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Examinando os efeitos de estressores climáticos na motivação de forrageamento e comunicação química em crustáceos decápodes

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Coorientação: Dr. Fernando Rafael De Grande

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SUMÁRIO

APRESENTAÇÃO

Os ambientes do entremarés são considerados ricos em diversidade e são frequentemente influenciados por variáveis bióticas e abióticas. Nesses habitats, as poças de maré em costões rochosos passam por variações significativas nas condições abióticas devido aos ciclos alternados de submersão e emersão. Em razão do seu tamanho reduzido, essas poças possuem baixa inércia térmica e respondem rapidamente às mudanças de temperatura, pH e níveis de oxigênio durante as marés baixas. Isso pode resultar em altas temperaturas, queda de pH e condições de hipóxia, criando ambientes próximos aos limites de sobrevivência para os organismos que as habitam. Considerando o cenário atual de mudanças do clima, é de suma importância entender como que os organismos que habitam esses ambientes irão responder a intensificação dessas variáveis. Apesar das poças de maré serem ambientes menores em relação à extensão total dos costões rochosos, é importante ressaltar que a dinâmica que ocorrem nesse habitat pode nos auxiliar no entendimento da relação entre as espécies e variáveis ambientais em diferentes níveis de complexidade dos organismos. As oscilações drásticas das variáveis abióticas fazem com que os organismos que habitam as poças de maré sejam um ótimo modelo para testarmos a interferência de estressores climáticos. As interferências negativas dessas relações podem gerar efeitos em cascata no ambiente, podendo ter impactos em níveis ecossistêmicos colocando em risco a diversidade biológica. Dessa forma, o primeiro capítulo da minha dissertação de mestrado inclui o projeto desenvolvido no Brasil (Processo FAPESP 2021/07565-0) durante o período de 01/08/2021 a 01/08/2023. A primeira fase do projeto realizado no Brasil foi a etapa de “Caracterização ambiental”, realizada nos costões rochosos da praia dos Milionários, em São Vicente-SP, com o objetivo de coletarmos dados abióticos de temperatura, pH e salinidade, a fim de definirmos as variáveis que foram utilizadas nos experimentos subsequentes no laboratório. Esta etapa ocorreu durante o verão, período do ano em que são registradas as maiores temperaturas. Após a coleta, os dados foram analisados e foram calculados os valores médios, mínimos e máximos, bem como a taxa de variação ao longo do tempo das variáveis temperatura, salinidade e pH. Foram escolhidos valores médios e projetados de temperatura de acordo com a etapa de caracterização ambiental, afim de definir os tratamentos do segundo experimento em laboratório. Como objetivo geral, nosso segundo experimento pretendia investigar a capacidade dos caranguejos de detectar predadores em um cenário de aumento de temperatura. Mais especificamente, buscamos entender se o aumento da temperatura poderia afetar a capacidade de reconhecimento e, consequentemente, expor os caranguejos a um maior risco de predação durante o forrageamento em poças de maré. Nosso resultados demonstraram que os caranguejos não perceberam a pista química do peixe *B. soporator*. Dessa forma, para dar andamento a investigação, o terceiro experimento teve como objetivo compreender o efeito do aumento de temperatura sobre a motivação de forrageamento do caranguejo. Nesse caso, ao invés de dispormos de tratamento com e sem a presença de pistas químicas do predador, nós tivemos tratamentos com temperatura média e temperatura de previsão. Nosso delineamento envolveu uma abordagem que considerou tanto a possibilidade dos caranguejos serem presas de predadores em poças de maré quanto a busca por alimento nesses ambientes, permitindo a obtenção de dados, mesmo que nosso modelo não tenha sido capaz de identificar previamente a presença do peixe. Nosso resultados demonstraram que o caranguejo *P. transversus* não identificou a pista química do predador *B. soporator* e o aumento da temperatura diminui significativamente sua motivação de forrageamento. Ao estudarmos um ambiente dinâmico como as poças de maré, foi importante levar em consideração o fato de que as principais variáveis relacionadas com as mudanças do clima ocorrem de maneira simultânea. Nesse sentido, o segundo capítulo da dissertação foi composto por um estudo no qual avaliamos como o aumento de temperatura e hipóxia, em poças de maré, podem influenciar a percepção química do caranguejo *Carcinus maenas* em relação a presença da presa *Mytilus edulis*. Esse estudo foi realizado durante o estágio de pesquisa no exterior, na Universidade de Bangor, País de Gales, Reino Unido, (Processo BEPE-FAPESP 2021/07565-0) sob a supervisão do Prof. Dr. Stuart Rees Jenkins, sendo um projeto associado ao estudo que compôs o primeiro capítulo da dissertação. Nossos achados demonstraram que o caranguejo *Carcinus maenas* foi capaz de detectar a presença da presa *Mytilus edulis* mesmo sob condições de aumento de temperatura e hipóxia induzida por nitrogênio. As conclusões desses dois estudos ajudaram a entender como as mudanças climáticas podem afetar as interações entre predadores e presas em costões rochosos. A incapacidade de *P. transversus* de detectar predadores e a resiliência de *C. maenas* em condições adversas destacam a importância de investigar as respostas específicas das espécies para prever mudanças na dinâmica ecológica desses ambientes.

Capítulo 1

Temperature-driven changes in foraging motivation of *Pachygrapsus transversus*

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ABSTRACT

Based on IPCC projections, an increase of up to 4°C in mean temperatures is expected by the end of the century (2100). Climate change poses significant threats to coastal ecosystems, with projected increases in temperature, salinity, and pH levels impacting trophic relationships and predator-prey dynamics. This study investigates the responses of coastal organisms to these stressors, particularly focusing on temperature variations in tide pools. Using field data, we characterized environmental variables such as temperature, pH, and salinity within tide pools, highlighting fluctuations and their potential ecological implications. Tide pools exhibited an average temperature of 35°C, the pH was 8.6, and the salinity was 34. At high diurnal tide, the average temperature was 31°C, the pH was 8.3, and the salinity was 33. Subsequently, we conducted experiments to assess the foraging behavior of the crab *Pachygrapsus transversus* in response to chemical cues from the predator *Bathygobius soporator* under variable temperature conditions. The results indicated that while chemical cues did not significantly influence the crab's foraging motivation, higher temperatures led to reduced foraging activity, suggesting potential alterations in ecological dynamics. This scenario is likely to lead to a decline in the crabs' survival rate, which could in turn alter the structure and function of tide pool ecosystems.

Keywords: tide pool dynamics, species response, coastal ecosystems, environmental stressors, climate change impacts

1 INTRODUCTION

Organisms inhabiting the intertidal zone demonstrate remarkable tolerance to extreme environmental conditions throughout the tidal cycle and during periods of diurnal low tide (Massa et al., 2009; Luck et al., 2010; Woolway et al., 2021). For example, species from tropical environments may face temperatures exceeding 50°C in the summer (Vinagre et al., 2018; Little et al., 2020). Consequently, rocky shore inhabitants contend with conditions near their physiological tolerance limits, typically ranging between 40-50°C for most species (Sunday et al., 2012; Culler et al., 2015; Xi et al., 2016; Vinagre et al., 2021). Indeed, considering their heightened susceptibility to temperature extremes compared to species in different habitats, intertidal organisms are highly vulnerable to climate change impacts (Coutinho et al., 2016). Tide pools, for example, are mesocosms that frequently occur on rocky shores and are influenced by environmental variations during the tidal cycle (Trussell, 2004; Vinagre et al., 2021). Due to their low thermal inertia, recent studies indicate that tide pools can experience extreme temperature variations, heating up quickly during low tide (Vinagre et al., 2021). In this sense, tide pools are considered an ideal microenvironment for studying the effects of temperature on trophic relationships, providing insights into the complexities of these dynamics within a specific habitat (Hawkins, 2008; Steckbauer et al., 2020). Exploring how temperature affects interactions in tide pools provides a detailed insight into how organisms cope with extreme conditions. Understanding these complexities not only sheds light on intertidal species resilience but also provides valuable insights into broader ecological dynamics, such as those found in rocky shore ecosystems.

