



**UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”**



Instituto de Biociências

Campus do Litoral Paulista

**INVESTIGATING THE ROLE OF MUD STRUCTURES IN FIDDLER
CRABS: DO CHIMNEYS HAVE A FEEDING FUNCTION?**

TAINÁ MOREIRA FERREIRA

São Vicente

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Trabalho de Conclusão de Curso apresentado ao curso de Ciências Biológicas com Habilitação em Biologia Marinha da Universidade Estadual Paulista "Júlio de Mesquita Filho" como requisito para obtenção do título de bacharel em Ciências Biológicas, sob orientação da Prof.^a Dr.^a. Tânia Marcia Costa.

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RESUMO

Construções são observadas em diversos grupos de animais, desempenhando um papel crucial na sobrevivência e no comportamento dos organismos que as produzem. Além disso, essas estruturas podem influenciar processos ecológicos, como o ciclo biogeoquímico de nutrientes e a hidrodinâmica do ambiente. Os caranguejos chama-maré são organismos que constroem estruturas sedimentares, com a função variando de acordo com a espécie, sexo e estágio de vida. O caranguejo-maré *Leptuca thayeri* constrói estruturas sedimentares em forma de chaminé na entrada de suas tocas, principalmente durante os meses reprodutivos. As chaminés são mais altas nas tocas construídas por fêmeas ovígeras em comparação com fêmeas não ovígeras e machos adultos. O objetivo deste estudo foi avaliar o tempo de forrageamento e o tempo dentro da toca despendido entre machos, fêmeas ovígeras e não ovígeras. Além disso, buscou-se observar se as chaminés poderiam ser usadas como uma estratégia que permitisse as fêmeas obterem alimento durante a incubação dos ovos. Um experimento foi conduzido em campo para avaliar a diferença no tempo dentro da toca e tempo forrageando entre machos, fêmeas ovígeras e não ovígeras. Chaminés foram coletados para estimar a concentração de fitoplâncton (parte de sua alimentação) em laboratório para comparar se o fitoplâncton dentro da toca é consumido. Medidas dos caranguejos (largura da carapaça) e chaminé (altura, diâmetro de abertura, diâmetro da estrutura) foram realizadas, além da densidade do sedimento, porosidade e conteúdo de água intersticial da toca. Foram observadas diferenças na altura da chaminé, sendo a das fêmeas ovígeras a mais alta, e no tempo de forrageamento entre machos, fêmeas ovígeras e não ovígeras, onde as fêmeas ovígeras não foram observadas forrageando. Não foram observadas diferenças nas características gerais do sedimento da chaminé e nas concentrações de fitoplâncton. Essas investigações contribuíram para a compreensão da construção de estruturas sedimentares em formato de chaminé e o comportamento de forrageamento durante o período reprodutivo, demonstrando que as chaminés não são uma estratégia alimentar alternativa durante a incubação e que as fêmeas não são observadas forrageando na superfície do sedimento.

Palavras-chaves: manguezal; fenótipo estendido; construções sedimentares; alimentação.

ABSTRACT

Constructions are observed in various animal groups, playing a crucial role in the survival and behavior of their builders. The structures can influence ecological processes, such as nutrient biogeochemical cycles and environmental hydrodynamics. Fiddler crabs are organisms that build sedimentary structures, with functions varying according to species, sex, and life stage. The fiddler crab *Leptuca thayeri* constructs chimney-shaped sedimentary structures at the entrance of its burrows, primarily during reproductive months. Chimneys are taller in burrows constructed by ovigerous females compared to non-ovigerous females and adult males. The aim of this study was to assess foraging time and time spent inside the burrow among males, ovigerous females, and non-ovigerous females. Additionally, we aimed to observe whether chimneys could be used as feeding strategies. A field experiment was conducted to evaluate differences in burrow-dwelling time and foraging time among males, ovigerous females, and non-ovigerous females. Chimneys were collected to estimate phytoplankton concentration (part of their diet) in the laboratory to compare phytoplankton consumption inside the burrow. Measurements of crab (size) and chimney (height, opening diameter, structure diameter) were taken, along with sediment density, porosity, and interstitial water content of the burrow. Differences in chimney height were observed, with those of ovigerous females being the tallest, and in foraging time among males, ovigerous females, and non-ovigerous females, where ovigerous females were not observed foraging. No differences were observed in general sediment characteristics of the chimney and phytoplankton concentrations. These investigations contributed to the understanding of chimney-like sedimentary structures and foraging behavior during the reproductive period, demonstrating that chimneys are not an alternative feeding strategy during incubation and that females are not observed foraging on the sediment surface.

Keywords: mangrove; extended phenotype; sedimentary constructions; feeding behavior.

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1. Introduction

Constructions are present in a wide variety of animal groups, from invertebrates like termites, which build and maintain their mounds for multiple generations, to vertebrates like beavers, which construct large dams that cause significant alterations in river flow and flooding (Hansell, 2008). These constructions are capable of performing various functions, such as protecting early stages of ontogenetic development (e.g., egg incubation, larval stage) (De Gasperin *et al.*, 2016), providing stability to abiotic factors (Roper, 1992), retention and capture of prey (Dawkins, 1982; Beleyur *et al.*, 2021). Hansell (2000) characterizes these constructions as part and extension of the organisms that built them. According to Dawkins (1982), the behavior responsible for constructing these structures building behavior can be considered a manifestation of the builders' genes, recognized as an "extended phenotype". The manifestation of this extended phenotype does not occur in isolation and individually, but it is indeed the expression of genes at the population level (Whitham, 2003).

