). social facilitation also influences the congener activity fiddler crab *L. pugilator* (Horst, 1995; Pope, 2000). In a field experiment it was shown that males of *L. pugilator* wave less when isolated than when in the presence of females (Pope, 2000a). On the other hand, in contrast to *L. uruguayensis*, the *L. pugilator* did not wave in treatments whose individuals were only males (Pope, 2000a). *Leptuca pugilator* can even respond to visual stimuli emitted by playing videos (Pope, 2000b). Two other species that can respond to visual stimuli emitted by video playback are *Uca tangeri* and *Austruca lactea* (Ler burford et al., 2000; Murai et al., 2022). In addition, males of *A. lactea* increase the frequency of waves when they identify wandering reproductive females at a distance of 50 cm through transparent fences and without the need, therefore, of direct physical contact (Murai et al., 2022).

Although visual signaling is recognized as important for fiddler crab communication (Zeil & hemmi, 2005; Hemmi et al., 2006; Murai & backwell, 2006; Sanches et al., 2017; Smithers, et al., 2019; Silva et al., 2022) other stimuli may be involved in this process (Crane, 1966; Pratt et al., 2005; Mowles, 2017; Takeshita et al., 2018). Many species of fiddler crabs also communicate through acoustic signals, i.e., vibrations transmitted through the sediment, produced by the drumming of chelipeds (Crane, 1966; Salmon, 1968; Takeshita & Murai, 2016; Mowles, 2017; Takeshita et al., 2018). It was observed, for example, that neighboring males of *L. pugilator* synchronized the exit of their burrows to the surface, suggesting that individuals in separate burrows can communicate without seeing each other through sound communication (Pratt et al., 2005). In addition, it was observed that males of *L. pugilaror* and *a. Lactea* drum the chelipeds inside their burrows, attracting females that are on the surface of the sediment (Salmon & Atsaides, 1968; Akeshita & Murai, 2016). The courtship of females in A. *lactea* and *a. mjoebergi* on the surface of the sediment involves multimodal signals, including visual signals emitted by the waving of chelipeds, the construction of sedimentary structures at the entrance of the burrow and sound signals emitted by the drumming of the chelipeds of the males (Mowles, 2017; Takeshita et al., 2018). Tactile signals produced during direct contact between individuals, using both chelipeds and/or ambulacral legs, may also be important for fiddler crab communication (Crane, 1966). touching and pushing, using a larger cheliped, are common threat signals among male fiddler crabs (Fogo et al., 2019; Arakaki, et al., 2019). During the night, when visual communication is limited, males of *L. pugilator* attract females by drumming the chelipeds, but when they are touched by them, they increase the frequency of this sound display (Salmon & Atsaides, 1968).

The use of multimodal signals may represent an adaptive strategy that allows *L. uruguayesis* males not to spend energy unnecessarily. Some species of fiddler crabs wave even when they are not viewing other individuals (De Grande et al., 2018; Murai et al. 2022). Males of *A. lactea* perform "Broadcast Waves" waving even when they are not directly viewing a mate, with the intention of attracting the attention of distant females before their rival competitors (Murai et al., 2022). In males of *L. uruguayensis*, the wave rate may be associated with heat dissipation, aiding in the thermoregulation of individuals (De Grande et al., 2021). Despite this, waving is an energetically costly process, implying a continuous oxygen debt and increasing lactic acid accumulation (Matsumasa & Murai, 2005; Mowles, 2017). Prolonged waving can reduce individuals' running speed performance, which consequently puts them in a situation of vulnerability to predation (Mowles, 2017). Furthermore, the waving movement increases the conspicuity of males increasing the risk of predation (Ribeiro et al. 2003). Therefore, male fiddler crabs must adjust their display of waves in a way that increases the chance of attracting females, minimizing the costs involved in producing the signal (Tina, 2020). When the cost of wave outweighs the potential benefits, some species may reduce wave rates depending on the level of competition and distance from reproductive females (Tina, 2020). For example, males tend not to expend energy signaling to females that are too far from being detected or decrease energy expenditure signaling at very low rates to any potential female (Tina et al 2018, Tina, 2020). In a context of high competition with rival males, *A. annulipes* and *A. perplexa* increase the waving speed in order to draw female attention to themselves, but they reduce the waving rate when competition is removed (Milner et al., 2011; Tina, 2020).