The interactions between prey and predators significantly shape the structure and function of various ecosystems, particularly influencing community composition and species distribution within their habitats (Kroeker et al. 2013; Asakura, 2021). In the marine environment, prey-predator interaction can occur in various ways, one of which is through chemical signals in the water. These signals can act as alerts or indicators, such as the presence of a predator or food, enabling marine organisms to interact with their environment more effectively (Hay, 2009). These signals are transmitted by predators or by conspecific or heterospecific prey, and can be perceived by both, depending on the environmental conditions these organisms face (Skelhorn & Rowe, 2016; Draper & Weissburg, 2019).

Within the tide pool system, it is possible to observe the existence of a complex trophic network between organisms (Mendonça et al., 2018). Among them, the crab *Pachygrapsus transversus* (Gibbes, 1850) is a semi-terrestrial species very common on rocky shores which is active during low tide (Abele et al., 1986; Christofoletti et al., 2010). To escape of predators *P. transversus* usually use small crevices in the rocks as shelter (Abele et al., 1986; Christofoletti et al., 2010). This crab species has a varied diet, feeding on macroalgae, small crustaceans and fish (Abele et al., 1986; Flores & Negreiros-Fransozo, 1999). Generally, *P. transversus* forages outside the water, on surfaces emerged from rocks during low tide (Abele et al., 1986). When foraging outside the water, their exposure to aquatic predators is reduced, as for example the frillfin goby *Bathygobius soporator* (Tomida et al., 2012; Soares, 2016). However, we have observed *P. transversus* foraging underwater in tide pools where, apparently, no predatory fish was present (personal observation). Given this observation, we hypothesize that early detection of predatory fish could confer an adaptive advantage for these crabs. Since the fish exhibits effective camouflage in its environment and tends to hide in tide pool crevices, and considering that chemical communication is a widely used strategy among aquatic organisms, we posit chemical perception as a potential means for the crab to identify the predator.

Little is known about the dynamics of temperature variation in tide pools and how this variation can affect the organisms that live there (Marangon et al., 2020). The present study can contribute to filling this gap in the literature, elucidating how coastal organisms will respond to this stressor, considering the forecasts for the end of the century. In a first experiment, we monitored in the field the temperature of tide pools during the summer. Then, we use the average rate of these variables as a reference to test the *P. transversus* ability to detect chemical cues from the tidepool predator *B. soporator* in the laboratory. After not observing a response from the crab *P. transversus* to the odor of the predator *B. soporator* in our second experiment, we conducted a third experiment focused exclusively on the effect of temperature increase. In this third test, we found that under elevated temperature conditions, there was a decrease in the crab's motivation to forage. These results indicate that, even in the face of the apparent lack of chemical perception of the crab to the predator, the temperature increase triggers a less active foraging behavior. This suggests that temperature increase can directly influence the foraging activities of the crab. We discussed the effects of the temperature-induced increase in foraging behavior in a species that seemed not to perceive the odor of its predator. These observations provide valuable insights into the complex interactions between temperature, foraging behavior, and predation, emphasizing the crucial importance of understanding adaptive responses in coastal environments, especially in light of projected climate changes.

2 MATERIAL AND METHODS

2.1 Study area

The study was conducted on the rocky shores of Praia dos Milionários (23°58'33.6"S 46°22'21.4"W), situated in the municipality of São Vicente/SP. The study area is characterized by a dissipative beach system with jagged rocky shores and a semidiurnal tidal regime (Farinnaccio et al., 2009). The average air temperature is 21.8°C, and the annual precipitation averages 2,148 mm (Climatempo, 2021).

2.2 Characterization of the environmental variables of the tide pool

To characterize the environmental variables in the tide pools of the rocky shores of Praia dos Milionários, we sampled water temperature, pH, and salinity. Samples were collected from five different tide pools during low tide and high tide periods on sunny days in the summer season. Measurements of temperature, salinity, and pH were taken hourly over 4 days between January 2022 and March 2022. Collection was consistently performed at the same tide pools. We categorized the collections into two groups: daytime high tide and daytime low tide, recording data separately for each. Regardless of the start and end times of sampling during high and low tides, the total period spanned approximately 3 hours per day. We defined low tide as values up to 0.6 m, when the tide pool was no longer connected to the sea, and high tide as values above 0.9 m, when connection was restored and pools were submerged. For temperature measurements, we used iButton Dataloggers (Hobo MX2201), placed at the bottom of the tide pools to detect and transmit temperature data every minute to the system. The pH of the tide pools was measured using a pH meter (Gehaka PG1400), and salinity was measured using a refractometer (Kasvi - ACT salinity refractometer), with both parameters sampled every hour for approximately 3 hours. Although *P. transversus* is a semi-terrestrial species, our focus was exclusively on water parameters. We based this decision on observations that crabs feed within the tide pools, suggesting that critical predator interactions may occur during foraging within these pools.

2.3 **Sampling of organisms**

We studied the species *Pachygrapsus transversus*. Adult crabs of both sexes were used, with a mean maximum carapace width ranging from 10 mm to 15 mm (Flores & Negreiros-Fransozo, 1999a). As a predator model, we selected the fish *Bathygobius soporator*, ranging in size from 7 cm to 10 cm in length. Both models were collected from rocky shores during low tide. Crabs were collected using active collection techniques and were housed and transported in 5L buckets with a thin layer of water and PVC pipes for shelter. Fish were captured in tide pools using baited fish traps and transported in 15L plastic buckets filled with seawater and portable aerators to the laboratory.

2.4 **Animal stock maintenance**

During the acclimation period of *P. transversus* and *B. soporator*, abiotic variables were strictly controlled, maintaining a temperature of 31°C, salinity at 35, and pH at 8.3, according to parameters observed during the field characterization phase (Section 6.1, Figure 4a). For the maintenance of *P. transversus*, we used 22-liter aquariums (40 x 24 x 23 cm), filled halfway with artificial seawater (11 liters). Each aquarium featured a 15 cm high concrete platform and a biological filter consisting of a 200 ml perforated container filled with biological media and shell gravel, to control nitrate levels (<1 ppm), ammonia (<0.5 ppm), and nitrite (<0.5 ppm) (adapted from Pereira et al., 2017). A PVC pipe shelter with small perforations, 60 mm in diameter and 15 cm in length, was connected to the upper region of the platform, containing a trapdoor (Figure 1). The shelter was positioned above the platform so it could be filled with up to 0.5 cm depth of artificial seawater (Figure 1). This allowed crabs, when conditioned to the shelter, to have contact with seawater without being completely submerged. To facilitate the tracking of crab movement, 5 cm by 5 cm quadrants were drawn on the outer glass surface of the aquariums. These same aquariums were used during the acclimation period and subsequent experiments.

Specimens of *B. soporator* were maintained in a 150-liter tank of artificial seawater, equipped with PVC pipes to simulate shelters, during a 15-day acclimation period. Additionally, we used 3 aerators (BOYU/sc-3500) for water circulation and oxygenation, along with a biological filter consisting of a 15-liter bucket filled with biological media and shell gravel to control nitrate levels (<1 ppm), ammonia (<0.5 ppm), and nitrite (<0.5 ppm) (adapted from Pereira et al., 2017). The photoperiod was kept constant with a 12-hour light and 12-hour dark cycle for both crabs and fish.

2.5 Chemical cue collection from the fish *Bathygobius soporator*

To collect the chemical cue from the fish, we used 22-liter aquariums (40 x 24 x 23 cm) to house two fish, following the ratio of 2g/L of fish (Barrilli et al., 2021), over a period of 15 days. The fish were fed once daily with 4% of their biomass (Menezes et al., 2003) using macerated pieces of the crab *P. transversus.* We chose to feed the fish with *P. transversus* instead of fish food because the diet of the fish can influence the production of chemical cues. Conventional feed could introduce specific odors into the water, interfering with the interpretation of results on chemical recognition between predator and prey (Ferrari et al., 2010). After the acclimation period, we performed a complete water change in the aquarium, leaving the fish for 72 hours in artificial seawater without feeding them during this period. This approach was adopted to ensure that the collection of the fish's chemical cue was not affected by other interfering substances in the production of the chemical cue. The collection of the fish chemical cue samples was conducted based on the methodology of previous studies (Miyai et al., 2016; Arvigo et al., 2019), which involved withdrawing 3.5 liters of water from the aquariums containing the chemical cue of the fish *B. soporator*, followed by dividing this water into 50 ml vials and storing them at -20°C.

2.6 Foraging motivation of the crab *Pachygrapsus transversus* in response to chemical cues from the predator *Bathygobius soporator*.