Fiddler crabs construct sedimentary structures and spend part of their lives in burrows on intertidal mudflats and sandflats. Each adult defends its burrow and surrounding area. They are active on the surface during low tide, feeding on bacteria, algae and detritus in the sediment surface (Zeil *et al.*, 2006). Burrows provide protection against predators and other crabs, sometimes with complex architecture, including tunnels and chambers, making it difficult for intruders to gain access (Wolcott, 1978; McLusky & Elliott, 2004). The narrow entrance acts as a barrier against larger enemies (Wolcott, 1978; McLusky & Elliott, 2004). These burrows help crabs maintain an optimal body temperature, regulate humidity, and prevent dehydration (Vernal & Terman, 1979; Smith & Bauner, 1984).

Fiddler crab burrows can also function as passive traps for suspended particles in the water column through turbulence generation (Depatra & Levin, 1989; Botto & Iribarne, 2000). There is a strong relationship between the effectiveness of traps in cylindrical shapes and the capture of particles from turbulent water flow systems (Palmer & Gust, 1985; Butman, 1986; Depatra & Levin, 1989; Yager *et al.*, 1993; Nickell & Atkinson, 1995; Botto & Iribarne, 2000). Burrows can induce turbulence in water flow creating areas of reduced velocity and vorticity, thereby increasing particle capture rates (Palmer & Gust, 1985; Butman, 1986; Depatra &

Levin, 1989; Yager *et al.*, 1993; Nickell & Atkinson, 1995; Botto & Iribarne, 2000). Areas with large concentrations of fiddler crabs are generally richer in organic matter, which may indicate the efficiency of burrows as traps for suspended particles (Botto & Iribarne, 2000; Escapa *et al.*, 2004). Evidence suggests that during the excavation and maintenance of their burrows, fiddler crabs remove sediments non-selectively to the surface (Katz, 1980; Montague, 1982; Botto & Iribarne, 2000; Mccraith *et al.*, 2003), allowing the transport of carbon (C) from deeper sediment layers to the tidal flow, exposing these particles to aerobic decomposition processes, altering the environment's biochemistry, and stimulating nutrient cycling, oxygenation, and particle distribution (Watling, 1991; Botto & Iribarne, 2000; Smith, 1991; Gutiérrez, 2006; Natálio *et al.*, 2017). These activities of fiddler crabs play an important role as ecosystem engineers (Kristensen, 2008), as they alter or maintain biotic and/or abiotic factors in the environment (Jones *et al.*, 1994), influencing not only nutrient and energy flow within the ecosystem they inhabit but also between ecosystems (Hastings *et al.*, 2007).

In addition of burrows, all genera of fiddler crabs build sediment structures above the sediment surface, with exceptions such as *Petruca panamensis* (Stimpson, 1859) and *Cranuca inversa* (Hofmann, 1874) (Pardo *et al.*, 2020). These sediment structures display a wide variety of shapes, functions, social contexts, and ecological roles not only among species but also between males and females of the same species (Christy, 1982; Pardo *et al.*, 2020). There are four types and two subtypes of sediment structures built by fiddler crabs: chimneys, hood (or shelter), which subcategorize into semi-domes and pillars, "mud balls," and "rim" (or lip) (Pardo *et al.*, 2020). Among them, chimneys are vertical wall-like structures that encircle the entire entrance of the burrow (Fig. 1) (Salmon, 1987). Currently, 26 species are known to build chimneys, including *Leptuca thayeri* (Gusmão-Júnior *et al.*, 2012; Pardo *et al.*, 2020).

Leptuca thayeri are active chimney constructors inhabiting muddy mangrove areas, thriving in both mesohaline and euryhaline environments within the middle intertidal zone (Kriegler, 2019). The species is found along the Western Atlantic coast of Florida, Gulf of Mexico, Cuba, Jamaica, Puerto Rico, Guatemala, Venezuela, Trinidad and Tobago, and Brazil (Crane, 1975; Thurman *et al.*, 2013; Farias *et al.*, 2014; Mantelatto *et al.*, 2020). In the Brazilian coastline, *Leptuca thayeri* is one of the species that occur continuously from Amapá to Santa Catarina being one of the three

most common species on the Brazilian coast (Thurman *et al.*, 2013). As a member of the fiddler crab group, *L. thayeri* exhibits apparent sexual dimorphism: females have both chelipeds of the same size, while males have one of the conspicuous chelipeds hypertrophied (Crane, 1975). Analyses of carbon and nitrogen isotopic ratios present in *L. thayeri* indicate a predominantly phytoplankton-based diet (Natálio, 2016). Experimental studies have corroborated these findings, showing that the species actively leaves its burrow during high tide, suggesting feeding on phytoplankton when submerged (De Grande *et al.*, 2018a).