The presence of other individuals did not affect the time that *L. uruguayensis* spent outside the burrow. O ther factors may be more important in determining the time *L .uruguayensis* spends outside the burrow. Burrows are used by fiddler crabs as shelter during high tide, and as a form of refuge from high temperatures and predators (Crane, 1975; Rossi & Chapman, 2003). For example, the time spent outside the burrow in *L. uruguayensis* may decrease according to the increase in ambient temperature and the moisture content of the sediment surface (Darnell, 2019; De Grande et al. 2021). Males of *L. uruguayensis* also decrease the time spent outside the burrow according to the risk of predation by the predatory crab *Chasmagnathus granulatus* (Daleo, 2003).

According to Reinsel & Rittschof (1995) the organic content, as well as the amount of water in the sediment, are determining factors for the crab to perform its activities outside the burrow. This happens because crabs are able to detect ideal environmental conditions through chemoreceptors present in the dactyl of their pereiopods (Reinsel & Rittschof, 1995). however, for *L. uruguayensis*, the organic matter of the environment does not influence its distribution in the mangrove, and there may be other biotic and abiotic factors influencing the time that the crab spends performing its activities in its habitat (De Grande et al., 2018). Some abiotic variables, such as physiological stressors, are able to modulate the activities that the crab performs when it is out of the burrow independently of sympatric interactions (Nobbs & Blamires, 2017). Thus, the difference between the time out of the burrow, in both experiments, may be related to the fact that the presence of individuals did not affect the number of scoops in the sediment, which means that the animal remains out of the den in most of his time eating (personal observation) and at times he takes the opportunity to wave.

In a laboratory experiment was observed that the feeding activity of *L. pugilator*, differently from the case of *L. uruguayensis* in the present study, was influenced by the presence of other individuals both by physical stimuli (i.e., physical interactions between conspecifics) as well as by visual stimuli (i.e. isolated individuals, but with the possibility of visualizing 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 form foraging herbs in the infratidal region, which is rich in food (Ens et al., 1993; Viscido & Wethey, 2002). Thus, for this species, the visual stimulus caused by individuals feeding could help in the formation of foraging herds and indicate feeding patches. On the other hand, *L. uruguayenis* is a sedentary species, which forages in a radius of a few centimeters around its burrow from where it is able to extract all its food (De grande et al., 2018; Arakaki et al. 2020). Since *L. uruguayensis* defend the borders of the territory around its burrow (Fogo, 2019; Arakaki et al. 2020), the presence of an individual feeding would not bring a direct benefit to another individual whose area of food is limited to its own territory. Probably, the decision of when to feed on *L. uruguayensis* involves other mechanisms, such as the chemical perception of substances in the sediment that indicate the presence of food.

Our findings bring practical consequences for the elaboration of studies that use *L. uruguayensis* as an experimental model. Since the presence of individuals can affect the activity of an animal, it is necessary to consider it as a source of variation to be controlled in experimental designs that use this organism as a model for the study. In this sense, studies that want to test the relationship of a certain predictor variable on a behavioral response variable of the fiddler crabs should consider whether the behavioral variable is influenced or not by the presence of other individuals. For example, in De Grande et al., 2021, it was tested whether temperature is associated with the wave rate of *L. uruguayensis* males in a field experiment. to test this hypothesis, the authors isolated *L. uruguayensis* males in arenas that prevented physical and visual contact with other neighboring crabs. Such a procedure, according to the present work, may have been really important to control the interference of the presence of other crabs on the tested variable of interest. In other situations, visual isolation of animals would not be so necessary. For example, studies that used the number of scoops on the sediment as a response variable as a function of abiotic predictor variables (i.e., sediment organic matter content, temperature, and humidity) took care to isolate focal animals from visual contact with others. Individuals, including studies with *L. uruguayensis* (e.g. De grande et al 2018a,b; Dyson et al., 2020; De grande et al., 2021).

However, since we demonstrated that *L. uruguayensis* does not change its feeding activity due to the presence of other individuals, this procedure could not have been performed, which could have saved time and resources during the research. on the other hand, in some situations, only the exposure of the visual stimulus may not be enough for the operationalization of the response variable. for example, it is common in choice experiments involving communication signals, such as the experiment on choosing a female or a rival for a certain morphological or behavioral trait, to provide only visual stimuli to the focal animal, typically individuals behind a transparent barrier, or video playback (Pope, 2000b; Ler burford et al., 2000; De Grande et al., 2018; Murai et al., 2022). However, visual communication alone may be insufficient for communication between fiddler crabs and information mediated by physical contact may be necessary variables, as demonstrated in the present study. In these cases, it is preferable that the focal animal has the possibility of interacting with the tested individuals (For an example, see: Arakaki et al., 2020). Despite these considerations regarding experimentation, the responses of fiddler crabs to the presence of other individuals, conditioning their main activities, may be species-specific. Expanding the knowledge about which stimuli and mechanisms are involved in the behavioral responses of different species of fiddler crabs is crucial for the use of these crustaceans as experimental models.