Our first experiment in laboratory aimed to testing the motivational behavior of the crab *P. transversus* in foraging in tide pools with the presence of chemical cue of a predatory fish (*B. soporator*). For this, the design included the odor of the predator (chemical cue) as a factor (fixed factor, with 2 levels: presence and absence) and the experiment was divided into two treatments: a) control, where the focal crab was isolated in an aquarium without the presence of predator chemical cue (N = 10); b) treatment where the focal crab was isolated in an aquarium with the presence of a chemical cue (odor) of the predator (N = 10). A food source was provided at the bottom of the aquarium (macroalgae *Ulva lactuca*) and the crab was kept inside a shelter with a trapdoor at the top of the platform. The aquarium water present at the top of the platform formed a water layer at the bottom of the hiding place of approximately 3 mm, sufficient for the crab to perceive chemical cue through its chemoreceptors present in the dactyls (Jacques, 2020). Before starting the experiment, the crabs were placed inside the shelter and confined for 30 minutes. At the end of the confinement step, the trapdoor was opened and we began observations hoping that the crab would not run the risk of leaving the surface of the aquarium to access the food in the treatment where the predator's chemical cue was present (Figure 1b). We wait 30s after the dispersion of the fish odor to open the trapdoor and begin to observe the behavior of the crab. The response variables chosen were: a) The latency time of the crab to leave the shelter after the stimulus of the chemical cue from the predator fish *B. soporator*; b) Displacement, measured in centimeters, using quadrant counting as a basis to track the distance traveled by the crab within the aquarium.

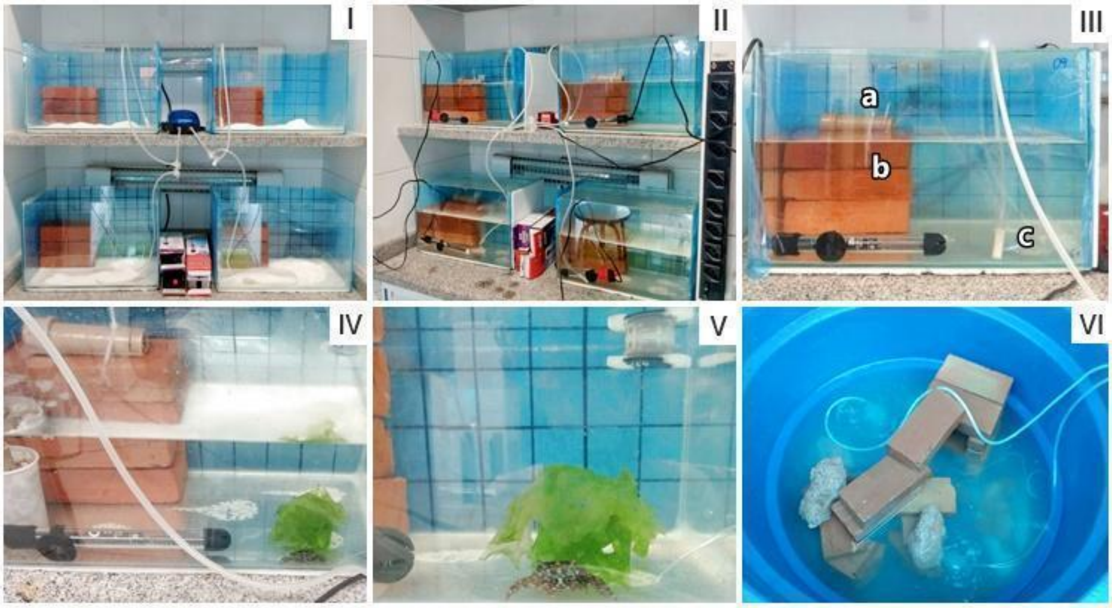

Figure 1. Experimental apparatus of the experiment on the characterization of the behavioral responses of the crab *Pachygrapsus transversus* in the presence of a chemical cue from the predator *Bathygobius soporator*. **I** - Assembly of aquariums with platforms. **II** - Experimental aquariums set up with aerators and thermostats to maintain the temperature. **III - a)** Shelter **b)** Platform, **c)** Aerator and place where the chemical cue of the fish was inserted. **IV** - Aquarium at the time of the experiment with the presence of crab and seaweed. **V**- Model animal and macroalgae. **VI**- Model animal stock with platforms.

2.7 **Effect of increased temperature on foraging motivation and consumption of the macroalga *Ulva lactuca* by the crab *Pachygrapsus transversus*.**

The third experiment consisted of two distinct treatments: 1) Average temperature of 31°C and 2) predicted temperature of 35°C, according to the RCP 8.5 scenario from the IPCC (2021) for temperature increase by the end of the century (Fig. 2). In these treatments, we assessed the foraging motivation and macroalgae consumption by the crab. The selected response variables were derived from the previous experiment and included: 1) The time taken by the crab to leave the shelter, indicating its exploratory behavior; 2) Displacement, representing the distance traveled by the crab within the experimental environment; and 3) *U. lactuca* consumption, quantifying the total macroalgae consumption by the crab during the experiment. The main focus of this second experiment was to test whether an increase in temperature leads to greater foraging motivation in the crab. This approach was adopted to investigate how the crab responds to thermal variations, even in the absence of predator chemical cue detection in the first test. This approach aims to provide additional insights into crab behavior in specific thermal scenarios, contributing to a more comprehensive understanding of its ecology and adaptation to environmental stressors.

The crabs were introduced into the experimental tanks and subjected to two temperatures over 5 days. After being allocated in the experimental tanks, equipped with a platform and shelter, the crabs roamed freely in the tank during the temperature exposure period. In the first two days, they were fed with *U. lactuca* macroalgae, followed by three days of fasting in preparation for foraging motivation and consumption observations. Before each observation, the crab was temporarily positioned inside the shelter, remaining there for 30 minutes. For macroalgae exposure, 0.8 mg of *U. lactuca* was added to each experimental tank, on the opposite side of the platform, at the bottom of the tank, while the crab remained inside the shelter. Observations started when the crab was released from the shelter, lasting for 25 minutes. To assess macroalgae consumption, crabs were left free in the experimental tanks for 12 hours, during which they could feed freely. After this interval, the macroalgae were removed for weighing. The wet weight of the macroalgae was measured on a precision scale before being placed in the experimental tanks and again after the 12-hour exposure period in the macroalgae consumption experimental stage.