On the southeast coast of Brazil, the *L. thayeri* reproduction is seasonal and occurs in the warmer months, between September and March, with a break between April and August (Costa *et al.*, 2006). The highest number of taller chimneys coincides with their reproductive periods when *L. thayeri* ovigerous females have eggs in maturation (Salmon, 1987). Both males, ovigerous and non-ovigerous females build chimneys (Shih *et al.*, 2005; Gusmão-Júnior *et al.*, 2012), but ovigerous females generally expend more energy in building larger and higher chimneys compared to non-ovigerous females and males (Gusmão-Júnior *et al.*, 2012).

Female crabs living in wetter areas or closer to rivers generally have larger and more numerous egg masses than crabs living in drier habitats (Henmi & Kaneto, 1989). Henmi & Kaneto (1989) observed that females with large egg masses are more susceptible to mechanical losses of eggs while performing surface behaviors, like walking and foraging, and thus, some species like *Leptuca pugilator* (Christy & Salmon, 1984) and *Austruca lactea* (Murai *et al.*, 1987) do not feed when ovigerous (Christy & Salmon, 1984; Murai *et al.*, 1987). Salmon (1987) noted that these species of ovigerous females defend and build burrows with a chimney shape near river margins, where they stay during the incubation period.

More specifically for the *L. thayeri*, Gusmão-Júnior *et al.* (2012) showed that ovigerous females are less frequently seen on the sediment surface, however, the feeding strategies of ovigerous females are still unknown. Salmon (1987) suggested that females of *L. thayeri* are capable of foraging in an area of 50 cm near their burrow, but much less frequently than non-ovigerous females and males. It is not known whether ovigerous females remain inside the burrow or if they are able to forage during the incubation period, which leads to considering other feeding strategies in such a vulnerable and energy demanding life-stage. If ovigerous females are less observed on the sediment surface and are the ones building the

higher chimneys, we could hypothesize that ovigerous females might be scoop sediment inside the burrow, and the chimney could serve as an extension of the foraging area. In this study, we aimed to assess whether the chimney could be used as a feeding strategy. Also, we investigated whether there are differences in foraging time and time spent inside the burrow among males, ovigerous, and non-ovigerous females.

Here, we aim (1) to evaluate the foraging time out of the burrow and the time spent inside the burrow among males, ovigerous females, and non-ovigerous females on the sediment surface; and (2) investigate whether the amount of microphytobenthos present in the sediment differs between chimney-shaped structures of different heights. We collected general information about the incubation period of *L. thayeri* and the chimneys features, including height, width, opening size. Also, sediment characteristics like porosity, density, and percentage of interstitial water were analyzed. Porosity, density and water content are characteristics that also can indicate the spaces between grains, which can serve as habitats for various microorganisms such as bacteria, microfauna and microphytobenthos. By analyzing these characteristics, we provide insights into the function of the chimneys, as well as differences in sedimentary structure characteristics between males, ovigerous, and non-ovigerous females.

2. Material and Methods

2.1. Study site

The research was conducted in a mangrove forest located in the municipality of Praia Grande, situated on the southern coast of São Paulo, Brazil (23° 59 '15"S, 46° 24' 23"W). The sediment composition is predominantly silt and clay with high organic matter content (Gusmão-Júnior *et al.*, 2012). In the species occurrence areas, densities range from 4.14 to 9.71 in July, and in the warmer months, from 4 to 6.04 individuals/m² (Checon & Costa, 2017). Our field experiment occurred in January and February 2024.

2.2. Foraging behavior

This experiment was conducted to assess differences in foraging rate outside burrows and time spent inside the burrow among males, ovigerous and

non-ovigerous females of *L. thayeri*. During the spring tide low water period, adult individuals (> 10 mm carapace length, according to Negreiros-Fransozo *et al.*, 2003) of males ($N = 10$), ovigerous ($N = 10$), and non-ovigerous females ($N = 10$) were selected from within their respective burrows and observed. Only burrows with visible chimneys were considered.

An observer was positioned 1 m from a focal animal's burrow/chimney. Then, a 5-minute acclimation period was allowed for the animal to become accustomed to the observer's presence. Subsequently, the time spent outside the burrow feeding was assessed for 10 minutes (De Grande *et al.*, 2018b). The time spent foraging was counted when the crab was picking at the sediment surface outside the burrow. On the other hand, the time inside the burrow was considered when the entire body of the crab was inside the chimney, making it impossible to observe. Additionally, the time the animals spent outside the chimney, but not foraging, such as when they were walking or waving, was also recorded but not considered here. For this study, only the time spent foraging and inside the burrow were considered and the duration of each behavior was recorded using a stopwatch. All observers received the same instructions and trials were taken prior to observations.

After the observations, measurements of height (h), width (w), and opening of the chimney were taken, the chimney's sediments were collected for further analysis, and the crabs were collected for carapace size measurement and egg mass verification. No chimney was repeated. Chimneys were collected and placed in dark jars, shielded from light for further laboratory analysis, and they were measured using a caliper (mm). A structure exposed above the sediment line was considered as a chimney (Fig. 1). Following the collection of the chimneys, the crabs were collected by digging out each burrow using a garden shovel. Data on the species and sex of the crabs, whether females carried eggs, and their carapace width (CW) were recorded. Carapace width was collected using a caliper. Crabs were classified as adults ($CW > 10$ mm) and juveniles ($CW \leq 10$ mm, Negreiros-Fransozo *et al.*, 2003).