6. Conclusion

Understanding how the activity of the fiddler crab can be modulated by abiotic and biotic factors contributes to filling important gaps in animal experimentation, such as, for example, whether this organism is capable of carrying out its activities even when isolated from other animals, or whether it can adapt well to a controlled laboratory environment or an experimental approach carried out in the field. Through the analysis of our results, we concluded that the fiddler crab *L. uruguayensis* should be used in a non-isolated way in experimental approaches that use behavioral predictor variables, especially in communication studies. Thus, when using multimodal stimuli in experiments, such as the interaction of conspecifics, will avoid problems during the performance and quantification of the activities of the animal model. in the case of studies that assess non-behavioral abiotic variables, it would be desirable to isolate the effects that the presence of conspecifics can bring to the study model through the mechanism of social facilitation.

7. References

Allen bj, rodgers b, tuan y, levinton js (2012). size-dependent temperature and desiccation constraints on performance capacity: implications for sexual selection in a fiddler crab. journal of experimental marine biology and ecology, 438, 93-99.

Alvares ca, stape jl, sentelhas pc, de moraes g, leonardo j, sparovek g (2013). köppen's climate classification map for brazil. meteorologische zeitschrift, 22(6), 711-728.

Arakaki jy, de grande fr, arvigo al, pardo jcf, fogo br, sanches fh, costa tm (2020). battle of the borders: is a range-extending fiddler crab affecting the spatial niche of a congener species?. journal of experimental marine biology and ecology, 532, 151445.

Backwell pr, passmore ni (1996). time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *uca annulipes*. behavioral ecology and sociobiology, 38(6), 407-416.

Backwell pr, christy jh, telford sr, jennions md, passmore j (2000). dishonest signalling in a fiddler crab. proceedings of the royal society of london. series b: biological sciences, 267(1444), 719-724.

Botto f, iribarne o (2000). contrasting effects of two burrowing crabs (*chasmagnathus granulata* and *uca uruguayensis*) on sediment composition and transport in estuarine environments. estuarine, coastal and shelf science, 51(2), 141-151.

Capparelli mv, mcnamara jc, thurman cl, pérez-ceballos r., gómez-ponce ma, cardoso-mohedanoa jg, moulatlet gm (2022). can tolerances of multiple stressors and calculated safety margins in fiddler crabs predict responses to extreme environmental conditions resulting from climate change? marine pollution bulletin, 179, 113674.

Chuang,a, peterson cr (2016). expanding population edges: theories, traits, and trade-offs. global change biology, 22(2), 494-512.

Citadin m, costa tm, netto sa (2016). the response of meiofauna and microphytobenthos to engineering effects of fiddler crabs on a subtropical intertidal sandflat. austral ecology, 41(5), 572-579.

Costa tm, fransozo ml (2002). population biology of *uca thayeri* rathbun, 1900 (brachyura, ocypodidae) in a subtropical south american mangrove area: results from transect and catch-per-unit-effort techniques. crustaceana, 75(10), 1201-1218.

Capparelli mv, bordon ic, araujo g, gusso-choueri pk, abessa dm, mcnamara jc (2019). combined effects of temperature and copper on oxygen consumption and antioxidant responses in the mudflat fiddler crab minuca rapax (brachyura, ocypodidae). *comparative biochemistry and physiology part c: toxicology & pharmacology*, *223*, 35-41.

Colpo kd, lópez greco ls (2017). temperature influences the reproduction of fiddler crabs at the southern edge of their distribution. invertebrate biology, 136(2), 171-183.

Checon hh, costa tm (2018). the importance of considering small-scale variability in macrobenthic distribution: spatial segregation between two fiddler crab species (genus *leptuca*) (decapoda, ocypodidae). iheringia. série zoologia, 108.

Checon hh, costa tm (2017). fiddler crab (crustacea: ocypodidae) distribution and the relationship between habitat occupancy and mouth appendages. marine biology research, 13(6), 618-629.

Crane (1975). fiddler crabs of the world: ocypodidae: genus *uca*. vol 1276, princeton university press.

Citadin m, costa tm, netto sa (2016). the response of meiofauna and microphytobenthos to engineering effects of fiddler crabs on a subtropical intertidal sandflat. austral ecology, 41(5), 572-579.