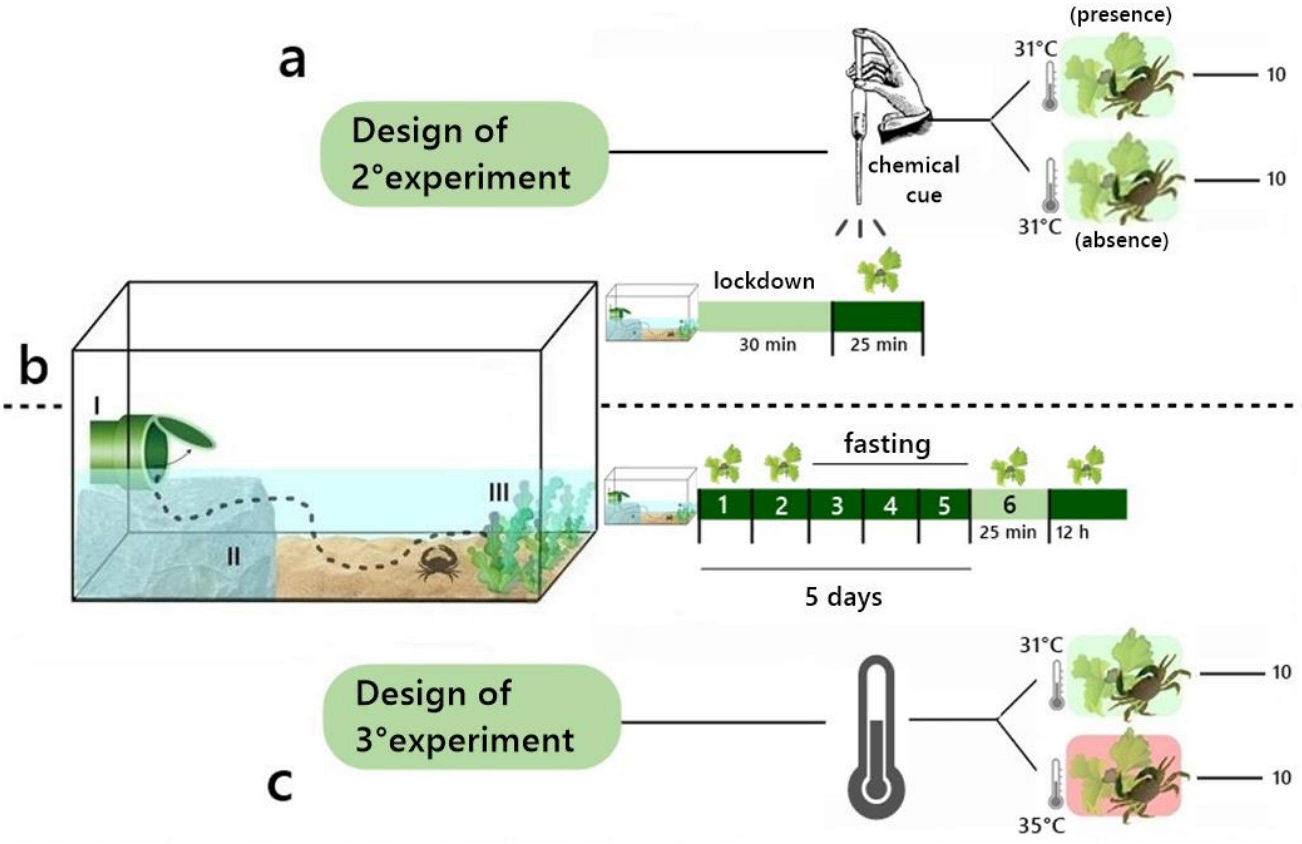

Figure 2. Representation of experimental designs in the laboratory. a) Experimental design of the 2nd experiment (section 2.6) with chemical cue as a fixed factor, with 2 levels: presence and absence. This experiment included a confinement stage of the crabs for 30 minutes, followed by an exposure stage to macroalgae during 25 minutes of observation. b) Configuration of the experimental aquarium used in both experiments. I - Shelter with trapdoor, II - Platform, III - Macroalgae. c) Experimental design of the 3rd experiment (section 2.7) with temperature as a fixed factor, with 2 levels: 31°C and 35°C. This experiment included a 5-day acclimation stage, with the first two days of feeding and the last three days of fasting, an exposure stage to macroalgae during 25 minutes of observation, and a prolonged exposure stage to macroalgae for 12 hours to assess consumption. In both experiments, each treatment was replicated 10 times, totaling 20 experimental units.

3 DATA ANALYSIS

For environmental characterization, each tide pool and sampling day was treated as an independent replicate. We calculated the average, minimum, and maximum values of temperature, pH, and salinity during both low and high diurnal tides. Additionally, we calculated the rate of change of these variables over time. In the second experiment, we aimed to identify whether the crab *P. transversus* could detect the chemical cue of the predator *B. soporator* through an experiment involving the presence and absence of the predator's chemical cue. The response variables used were latency in seconds and displacement in cm. We assessed the assumptions of normality and homoscedasticity of the data using the Shapiro-Wilk and Breusch-Pagan tests, respectively. Analyses were performed using generalized linear mixed models (GLMM) in R software version 3.6.3. Displacement did not meet the assumptions of normality and homoscedasticity and was tested with a model adjusted for a Poisson distribution and its logarithmic link function. The time it took for the crab to leave its shelter was tested with a model adjusted for a Gaussian distribution, as this is suitable for data with a normal distribution.

In the third experiment, we analyzed the impact of increased temperature on the foraging motivation and ingestion of macroalgae by the crab *P. transversus*, focusing on the variables of latency, displacement, and consumption of *U. lactuca.* Initially, we assessed the assumptions of normality and homoscedasticity of the data using the Shapiro-Wilk and Breusch-Pagan tests, respectively. Prior to the main analysis, we also performed the Wald test, considering crab size as a covariate to evaluate whether variability in size could potentially act as an interfering factor concerning the variables of latency, displacement, and macroalgae consumption. Latency was tested with a model adjusted for a Gaussian distribution. Displacement was tested with a model adjusted for a Poisson distribution. *Ulva lactuca* consumption was tested with a model adjusted for a Gaussian distribution. Analyses were performed using generalized linear mixed models (GLMMs) in R software version 3.6.3. A significance level of $\alpha = 0.05$ was adopted for all tests.

4 RESULTS

4.1 Characterization of the environmental variables of the tide pool

During sampling at low diurnal tide, an average temperature (± standard deviation) of 35°C ± 3.5°C was recorded (Figure 3a), while during the high diurnal tide period, the average temperature was 31°C ± 4.1°C (Figure 3b). During low tide, the temperature showed an average maximum of 40°C ± 1.3 and an average minimum of 29°C ± 1.3°C. During high tide, the temperature showed an average maximum of 38°C ± 2.1°C and an average minimum of 29°C ± 2.5°C. The average rate of temperature variation over the sampling period was 1°C per hour (Figure 3a,b). Regarding pH, during sampling at low tide, an average pH of 8.6 ± 0.09 was recorded (Figure 3a); during the high diurnal tide period, an average pH of 8.3 ± 0.4 was recorded (Figure 3b). During low diurnal tide, the pH showed an average maximum value of 9.4 ± 0.1 and an average minimum of 7.8 ± 0.2. During high diurnal tide, the pH showed an average maximum value of 9.1 ± 0.1 and an average minimum of 7.9 ± 0.1. The pH variation occurred at a rate of approximately one unit per hour during the sampling period (Figure 3a,b). With regard to salinity, during sampling at low diurnal tide, an average of 34 ± 1.1 was recorded (Figure 3a), and during the high diurnal tide period, the average salinity was 33 ± 0.6 (Figure 3b). During low diurnal tide, the salinity showed an average maximum value of 36 ± 1.3 and an average minimum of 31 ± 1.0. During high diurnal tide, the salinity showed an average maximum value of 37 ± 1.5 and an average minimum of 28 ± 1.3. The salinity variation occurred at a rate of approximately one unit per hour during the sampling period (Figure 3a,b).