Figure 1: Sedimentary structure in chimney shape built by male (A), non-ovigerous (B) and ovigerous female (C) *Leptuca thayeri*.

2.3. Phytoplankton concentration in chimney sediment

Since ovigerous females are rarely observed outside their burrows, it is possible that they feed on sediment within their own burrow and its associated chimney. Here we investigate whether there are differences in the amount of chlorophyll *a*, an important component of the diet of this species provenient of microalgae, between males, ovigerous and non-ovigerous females in their respective chimneys. To assess the amount of microalgae/microphytobenthos present in the chimney sediment of males (N = 10), ovigerous (N = 10), and non-ovigerous females (N = 10), the concentration ($\mu\text{g/g}$) of chlorophyll *a* was quantified.

A subsample of sediment from the chimney collected in the field (approximately 0.5 g each) was transferred into a test tube containing 5 ml of 90% ethanol. The test tubes were shaken for about 30 seconds. The sediment was then extracted overnight in darkness (at 4°C). After the extraction was completed, the test tubes were centrifuged for 30 minutes at 3000 rpm. The absorbance of the supernatant was measured at 665 and 750 nm before and after acidification with one drop of 2 M HCl and mixing. The chlorophyll *a* content was calculated according to the method of Parsons *et al.* (1984).

2.4. Sediment characteristics

Sediment density, porosity, and water content are crucial parameters for understanding microbial ecology in sedimentary environments. Sediment density is influenced by both water content and porosity, the latter referring to the volume of

void spaces within the sediment. These void spaces are significant as they may be occupied by organisms such as phytoplankton, which form part of the diet for the crab species *Leptuca thayeri*. To determine sediment density between males (N = 10), ovigerous (N = 10), and non-ovigerous females (N = 10), a 5-ml plastic syringe, pre-weighed, was filled to the 5-ml mark with sediment, carefully ensuring no air was trapped. The syringe was subsequently re-weighed to calculate the weight of the sediment. Density was then calculated using the formula: $d = m/5$ (g cm⁻³), where 'm' represents the sediment weight (the final weight of the syringe minus the weight of the empty syringe). After weighing, the sediment was oven-dried at 60 °C to determine water content, comparing the pre-dried wet weight to the dry weight after approximately 24 hours. Water content (β) was calculated using the formula: $\beta = [(ww - dw)/ww] \cdot 100$ (%), where 'ww' denotes the wet weight, 'dw' denotes the dry weight, and the weight of the aluminum tray was subtracted. Porosity (f) was subsequently calculated with the formula: $f = \beta \cdot d/100$.

3. Statistical Analyses

The normality and homogeneity of variances were assessed using the Shapiro-Wilk and Bartlett tests, respectively. The tests indicated that the chimney high and the data of behavior experiments exhibit homogeneous variances but did not conform to a normal distribution.

Regarding the behavioral experiment data, ovigerous females were not observed outside the chimney, meaning all results were zero. Therefore, there were no results to compare against the averages. Consequently, we opted to compare the female and male treatments using a t-test.

A General Linear Model (GLM) test was used to identify significant differences and compare the chimney high among the treatments. Chimney width and opening was applied to observe if statistics differences are present using a Variance Analysis (ANOVA). The Tukey post hoc test was used to identify significant differences and compare the treatments.

The non-parametric Kruskal-Wallis test was employed to determine if there was a statistical difference in the chlorophyll a concentrations. Porosity, density, water content, and phytoplankton concentration exhibit homogeneous variances and a normal distribution. In this case, a Variance Analysis (ANOVA) was applied to observe statistical differences. The Tukey post hoc test compares the treatments. In

all tests, a significance level (α) of 0.05 was utilized. All tests were performed in the open-source software R.

4. Results

4.1. Foraging Behavior

The carapace width (CW) in *Leptuca thayeri* males was $21,0 \pm 0,6$ (mean and standard deviation) and chelipod was $17,5 \pm 0,05$. In ovigerous females, the CW was $17 \pm 0,05$ and non-ovigerous females was $15,8 \pm 0,02$.

Males (5.20 ± 4.0 min) and non-ovigerous females (6.21 ± 3.8 min) of *Leptuca thayeri* have spent similar amounts of time foraging outside burrows ($t_{18} = 0.57541$, $p = 0.57$).

Ovigerous females were not observed foraging outside the burrow and, therefore, were the group observed spending the most time inside the burrow (9.41 ± 1.9 min). Non-ovigerous females spent an average of 4.02 ± 3.6 minutes inside the burrow, while males spent 4.11 ± 4 minutes. A t-test indicated no statistically significant difference between these two groups ($t(18) = -0.056379$, $p = 0.96$).