Daleo p, ribeiro p, iribarne o (2003). the sw atlantic burrowing crab *chasmagnathus granulatus* dana affects the distribution and survival of the fiddler crab *uca uruguayensis* nobili. journal of experimental marine biology and ecology, 291(2), 255-267.

Darnell, mz, nicholson hs, munguia p (2015). thermal ecology of the fiddler crab *uca panacea*: thermal constraints and organismal responses. journal of thermal biology, 52, 157- 165.

Detto t, backwell pr., hemmi jm., zeil, j (2006). visually mediated species and neighbour recognition in fiddler crabs (uca mjoebergi and uca capricornis). proceedings of the royal society b: biological sciences, 273(1594), 1661-1666.

Detto t, jennions md, backwell, pr (2010). when and why do territorial coalitions occur?

experimental evidence from a fiddler crab. the american naturalist, 175(5), e119-e125.

Dyson ml, perez dm, curran t, mccullough el, backwell pr (2020). the role of claw color in species recognition and mate choice in a fiddler crab. behavioral ecology and sociobiology, 74(10), 1-11.

De Grande fr, colpo kd, queiroga h, cannicci s, costa tm (2018a). contrasting activity patterns at high and low tide in two brazilian fiddler crabs (decapoda: brachyura: ocypodidae). journal of crustacean biology, 38(4), 407-412.

De Grande fr, granado p, sanches fhc, costa tm (2018b). organic matter affects fiddler crab distribution? results from field and laboratorial trials. estuarine, coastal and shelf science, 212, 138-145.

De Grande fr, arakaki jy, marochi m, costa tm (2021a). cold water temperatures define the poleward range limits of south american fiddler crabs. estuarine, coastal and shelf science, 260, 107494.

De Grande fr, fogo br, costa tm. (2021b). losers can win: thermoregulatory advantages of regenerated claws of fiddler crab males for establishment in warmer microhabitats. journal of thermal biology, 99, 102952.

De Grande fr, granado p, costa tm (2021c). size-at-age or structure shift: which hypothesis explains smaller body size of the fiddler crab *leptuca uruguayensis* in northern populations? estuarine, coastal and shelf science, 254, 107358.

Fogo br., sanches fh, costa tm (2019). testing the dear enemy relationship in fiddler crabs: is there a difference between fighting conspecific and heterospecific opponents?. behavioural processes, 162, 90-96.

Galef bg, laland kn (2005). social learning in animals: empirical studies and theoretical models. bioscience, 55(6), 489-499.

Galef bg (1988). imitation in animals: history definition, and interpretation of data from the psychological laboratory. in: social learning. psychological and biological perspectives (ed. by t. r. zentall & g. galef), pp. 3-28. hillsdale, new jersey: lawrence erlbaum.

Herman cp (2015). the social facilitation of eating. a review. appetite, 86, 61-73.

Hill ps (2001). vibration and animal communication: a review. american zoologist, 41(5), 1135-1142.

Hill ps (2009). how do animals use substrate-borne vibrations as an information source? naturwissenschaften, 96(12), 1355-1371.

Horst rw (1995). feeding and social facilitation in the sand fiddler crab, uca pugilator. bios, 79-85.

Koga t, backwell pr, christy jh, murai m., kasuya e (2001). male-biased predation of a fiddler crab. animal behaviour, 62(2), 201-207.

Krumme u, keuthen h, saint-paul u, villwock w (2007). contribution to the feeding ecology of the banded puffer fish *colomesus psittacus* (tetraodontidae) in north brazilian mangrove creeks. brazilian journal of biology, 67, 383-392.

Kim tw, lee jh, choe jc (2017). not all crabs are created equal: diverse evolutionary paths of female preferences for courtship structures in fiddler crabs (genus uca). behavioral ecology and sociobiology, *71*(2), 33.

Kristensen e (2008). mangrove crabs as ecosystem engineers; with emphasis on sediment processes. journal of sea research, 59(1-2), 30-43.

Kristensen e, alongi dm (2006). control by fiddler crabs (*uca vocans*) and plant roots (*avicennia marina*) on carbon, iron, and sulfur biogeochemistry in mangrove sediment. limnology and oceanography, 51(4), 1557-1571.

Kristensen e, penha-lopes g, delefosse m, valdemarsen t, quintana co, banta gt (2012). what is bioturbation? the need for a precise definition for fauna in aquatic sciences. marine ecology progress series, 446, 285-302.