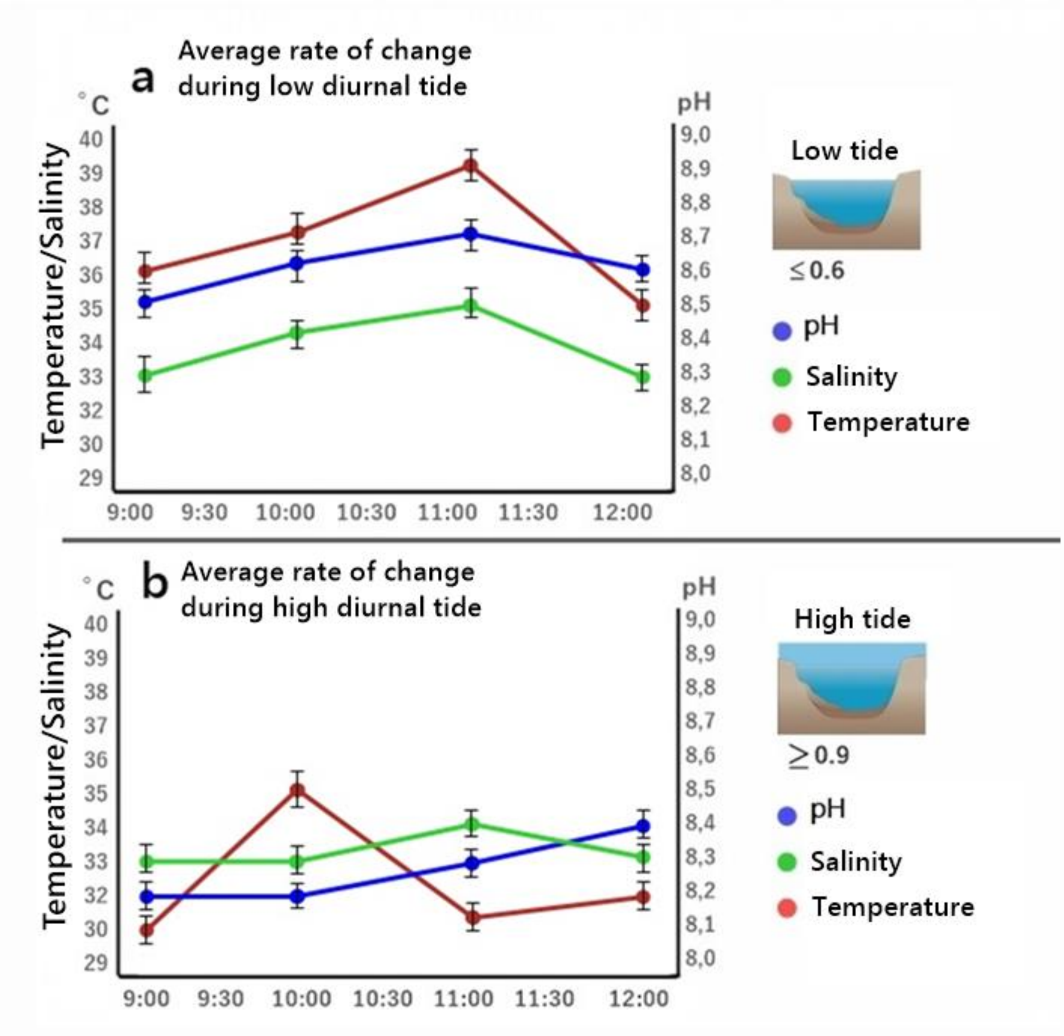

Figure 3. Abiotic variables of 5 tide pools from the rocky shores of Praia dos Milionários, located in the municipality of São Vicente/SP, during the environmental characterization stage in the summer season from January 2022 to March 2022. a) Average rate of variation over time during low diurnal tide. b) Average rate of variation over time during high diurnal tide.

4.2 Foraging motivation of the crab *Pachygrapsus transversus* in response to chemical cues from the predator *Bathygobius soporator*.

The displacement in cm of the crabs exposed to the predator's chemical cue did not differ from those not exposed to this stimulus (Table 1; Figure 4). In the control treatment, the crabs exhibited an average displacement (± standard deviation) of 171.45 ± 166.29 centimeters during a 25-minute period after the introduction of 50 ml of distilled water into the aquariums. In the treatment with the chemical cue, the crabs presented an average displacement of 151.6 ± 137.19 centimeters during the same period after the introduction of 50 ml of *B. soporator's* chemical cue. Similarly, latency in treatments with and without the chemical cue stimulus showed no significant difference (Table 1; Figure 4). The latency duration in seconds in the control treatment was 249 ± 382.98 during 25 minutes of exposure to 50 ml of distilled water. In the chemical cue exposure treatment, this duration was 206.16 ± 330.05 seconds during the same 25-minute exposure period.

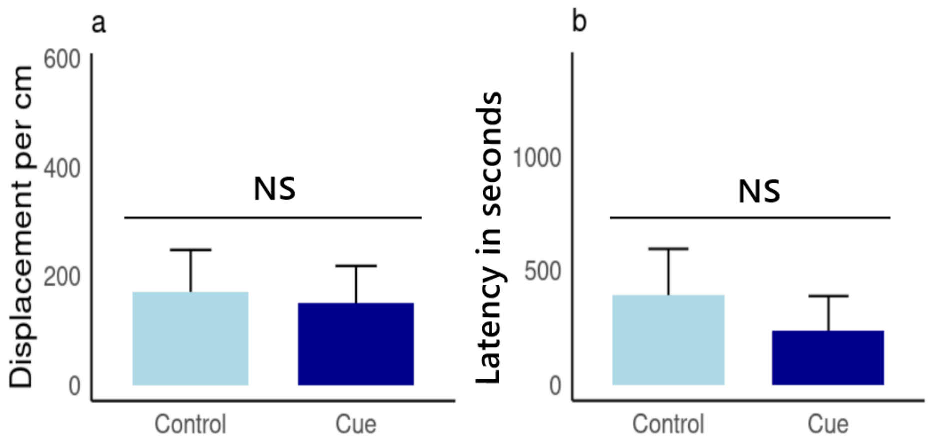

Figure. 4 Behaviors observed in *Pachygrapsus transversus* in treatments with the presence and absence (control) of the chemical cue of *Bathygobius soporator*. a) Mean ± standard deviation of the displacement of *P. transversus* in centimeters in both treatments during observations. b) Mean ± standard deviation of the time in seconds that *P. transversus* takes to leave the shelter during observations. The abbreviation NS and the horizontal lines indicate that there was no statistical significance (***p* = 0.7581; *p* = 0.6756**).

Table 1. Generalized Linear Model (GLM) Comparisons for response variations of the crab *Pachygrapsus transversus* in the experiment with the presence and absence of a chemical cue from the fish *Bathygobius soporator* (Odor Source). Significance level adopted: $p<0.05$.

Response variables	*df*	Deviance	Df Resid	Dev	*p*
Displacement per cm	1	15.832	17	1.368	0.7581
Latency (sec)	1	0.0632	17	2.262	0.6756

4.3 Effect of increased temperature on foraging motivation and consumption of the macroalga *Ulva lactuca* by the crab *Pachygrapsus transversus*.

The displacement of the crabs did not show significant differences between the treatments (Table 2; Figure 5a). In the treatment with a temperature of 31°C, the crabs exhibited an average displacement of 59.9 ± 83.0 centimeters during observations. In the treatment with a temperature of 35°C, the crabs exhibited an average displacement of 29.6 ± 35.9 centimeters during observations. However, latency showed a significant difference between the treatments (Table 2; Figure 5b). The latency in seconds in the 31°C treatment was 67.3 ± 94.0 during observations. The latency in seconds in the 35°C treatment was 12.4 ± 23.6 during observations. The feeding motivation of the crab *P. transversus* at different temperatures also showed a significant difference between the treatments for macroalgae consumption (Table 2; Figure 5c). The mean consumption of *U. lactuca* in the 31°C treatments was 0.228 ± SD 0.131 mg over a 12-hour period. The mean consumption of *U. lactuca* in the 35°C treatments was 0.099 ± SD 0.110 mg over a 12-hour period. The results of the Wald test indicated that the size of the crabs (carapace width in mm; mean size of 15.8 ± 2.0 mm CW) did not have a significant effect on macroalgae consumption (Table 3).

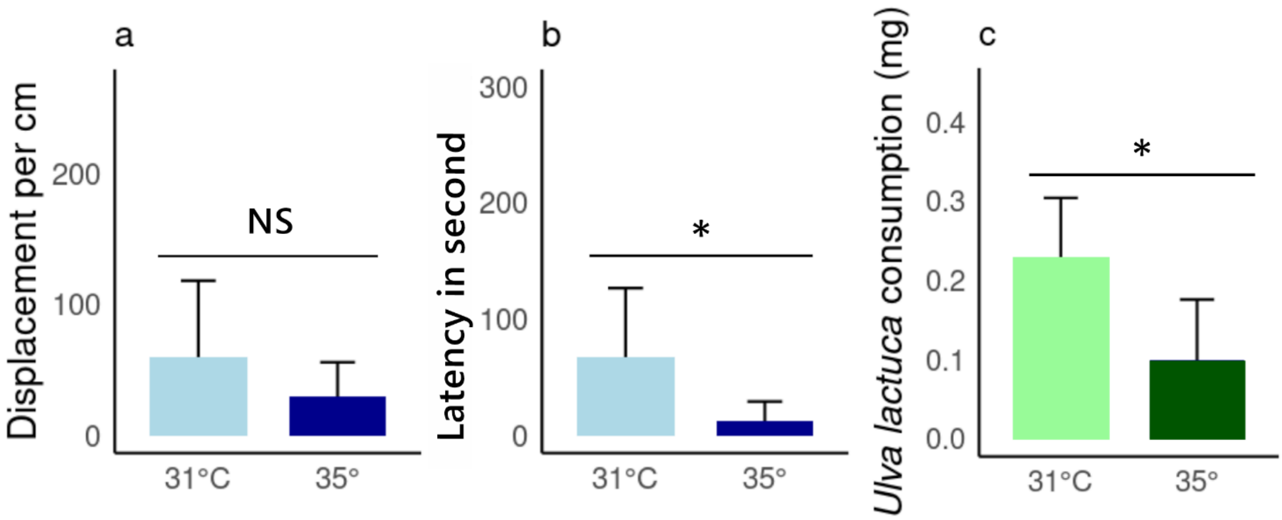

Figure. 5 Behaviors observed in *Pachygrapsus transversus* in treatments with temperatures of

31°C and 35°C. a) Mean ± standard deviation of displacement of *P. transversus* in centimeters during observations. The horizontal bar and NS indicate no statistical significance (**p = 0.7581**). b) Mean ± standard deviation of the time in seconds that *P. transversus* takes to leave the shelter during observations. The asterisk and horizontal bars indicate statistical significance (**p = 0.0124***). c) Mean ± standard deviation of consumption of the macroalga *Ulva lactuca* in milligrams during observations. The asterisk and horizontal bars indicate statistical significance (**p = 0.0237***).