4.2. Chimney characteristics across sex and reproductive stage

Overall the *Leptuca thayeri* chimney structure (width and opening) was similar among male (width = 3.39 ± 1 cm; opening = $1,71 \pm 1$ cm), ovigerous (width = 3.53 ± 0.6 cm; opening = 1.73 ± 0.5 cm) and non-ovigerous females (width = 4.05 ± 0.6 cm; opening = 1.76 ± 0.4 cm) (Fig. 3 and Fig. 4, no statistical differences were observed between sexes (width = ANOVA: $F = 1.92$, $MS = 1.1910$, $p = 0.166$; opening = ANOVA: $F = 0.026$, $MS = 0.0062$, $p = 0.975$). However, the chimney height differs (Fig. 1), being higher in ovigerous females (2.37 ± 0.7 cm) than males (1.18 ± 0.5 cm) and non-ovigerous females (1.56 ± 0.4 cm) (Δ Deviance = 4.223, $Df = 2$, $p < 0.001$).

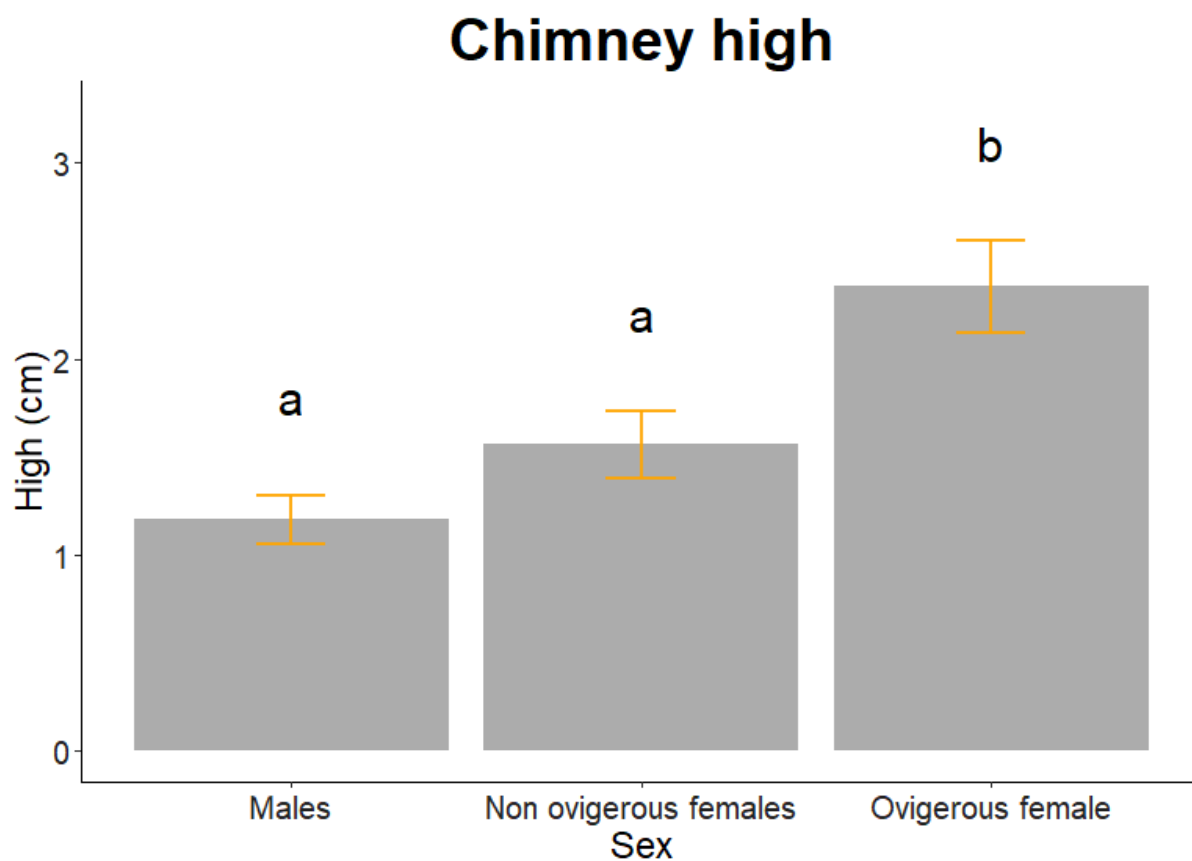


Figure 2: Mean high of the chimney-shaped sedimentary structure built by individuals of the species *Leptuca thayeri*. The different letters above the mean bar indicate that there was a difference between the treatments (Tukey's test, $P < 0.05$). The bars above the mean indicate the standard deviation.