Kurta a (1982). social facilitation of foraging behavior by the hermit crab, *coenobita compressus*, in costa rica. biotropica, 132-136.

Murai m, backwell, pr (2006). a conspicuous courtship signal in the fiddler crab *uca perplexa*: female choice based on display structure. behavioral ecology and sociobiology, 60(5), 736-741.

Milner rn, jennions md, backwell pr. (2010). eavesdropping in crabs: an agency for lady detection. biology letters, 6(6), 755-757.

Milner rn, jennions md, backwell pr (2012). mantendo as aparências: os caranguejos violinistas machos acenam mais rápido na multidão. biology letters, 8 (2), 176-178.

Marochi mz, de grande fr, pardo, jcf, montenegro, á, costa tm (2022). marine heatwave impacts on newly-hatched planktonic larvae of an estuarine crab. estuarine, coastal and shelf science, *278*, 108122.

Masunari s, martins sb, marochi mz, serra ws, scarabino f (2017). morphological variability in populations of the fiddler crab *leptuca uruguayensis* (nobili, 1901) (crustacea, decapoda, ocypodidae) from south america. brazilian journal of oceanography, 65(3) 373- 381.

Mowles sl, ord tj (2012). repetitive signals and mate choice: insights from contest theory. animal behaviour, 84(2), 295-304.

Milner rn, jennions md, backwell pr (2011). keeping up appearances: male fiddler crabs wave faster in a crowd. biology letters, *8*(2), 176-178.

Mowles sl, jennions m, backwell pr (2017). multimodal communication in courting fiddler crabs reveals male performance capacities. royal society open science, 4(3), 161093.

Nabout jc, bini lm, diniz-filho ja (2010). global literature of fiddler crabs, genus *uca* (decapoda, ocypodidae): trends and future directions. iheringia. série zoologia, 100(4), 463-468.

Nielsen oi, kristensen e, macintosh dj (2003). impact of fiddler crabs (*uca* spp.) on rates and pathways of benthic mineralization in deposited mangrove shrimp pond waste. journal of experimental marine biology and ecology, 289(1), 59-81.

Nobbs m, blamires sj (2017). fiddler crab spatial distributions are influenced by physiological stressors independent of sympatric interactions. journal of experimental marine biology and ecology, 491, 19-26.

Perez dm, christy jh, backwell pr (2016). choosing a mate in a high predation environment: female preference in the fiddler crab uca terpsichores. ecology and evolution, 6(20), 7443-7450.

Penha-lopes g, bartolini f, limbu s, cannicci s, kristensen e, paula j (2009). are fiddler crabs potentially useful ecosystem engineers in mangrove wastewater wetlands?. marine pollution bulletin, 58(11), 1694-1703.

Posey mh (1987). influence of relative mobilities on the composition of benthic communities. marine ecology progress series. oldendorf, *39*(1), 99-104.

Popper an, salmon m, horch kw (2001). acoustic detection and communication by decapod crustaceans. journal of comparative physiology a, 187(2), 83-89.

Prefeitura municipal da cidade de praia grande/sp. disponível em:
<http://www.praiagrande.sp.gov.br/praiagrande/index-.asp?cd_pagina=77> acesso em: 27 de abril de 2019.

Rozas lp, lasalle, mw (1990). a comparison of the diets of gulf killifish, fundulus grandis baird and girard, entering and leaving a mississippi brackish marsh. estuaries, 13(3), 332- 336.

Reinsel ka (2004). impact of fiddler crab foraging and tidal inundation on an intertidal sandflat: season-dependent effects in one tidal cycle. journal of experimental marine biology and ecology, 313(1), 1-17.

Ryan mj, cummings me (2005). animal signals and the overlooked costs of efficacy.