Table 2. Comparisons of the Generalized Linear Mixed Model (GLMM) for the response variables of the foraging motivation experiment at different temperatures. Adopted significance level: p<0.05 (* indicates statistical significance).

Response variables	*df*	Deviance	*df* Resid	Dev	*p*
Displacement	1	0.46254	17	82.881	0.7581
Latency	1	616.216	17	1775359	**0.0124***
Ulva lactuca consumption	1	0.06327	17	2.262	**0.0237***

Table 3. Wald test to assess the significance of the covariate 'carapace size' on crab consumption in the generalized linear regression model. Significance level adopted: p < 0.05.

Response variables	*Res. df*	*df*	*Chisq*	*p*
Displacement	17	1	0.1979	0.6564
Latency	17	1	2.0098	0.1563
Ulva lactuca consumption	16	1	0.2906	0.5898

5 DISCUSSION

To investigate the effect of temperature increase on chemical communication and foraging motivation in tide pools, this study examined the behavior of the crab *P. transversus* in response to the chemical cue of the predator *B. soporator*, a common fish in these environments. The results showed that the crab did not recognize the predator's chemical cue, indicating limitations in chemical recognition. However, it was observed that the temperature increase significantly reduced the foraging activity of the crab. The results obtained in the environmental characterization phase demonstrated that the temperature variation rate in tide pools can reach extreme levels, close to the thermal limits of some coastal species (Somero, 2002; Pörtner, 2002). These findings suggest that *P. transversus* is already living under current thermal stress conditions, which are expected to intensify in the coming decades with the projected temperature increase in the climate scenario.

The approach conducted in laboratory during our second experiment revealed that the predator's chemical cue was not identified as an alert signal by the crab. Previous studies across different taxa, including other crab species, have demonstrated the capacity for multimodal communication, utilizing various forms of external stimulus identification (Partan & Marler, 2005; Wilke, 2021). One of the ways to identify aspects of the environment is through chemical stimuli, especially in aquatic environments (Skelhorn & Rowe, 2016; Draper & Weissburg, 2019). However, the lack of response to the chemical cue of the fish *B. soporator* may also indicate a potential limitation in the crab's chemical recognition or a limitation related to the stimulus itself.

The controlled setup of a laboratory environment may not have accurately reproduced the complex behavioral and sensory apparatus found in the species' natural habitat. This discrepancy could have influenced the response to the simulation of an isolated stimulus, as discussed by Brown et al. (2013). In the case of *P. transversus*, exclusive dependence on chemical cues may not be sufficient to trigger effective anti-predatory responses. Breithaupt and Thiel (2010) and Hebets and Rundus (2010) emphasized that multiple stimuli, such as visual and vibratory cues, play important roles in communication and defensive behavior across various taxa, including crustaceans. These additional stimuli can provide contextual information that enhances the crab's ability to recognize and react to predatory threats (Breithaupt & Thiel, 2010; Hebets & Rundus, 2010). Therefore, the intensity and complexity of the chemical cue may have been insufficient to stimulate a behavioral response in the crab. Future studies are recommended to use more potent stimuli by combining different types of cues to assess whether a multimodal approach enhances the effectiveness of anti-predatory responses in decapod crustaceans. Considering the validity of the experiment, the crab's inability to detect the predator could lead to significant ecological implications for the species, altering local survival patterns and population dynamics. Similar observations have been noted in other studies, such as that by Moretto & Taylor (2023), which demonstrated changes in crab foraging behavior under different environmental conditions.

The failure to recognize the presence of a predator can expose prey to greater risks, increasing their vulnerability (Partan & Marler, 2005; Wilke, 2021). Hermit crabs, for instance, significantly alter their foraging behavior in response to predator chemical cues (Trussell et al., 2006). When exposed to predator chemical cues, hermit crabs reduce foraging time and increase hiding time (Trussell et al., 2006). When prey cannot detect a predator, they may continue foraging in dangerous areas, becoming easy targets (Ferrari et al., 2010). Furthermore, continuous exposure to predatory risks without the ability to detect and avoid them can lead to a decrease in species survival rates (Schmitz, 2008). For example, Weissburg et al. (2014) demonstrated that prey failing to recognize predator chemical cues exhibit riskier behaviors, resulting in higher predation rates. This can also affect ecological balance, as a decrease in prey population can cascade effects throughout the entire community (Schmitz, 2008; Ripple et al., 2014).

In the present study, the experiment on foraging motivation at different temperatures revealed that crabs maintained at 31°C exhibited significantly higher motivation to forage in the water and feed compared to those subjected to 35°C. Consistent with our findings, the study by Moretto & Taylor (2023) found that at the control temperature (8°C), the brown box crab *Lopholithodes foraminatus* showed increased foraging motivation and reduced latency compared to the elevated temperature (15°C). Conversely, another study on red king crab *Paralithodes camtschaticus* indicated that while food consumption increased with temperature, higher temperatures led to increased mortality (Windsland, 2015). Different crab species may exhibit varied responses to temperature increases, highlighting the complexity of ecological interactions in aquatic environments under climate change. Contrary to our results, previous studies have indicated an increase in the metabolism of marine invertebrates with rising temperatures (Moretto & Taylor, 2023; Vianna et al., 2020). These findings suggest that while temperature can increase metabolism up to a certain point, the opposite effect may occur beyond the optimal threshold (Pörtner, 2010; Somero, 2011). Changes in feeding behavior induced by temperature may be common among marine crabs, driven by metabolic demands and the efficiency of neuromuscular activity (Bennett, 1985; Moretto & Taylor, 2023).

Under conditions of temperature above the thermal optimum, circulation and ventilation may become insufficient to meet the increasing demand for oxygen (Pörtner et al., 2006). As temperature rises, so does the oxygen demand, but aerobic metabolism is only sufficient up to a certain point (Lagerspetz & Vainio, 2006; Azra et al., 2020). Beyond this critical point, animals may resort to anaerobic metabolism, allocating energy resources for survival, which reduces the energy available for other activities such as foraging (Pörtner et al., 2006). Therefore, at elevated temperatures, foraging capacity may significantly decrease as crustaceans need to prioritize basic vital functions (Lagerspetz & Vainio, 2006; Azra et al., 2020). Our results indicate that crabs subjected to the 35°C treatment may have experienced extreme thermal stress, which likely reduced their metabolic activity and consequently their foraging behavior and ingestion in these treatments. It is important to note that the 35°C temperature corresponds to the average observed in the tide pools during the environmental characterization stage, suggesting that *P. transversus* crabs may already be experiencing the consequences of temperature increase in their natural habitat.

The influence of temperature on foraging motivation and food intake can shape exploratory behavior and the distribution of animals in their natural habitat (Lagerspetz & Vainio, 2006; Azra et al., 2020; Moretto & Taylor, 2023). When an animal ceases foraging or reduces its foraging activity, this can have several implications for habitat and ecological dynamics (Schmitz et al., 2004; Ripple & Beschta, 2004; Ripple et al., 2014). Reduced foraging can lead to decreased predation pressure on macroalgae and other food resources, allowing these populations to grow and alter the environment's composition (Schmitz et al., 2004). Moreover, this change can affect other herbivores and competitors, potentially granting them greater access to resources previously utilized by the animal that reduced its foraging activity (Beckerman & Schmitz, 1997). Decreased foraging can also impact the physical condition of the animal, reducing its long-term reproductive capacity and survival (Brown & Mitchell, 1997). When this reduction in foraging occurs due to high temperatures, behavior initially responding to perceived predation risk, where animals prioritize safety over feeding, shifts to responding to climate-related consequences stemming from anthropogenic impacts (Root et al., 2003; Parmesan, 2006). This behavioral shift may reflect the animals' adaptation to new environmental pressures such as climate change, which alter resource availability and predator dynamics within the ecosystem (Cahill et al., 2013). Such behavioral changes can therefore have cascading effects throughout the food web, influencing ecosystem structure and function (Ripple & Beschta, 2004). In this context, selective pressures in semi-terrestrial environments, such as local climatic conditions, may play a crucial role in the adaptability of crabs in their quest for resources at different tidal phases. Understanding these adaptations is critical for predicting how species will respond to future environmental changes (Williams et al., 2008; Hof et al., 2011).