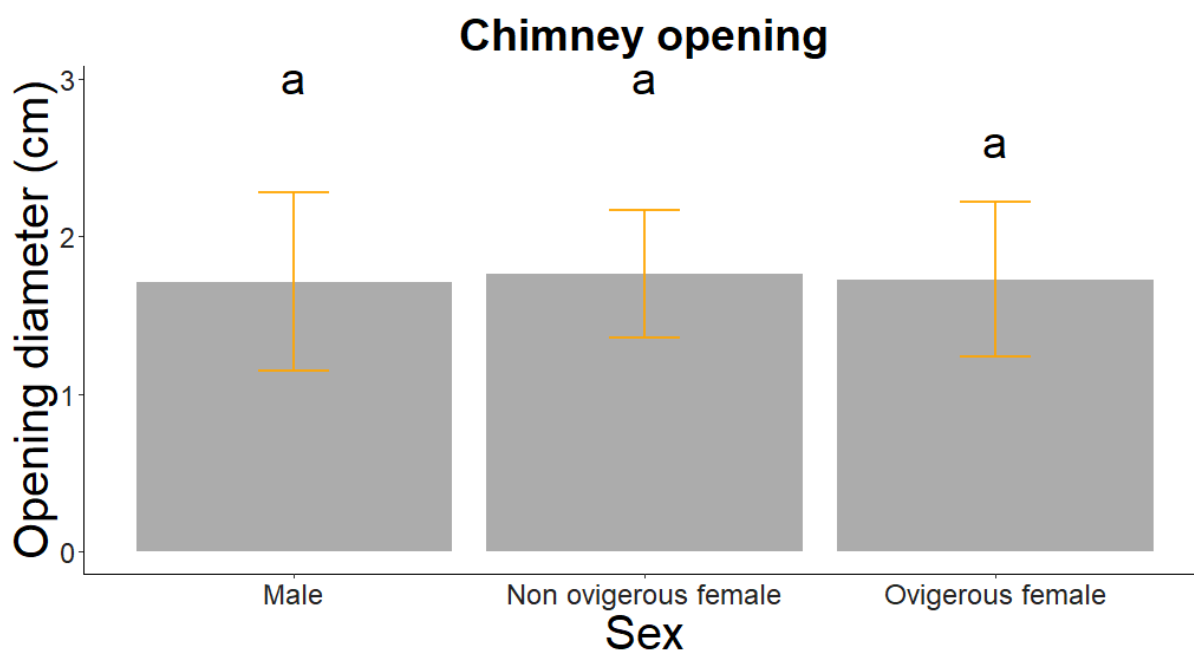


Figure 3: Mean opening diameter of the chimney-shaped sedimentary structure built by individuals of the species *Leptuca thayeri*. The identical letters above the mean bar indicate that there was no difference between the treatments (Tukey's test, $P < 0.05$). The bars above the mean indicate the standard deviation.



Figure 4: Mean width of the chimney-shaped sedimentary structure built by individuals of the species *Leptuca thayeri*. The identical letters above the mean bar indicate that there was no difference between the treatments (Tukey's test, $P < 0.05$). The bars above the mean indicate the standard deviation.

4.3. Phytoplankton concentration in chimney sediments

The concentration of phytoplankton in the sediment of the *Leptuca thayeri* chimneys in males was $5 \pm 2.3 \mu\text{g/g}$, non-ovigerous females was $5 \pm 1.9 \mu\text{g/g}$ and ovigerous female was $4.12 \pm 2.5 \mu\text{g/g}$ (Fig. 4). No statistical differences were observed between males, ovigerous and non-ovigerous females (ANOVA: $F = 2.752$, $MS = 0.13812$, $p = 0.0817$).

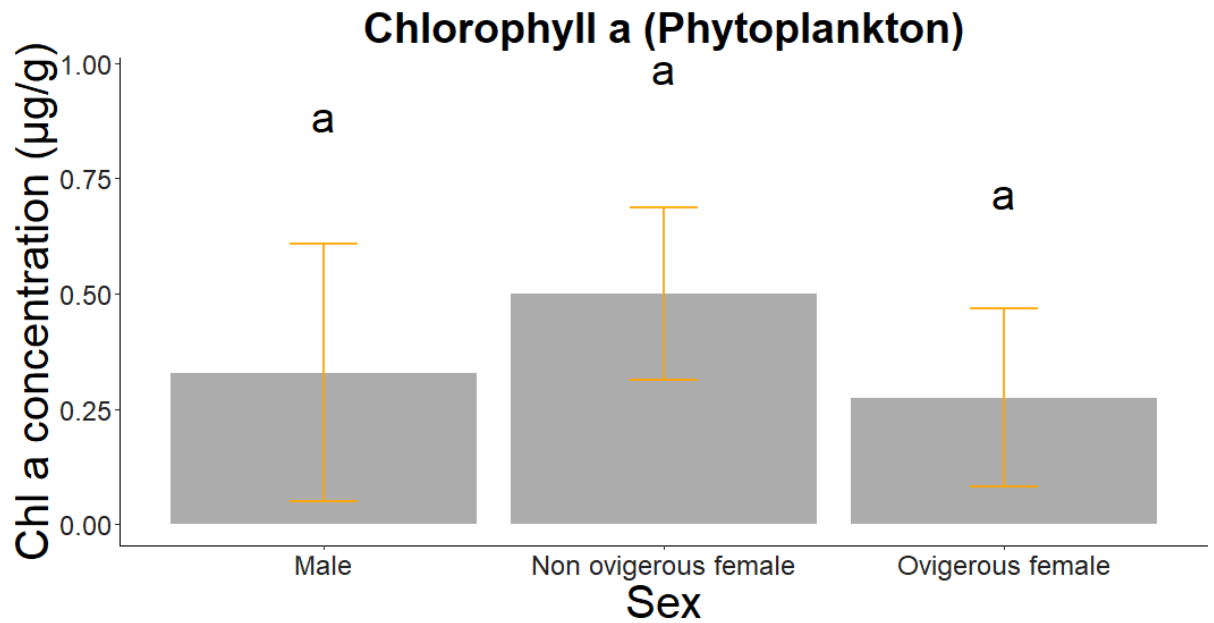


Figure 4: Mean chlorophyll a concentration of the chimney sediment built by individuals of the species *Leptuca thayeri*. individuals of the species *Leptuca thayeri* (Tukey's test, $P < 0.05$). The bars above the mean indicate the standard deviation.