evolution, *59*(5), 1160-1162.
Rosenberg ms (2002). fiddler crab claw shape variation: a geometric morphometric analysis across the genus *uca* (crustacea: brachyura: ocypodidae). biological journal of the linnean society, 75(2), 147-162.
Rossi f, chapman mg (2003). influence of sediment on burrowing by the soldier crab *mictyris longicarpus* latreille. journal of experimental marine biology and ecology, 289(2), 181-195.
Roberts l (2021). substrate-borne vibration and sound production by the land hermit crab coenobita compressus during social interactions. the journal of the acoustical society of america, 149(5), 3261-3272.
Roberts l, laidre me (2019). get off my back: vibrational assessment of homeowner strength. biology letters, 15(4), 20180819.
Ribeiro pd, iribarne oo, daleo p (2005). the relative importance of substratum characteristics and recruitment in determining the spatial distribution of the fiddler crab *uca uruguayensis* nobili. journal of experimental marine biology and ecology, *314*(1), 99-111.
Rosenberg ms (2014) contextual cross-referencing of species names for fiddler crabs (genus uca): an experiment in cyber-taxonomy. plos one 9(7): e101704.
Sanches fh, costa tm, barreto re, backwell, pr. (2018). the cost of living in mixed species populations: a fiddler crab example. journal of experimental marine biology and ecology, 500, 30-33.
Silva dja, erickson mf, santos r, pessoa dma (2022). thin-fingered fiddler crabs display a natural preference for uv light cues but show no sensory bias to other hypertrophied claw coloration. behavioural processes, 104667.
Rozas lp, lasalle, mw (1990). a comparison of the diets of gulf killifish, fundulus grandis baird and girard, entering and leaving a mississippi brackish marsh. estuaries, 13(3), 332- 336.
Saher nu, qureshi na (2017). construction of earthen structure as a sexual signals in the fiddler crabs. international journal of aquatic biology, *5*(1), 40-46.
Sanches fhc, costa tm, barreto re, backwell pr (2017). faster male displays and less complex choice are more attractive to female fiddler crabs as they reduce search costs. animal behaviour, *124*, 119-123.
Sanford e, holzman sb, haney ra, rand dm, bertness md (2006). larval tolerance, gene flow, and the northern geographic range limit of fiddler crabs. ecology, 87(11), 2882-2894.
Skov m, vannini m, shunula j, hartnoll r, cannicci s (2002). quantifying the density of mangrove crabs: ocypodidae and grapsidae. marine biology, 141(4), 725-732.
Shih ht, ng pk, davie pj, schubart cd, türkay m, naderloo r, liu my (2016). systematics of the family ocypodidae rafinesque, 1815 (crustacea: brachyura), based on phylogenetic relationships, with a reorganization of subfamily rankings and a review of the taxonomic status of *uca* leach, 1814, sensu lato and its subgenera. raffles bulletin of zoology, 64: 139–175.
Spivak ed, gavio ma, navarro ce (1991). life history and structure of the world's southernmost *uca* population: *uca uruguayensis* (crustacea, brachyura) in mar chiquita lagoon (argentina). bulletin of marine science, 48(3), 679-688.
Takeshita f, murai m, matsumasa m, henmi y (2018). multimodal signaling in fiddler crab: waving to attract mates is condition-dependent but other sexual signals are not. behavioral ecology and sociobiology, *72*(9), 140.
Thurman cl, faria sc, mcnamara jc (2013). the distribution of fiddler crabs (*uca*) along the coast of brazil: implications for biogeography of the western atlantic ocean. marine biodiversity records, page 1 of 21; vol. 6.
Truchet dm, ardusso mg, forero-lópez ad, rimondino gn, buzzi ns, malanca f, fernández-severini md (2022). tracking synthetic microdebris contamination in a highly urbanized

estuary through crabs as sentinel species: an ecological trait-based approach. science of the total environment, *837*, 155631.

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Biociências
Câmpus do Litoral Paulista

unesp IB-CLP

PARECER FINAL DO TRABALHO DE CONCLUSÃO DE CURSO

APRESENTAÇÃO REMOTA

Discente: CAROLINA GUARDINO MARTINS

Título: *"Mais importante do que ver é sentir: A influência de sinais multimodais de coespecíficos nas atividades do caranguejo-chama-maré Leptuca uruguayensis"*

Orientadora: Profa.Dra. Tânia Marcia Costa

Curso/Habilitação: Bacharelado em Ciências Biológicas/Gerenciamento Costeiro

COMISSÃO EXAMINADORA	CONCEITO
Profa. Dra. Tânia Marcia Costa	APROVADA
Dr. Rafael Campos Duarte	APROVADA

CONCEITO FINAL:

A Comissão Examinadora abaixo assinada conclui que a discente **Carolina Guardino Martins** obteve o seguinte conceito:

[X] APROVADO [ ] REPROVADO

São Vicente, 19 de janeiro de 2023.

Profa.Dra. Tânia Marcia Costa
(Orientadora)

Dr. Rafael Campos Duarte

Instituto de Biociências - Câmpus do Litoral Paulista
Praça Infante D. Henrique s/nº - CEP 11330-900 - São Vicente (SP) - Brasil
Tel. (13) 3569-7100 - graduacao.clp@unesp.br