6 CONCLUSION

This study revealed that the crab *P. transversus* does not recognize the predator *B. soporator's* chemical cue and that increased temperature significantly reduces its foraging activity. The inability to detect predators increases the crabs' vulnerability to predation, while reduced foraging activity due to thermal stress compromises their ability to feed adequately. Our findings indicate that *P. transversus* is already experiencing thermal stress conditions in its habitat, which may intensify with the predicted temperature increases under the IPCC's RCP 8.5 climate scenario. This scenario will result in a decrease in the crabs' survival rate, potentially altering the structure and function of tide pool ecosystems. Reduced foraging will lead to decreased predation pressure on macroalgae and other food resources, altering habitat composition, which can significantly affect species' population dynamics and local biodiversity.

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Capítulo 2

Tide pool challenges: Unraveling the combined impact of temperature and hypoxia on chemical perception in *Carcinus maenas*

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ABSTRACT

The marine environment is highly vulnerable to climate change impacts. IPCC projections suggest a potential 4°C temperature increase by 2100, impacting marine ecosystems profoundly. Coastal areas, already subject to daily temperature, salinity, oxygen, and pH fluctuations, face early and critical changes. Rising temperatures reduce oxygen solubility, potentially causing hypoxia in habitats like tidal pools. These climate stressors collectively affect marine organism communities, altering predator-prey dynamics and chemical signal detection. Understanding these interactions is crucial amid climate change challenges in intertidal zones. This study specifically examined the impact of the interaction between temperature and hypoxia on the chemical communication of the crab *Carcinus maenas*. Utilizing the mean rate of temperature change and dissolved oxygen levels in UK tidepools, we evaluated and categorized the crabs' motivation to feed in tidepools containing chemical cues from mussels. Our findings indicated that the crab *C. maenas* did not alter its ability to detect *Mytilus edulis* prey when exposed to the predicted increase in temperature and hypoxia anticipated for the end of the century.

Keywords: Climate change, multiple stressors, chemical communication, marine organisms.

1 INTRODUCTION

The intertidal zones are regions sensitive to climate change (Helmuth et al., 2006; Sorte et al., 2010; Pinsky et al., 2019) since they are located in the transition between terrestrial and marine environments and, therefore, undergo changes both due to exposure to sea water and air (Helmuth et al., 2006). It has been projected that there will be an average increase in ocean temperature of 2°C and 4°C above pre-industrial levels (Pinsky et al., 2019; IPCC, 2021). Increased temperature combined with other stressors such as hypoxia can limit the behavioral and physiological responses of coastal organisms that already live in extreme conditions (McBryan et al., 2013; Young & Gobler, 2020).

During a hypoxia event there is a drop in available oxygen levels in the water due to decreased oxygen solubility and increased stratification of the water column (Gruber, 2011; Breitburg et al., 2018; Young & Gobler, 2020). Hypoxia is an interference factor in the physiology of fish and marine invertebrates, and can cause disorders that include reduced mobility and greater vulnerability to diseases (Thomas et al., 2019). Animals that live in areas that have a wide variation in oxygen levels need to regulate their metabolism so that their bodily functions are maintained (Zheng, 2022). At an individual level, hypoxia can affect the life history of marine organisms in different ways, including growth rate and increased vulnerability to disease (Long et al., 2013). When analyzed at the population level, there is habitat loss for bottom species, habitat compression for pelagic species, migrations, decrease in biomass and species richness (Rosenberg, 2008; Rabalais et al., 2010). In crustaceans, for example, a decrease in food consumption was observed when *Balanus amphitrites* were subjected to hypoxia (Desai & Prakash, 2009). The increase in temperature and hypoxia is a major challenge for marine organisms to compensate for the energy instability that these stressors can cause in their metabolism (Young & Gobler, 2020). Since the 1940s, empirical and theoretical studies have shown a probable synergy between temperature and hypoxia in fish, which could lead to deleterious effects on populations (Fry & Hart, 1948; Pörtner, 2005; Pörtner & Farrell, 2008; Pörtner & Peck, 2010; McBryan et al., 2013). Hypoxia is a potentially important stressor in marine communities but more studies are still needed with this environmental variable, both independently and in interaction with other existing stressors (Young & Gobler, 2020).

The effects of interactions between biotic and abiotic variables can occur in different ways. When a variable potentiates the effect of another variable there is a synergistic effect between them (Przeslawski et al., 2015; Ong et al., 2017; Steckbauer et al., 2020; Pardal et al., 2021). The combination of stressors can also lead to additive and antagonistic effects (Steckbauer et al., 2020; Pardo & Costa, 2021). In the case of additive effects, they occur when the association between variables has the same effect as the sum of its individual effects, whereas in antagonism one of the variables attenuates or reduces the effect of the other (Steckbauer et al., 2020). In a study with more than one stressor with freshwater *Danio rerio* (zebrafish) larvae, it was observed that the larvae showed metabolic plasticity at a temperature increase of 3°C, but when exposed to hypoxia, there was a greater sensitivity to change in temperature. This temperature sensitivity is an example of synergy between stressors, which can lead to a bioenergetic imbalance (Pan & Hunt, 2017).

Tidal pools are mesocosms that frequently occur on rocky shores and are influenced by environmental variations during the tidal cycle (Trussell, 2004; Vinagre et al., 2021). Due to low thermal inertia, recent studies show that the temperature variation in tide pools can reach extreme levels, since these are environments that heat up faster when exposed to low tide (Vinagre et al., 2021). In addition to being environments that present superheating when there is sunlight, another phenomenon that occurs in tide pools is severe hypoxia at night (Todgham et al., 2005; McArley, et al., 2020). The temperature increases during diurnal low tide cause an increase of the demand for oxygen by bacteria, phytoplankton and animals, which leads to a drastic drop in dissolved oxygen in the tide pool when there is no more solar radiation (McBryan et al., 2013). The effects of high temperatures on the ability of organisms to maintain oxygen supply are the main interference factors in regulating the thermal limits of species. (Pörtner & Peck 2010; McBryan et al., 2013; McArley et al., 2020). Thus, organisms that inhabit tide pools may face temperatures that are already close to their physiological tolerance limits, which may limit their ability to survive (Sunday et al., 2012; Culler et al., 2015; Xi et al., 2016; Vinagre et al., 2021). Among them, the green crab *Carcinus maenas* (Linnaeus, 1758) is a species of decapods of the family Carcinidae very common on rocky shores, active during high tide (Warman & Naylor, 1995). The green crab is native from European waters to the Baltic Sea and North Africa (Cohen et al., 1995), however this species has already spread to other continents, being considered one of the main invasive species (Lowe et al., 2000). It is a generalist predator, and its diet consists mainly of bivalve mollusks, polychaetas, small crustaceans and even crabs of the same species (McGaw et al., 2011). In a climate change scenario, the predicted variations in temperature and oxygen levels by the end of the century could significantly impact coastal zones, including habitats where crabs reside. Temperature increases can lead to reduced oxygen solubility in water, potentially causing hypoxia in confined environments like tidal pools (Pörtner et al., 2006). These stressors, acting synergistically, may impair the chemical perception abilities crucial for survival in marine organisms, such as crabs (Skelhorn & Rowe, 2016; Draper & Weissburg, 2019). The ability to perceive chemical cues is vital for predator detection, foraging behavior, and overall survival in aquatic environments (Lagerspetz & Vainio, 2006; Azra et al., 2020). Therefore, the survival of marine organisms can be significantly influenced by the synergistic effects of temperature and hypoxia, highlighting the vulnerability of species in changing marine ecosystems (Pörtner et al., 2006; Skelhorn & Rowe, 2016; Draper & Weissburg, 2019). Depending on the degree and interaction between stressors, the effects may interfere with biological mechanisms, such as prey and predator detection (Draper & Weissburg, 2019).