4.4. Sediment characteristics

The sediment porosity or spaces between the grains of *Leptuca thayeri* species in males was 0.59 ± 0.19 , in ovigerous females was 0.48 ± 0.09 and non-ovigerous females was 0.61 ± 0.15 . There were no significant statistical differences between treatments (ANOVA: $F = 2.739$, $MS = 0.05663$, $p = 0.0826$).

The analysis of the sediment density of the chimneys observed that males was 1.15 ± 0.09 g/cm³, ovigerous females was 1.11 ± 0.06 g/cm³ and non-ovigerous females was 1.12 ± 0.04 g/cm³. The average density was very similar among groups, with no statistical differences observed (ANOVA: $F = 1.045$, $MS = 0.004325$, $p = 0.366$).

The analysis of the percentage of water between the grains, or interstitial water in chimneys of males was $44.215 \pm 18.99\%$, in ovigerous females was $41.943 \pm 9.69\%$ and non-ovigerous females was $52.055 \pm 18.25\%$. No significant statistical difference was observed between treatments (ANOVA: $F = 1.072$, $MS = 281.5$, $p = 0.357$).

5. Discussion

Feeding is a vital behavior to gain the energy required for various activities, particularly reproduction. Animals tend to spend less time feeding and more time on reproductive activities when the chances of selecting a mate and achieving reproductive success are higher, such as during the breeding season (Schoener, 1971). Conversely, they allocate more time to feeding and less to reproduction when the chances of reproductive success are lower, typically observed during the non-breeding season (Schoener, 1971; Caravello & Cameron, 1991). Sex, body size, and parental investment are significant factors that influence behavioral decisions in animals (Salmon & Hyatt, 1983). Males devote more time to reproductive activities compared to females (Salmon & Hyatt, 1983). Females spend more time feeding to gather energy for producing eggs and increasing the number of egg clutches (Salmon & Hyatt, 1983), while males focus more on feeding to gain energy for courtship displays (Milner *et al.*, 2012). In our foraging behavior experiment, ovigerous females spent the entire observation period inside the burrow, while females and males spent most of their time foraging. While Salmon (1987) noted that ovigerous females of this species were able to forage in an area near their burrows, here no ovigerous female was observed foraging outside their burrows. This suggests potential variability in foraging behavior among different populations or under different environmental conditions, like availability of food or temperature.

Murai *et al.* (1987) observed that the intertidal zone where a species lives can influence the duration of the incubation season. Comparing other species of Ocypodidae, such as *Marsupenaeus japonicus*, *Scopimera globosa*, and *Ilyoplax pusillus*, differences are observed in incubation strategies and foraging behavior. These differences appear to be related to the environmental conditions of the species' habitats, such as sediment type and food availability. Species in lower intertidal zones, with finer sediments and rich in organic matter, such as *M. japonicus*, have longer incubation seasons, while species in higher zones, like *S. globosa* and *L. pusillus*, have shorter incubation periods. This is likely due to incubation taking place in burrows, making it advantageous to lay eggs during the summer. The shorter incubation period at higher temperatures allows these species to temporarily forgo foraging for a shorter duration. *Leptuca thayeri* lives in the mid-intertidal zone, where sediment grains are still fine and rich in organic matter. In this study, many females had eggs in intermediate and final stages of development, suggesting that embryonic

development in this species occurs rapidly, the same pattern was observed in Costa & Negreiros-Fransozo (2006) study. So, ceasing to feed for a brief period to ensure the success of eggs hatching can be an interesting strategy. Also, could resemble the behavior observed by Murai *et al.* (1987) for species with short incubation periods, and the behavior works like species *L. pugilator* (Christy & Salmon, 1984) and *A. lactea* (Murai *et al.*, 1987) who do not forage during the egg incubation period.

In our study, the individuals observed in the field occupied a shaded area covered by trees, with the presence of pneumatophores and other fiddler crab populations. In contrast, the individuals observed by Salmon (1987) occupied an area where a clearing had been recently made, with the presence of trash and oyster shells. Here, in an environment that retained more characteristics of its original state, ovigerous females were observed incubating throughout the period within the chimney. In Salmon's (1987) study, where the environment had significant alterations, females were observed foraging. We can suggest, therefore, that *L. thayeri* may have a mixed incubation strategy, adopting alternative strategies when the environment undergoes changes or alterations. Thus, not foraging during incubation might be feasible, given the short incubation period of the egg mass (Salmon, 1987).

Salmon's (1987) study observed that behavior for a few hours of videos, while our study observed individuals for 10 minutes in the field, potentially resulting in ovigerous females not being observed foraging due to a methodological limitation. It is worth noting too that only one female was observed leaving the burrow for a few seconds before re-entering, but it did not engage in foraging behavior. Despite Salmon (1987) observing ovigerous females foraging outside the burrow, even with longer observation times and a much larger number of individuals observed, the occurrence of ovigerous females foraging was very low. This corroborates with the behavior found in our study, which showed a very low occurrence of ovigerous females on the sediment surface.

In the comparison of the height of the chimneys the ovigerous females differed from the other treatments, demonstrating, as observed in previous studies (Slatyer *et al.*, 2008; Gusmão-Júnior *et al.*, 2012), that ovigerous females build higher chimneys. However, the width and size of the opening did not differ. The characteristics of the sediment composing the chimney were also not different, as the sediment originates from the same mid-intertidal zone. The hypothesis that females feed by foraging the sediment inside the chimney was tested by chlorophyll concentration and was

refuted. If ovigerous females were foraging phytoplankton present in the chimney, the concentration found in the sediment of ovigerous females' chimneys would be lower than in the males and non-ovigerous females chimneys, which was not observed. The characterization of sediment porosity also refuted the hypothesis that there could be differences between chimneys of males and non-ovigerous females chimneys comparing with the ovigerous females chimneys, as there would be more spaces between the grains if microphytobenthos were being consumed by ovigerous females, which was also not observed. Since all chimneys originate from sediment of the same source, variables such as density and percentage of interstitial water also did not differ among the treatments. It is important to note that this species is also capable of feeding during high tide (De Grande, 2018), and our study only evaluated foraging behavior during low tide. This raises questions about the relevance of this feeding behavior in the species' feeding habits. All the characteristics for evaluating whether the chimneys could be an alternative feeding strategy during the incubation period were refuted.

Slatyer *et al.* (2008) investigated the species *Leptuca capricornis* to determine if chimneys conceal the burrow entrance from intruders who might otherwise usurp the burrow. The study observed that chimneys reduce the rate at which intruders locate burrow entrances, not because intruders avoid chimneys—indeed, they frequently approach and feed on the structure—but because they do not associate a chimney with a burrow entrance, which likely reduces burrow loss rates. Slatyer *et al.* (2008) also discusses that chimney owners were more likely to be females. He suggests that females may benefit more from disguising their burrows, as they have a lower ability to fend off intruding males (deRivera *et al.*, 2003). It was also observed that chimney builders fed less than non-owners, indicating that well-fed crabs, needing to forage less, may better afford the time and energy costs associated with chimney construction. Chimneys in *Leptuca subcylindrica* may act as temperature or humidity regulators (Thurman, 1984), the only species so far where the adaptive value of chimneys is determined. This temperature and humidity regulation function was also proposed by Gusmão-Júnior (2012) for chimneys in *Leptuca thayeri*. Our study suggested a potential feeding function: we hypothesized that the chimney could act as a food stock for ovigerous females, which are less frequently observed foraging on the sediment surface. However, this hypothesis was not corroborated, in

other words, our work did not find sufficient evidence to indicate the chimney as a structure serving a feeding function.

6. Conclusion

Construction of chimneys cannot be considered an alternative feeding strategy for obtaining food during egg incubation. Particularly, considering that the species has a foraging period for energy accumulation and the brief incubation period, not foraging during this time could be a strategy to ensure development and to avoid mechanical egg loss during surface behavior, given its large egg mass. Additionally, the chimney did not prove to be a feeding strategy within the burrow, as the chlorophyll *a* stock resulting from phytoplankton, which is part of *L. thayeri* diet, did not differ between treatments. This study has highlighted the importance of studying potential reproductive and feeding strategies and has contributed to understanding the function of the chimney structure. Future observations on chimney functions are desirable to complement the knowledge of a key species in an ecologically important environment.

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PARECER FINAL DO TRABALHO DE CONCLUSÃO DE CURSO

Discente: TAINÁ MOREIRA FERREIRA

Título: "Investigating the role of mud structures in fiddler crabs: do chimneys have a feeding function?"

Orientador: Profa. Dra. Tânia Marcia Costa

Curso/Habilitação: Bacharelado em Ciências Biológicas/Biologia Marinha

COMISSÃO EXAMINADORA	CONCEITO
Profa. Dra. Tânia Marcia Costa	APROVADA
MSC Marcio Camargo Araújo João	Aprovado

PARECER:

Declaro que a discente Tainá Moreira Ferreira, bem como seu trabalho de conclusão de curso, estão aptos a serem aprovados e a discente tem mérito para ser graduada como Bacharel em Ciências Biológicas, com habilitação em Biologia Marinha.

CONCEITO FINAL:

A Comissão Examinadora abaixo assinada conclui que a discente **Tainá Moreira Ferreira** obteve o seguinte conceito:

☒ APROVADO

☐ REPROVADO

São Vicente, 01 de julho de 2024.


Profa. Dra. Tânia Marcia Costa


MSC Marcio Camargo Araújo João