This type of interference can have knock-on consequences which impact community and population level processes (Zhang et al., 2010a). Intensification of the trophic cascades, for example, can result in the loss of biodiversity in marine environments, contributing to the expansion of dead zones (Altieri, 2015). Understanding to what extent the interaction of stressors can affect mechanisms such as chemical perception can provide us with information about the long-term consequences of stressors arising from climate change. In the present study, we evaluated whether there were synergistic interferences of temperature and hypoxia on the chemical perception performance of the crab *C. maenas* in tide pools. Understanding how various climate stressors can affect interactions between species, such as prey-predator relationships, can help us more robustly predict how organisms within an ecosystem will respond to these variations in the future (Woodward et al., 2010; Asakura, 2021). The use of chemical cue to assess predation risks in marine organisms is a commonly used method (Hay, 2009; Morishita & Barreto, 2011; Barreto et al., 2013) that allows understanding how environmental stressors interfere in the relationships between these organisms. Thus, to test our hypothesis, the experimental approach will analyze whether *C. maenas* can chemically identify the presence of prey *Mytilus edulis* under conditions of increased temperature and hypoxia.

2 MATERIAL AND METHODS

2.1 **Animal model collection and stock maintenance**

The study was conducted in the Menai Strait (53°13'35.5"N 4°09'34.6"W), located in the city of Bangor/Wales. To collect *C. maenas*, a pot trap with pieces of fresh mussels of species *M. edulis* was placed during high tide to attract crabs. The trap was left until a 6-hour tide cycle had passed before it could be removed and the animals collected. Sixty crabs were selected to carry out the experiment with approximately 10 cm carapace width. The crabs were kept in containers (370mm x 270mm x 320mm) with sea water, constant aeration and with the presence of some stones that served as shelter during acclimatization. The variables temperature and oxygen level were controlled during the acclimatization period. The temperature of the containers was controlled with thermostats at a level appropriate for the experimental treatment (see below) and oxygen levels were maintained at 100% saturation with aerators. The crabs remained under these conditions for a week and were fed every 48 hours with the soft tissue of *M. edulis*.

2.2 **Observation of the natural behavior of the crab *Carcinus maenas* when exposed to the chemical cue of the prey *Mytilus edulis***

Before starting the experiment with temperature and hypoxia stressors, we performed a categorization of the natural behavior of the crab *C. maenas* when exposed to the presence of a chemical cue of *M. edulis*. For this, 10 crabs that had been fasting for 72 hours before the introduction of the chemical cue were separated. To present the chemical cue to the crabs, pots with holes were made where we introduced the open mussels. The pots were opaque, preventing the crabs from being able to identify the prey through sight, leaving only the possibility of identification through the chemical cue of the mussels that was dispersed in the water. Each crab was individually observed for 5 minutes after insertion of the pot with the mussels. In order to classify the perception of the crabs in relation to the chemical cue of the prey, during the 5 minutes of exposure of the chemical cue, the following behaviors were identified: 1- Latency, as the first movement of the dactyls after insertion of the chemical cue, 2- Approaching the source of the chemical cue. The categorization of the natural behavior of the *C. maenas* crab was important for choosing the main response variables that would be analyzed in the main experiment. Thus, we only worked with variables that had the potential to indicate some variation in the behavior of the crab when exposed to the chemical cue of the prey of *M. edulis* in a context of stress. The variables chosen were: a) Latency; and b) Proximity to chemical cue source. Such variables were used in the experimental planning where we included temperature and hypoxia factors, both stressors of interest to this project.

2.3 Evaluation of the chemical perception of *Carcinus maenas* when exposed to the odor of the mollusk *Mytilus edulis* in a scenario of high temperature and hypoxia

After testing whether the crab is able to identify the chemical cue of the predator and which behaviors were associated with this perception (section 2.2), tests were performed with the objective of analyzing the effect of the stressors temperature increased and hypoxia on the crab's perception capacity when exposed to the chemical cue of a common prey (*M. edulis*). The latency and chemical cue source approximation variables were measured using a stopwatch. Thus, as soon as the container with *M. edulis* was inserted in the experimental container of each treatment, the time that the crab took to perform the first body movement (dactyls movement) began to be recorded (subsequently referred to as “Latency");

and the time it took the crab to reach the prey chemical cue source (later referred to as "proximity to chemical cue source"). Each response variable was counted during 5 minutes of observation. In all, 4 treatments were tested, with chemical cue exposure as a fixed factor: 1) Medium temperature (14°C) + hypoxia (2mg/l), 2) Medium temperature + normoxia (10mg/l), 3) High temperature (18°C) + hypoxia (2mg/l), 4) High temperature (18°C) + normoxia (10mg/l). In all, 10 replicates were performed for each of the aforementioned treatments. At the end of the experiment, all crabs were fed and released at the place where they were collected.

We use an average sea temperature variation rate of 14°C according to the rates of the warmest months (April to August) and a projection of four more degrees above the average (18°C) according to the RCP-8.5 of the IPCC for the end of the century (IPCC, 2021). For treatments with hypoxia induction, we used a rate of dissolved oxygen in water of 2.0 mg/l (20%); control treatments were maintained at a rate of 10 mg/l (100%) of dissolved oxygen. The oxygen rates were chosen according to the predicted oxygen levels at the end of the century (Isensee et al., 2016; Jane et al., 2021). The treatments without hypoxia induction were maintained with constant aeration of oxygen. To achieve an oxygen level of 2 mg/l in the hypoxia-induced treatments, nitrogen gas was bubbled into the experimental containers for 30 minutes using a pneumatic hose attached to a cylinder (adapted from Jie et al., 2021). The treatments without hypoxia induction were kept aerated. The pre-experimental procedures were characterized by the acclimatization of the crabs to the experimental temperatures. For this, 10 crabs were acclimatized to the average temperature of 14 and 10 crabs were acclimated to the predicted temperature of 18. Acclimatization prior to established temperatures was carried out since the objective of the study is to evaluate a behavioral response within the temporal context of change in temperature. temperature towards the end of the century. During acclimatization, the crabs were fed twice a week with one opened shell of *M. edulis* each. At the end of the week, they underwent a 72-hour fasting period before the start of the experiment. To carry out the tests, the crabs were placed individually in 22-liter containers (40 x 24 x 23 cm) containing 11 liters of artificial marine water. The temperature was maintained using a thermostat and a temperature regulation sensor. Oxygen saturation and water circulation were maintained with aerators. To collect prey chemical cue, 3 fresh *M. edulis* were separated for each replica, their shells were opened and the internal contents were inserted into a small container (12 cm in diameter) with holes. In this way, the liquid content that was inside the shell of the mollusk can come into contact with the water in the experimental container, allowing the contact of chemical cue with the chemoreceptors present in the crab's setal system (Jacques, 2020).

3 DATA ANALYSIS

We investigated the effects of increased temperature and decreased oxygen levels on the behavior of the marine crab *Carcinus maenas* when exposed to the chemical cue of the mussel *Mytilus edulis*. We were interested in identifying whether the stressor variables, specifically the 4°C temperature increase and 20% oxygen decrease, were significantly related to the latency and time it took the crab to reach the chemical cue source. We used the generalized linear model (GLM) to examine the relationship of our response variable, the latency and time to chemical cue, and the fixed-effect predictor variables of temperature and oxygen level. The full model was tested with interaction and we used simulated residuals to visually assess model fit by plotting the residuals against each predictor variable. Data were adjusted for negative binomial distribution for our response variable, since latency is a continuous variable. We hypothesize that there may be a synergistic interaction between temperature and oxygen level, where temperature may have a greater effect on latency and time to prey chemical cue compared to the isolated effect of this predictor variable. The analyzes were implemented using the statistical software package R (R version 3.6.3), the graphs were made using the "gtsummary" and "ggplot2" package. We adopted a minimum significance level of $p < .05$ in all analyses.

4 RESULTS

4.1 **Observation of the natural behavior of the crab *Carcinus maenas* when exposed to the chemical cue of the prey *Mytilus edulis***

The latency in seconds in the treatments in which the crabs were exposed to the chemical cue of the prey showed a significant difference to the treatments in which the crabs were not exposed to the stimulus (Table 1; Fig 1, a). The latency in seconds in the treatments in which the crabs were exposed to the chemical cue of the prey had an average of 193 ± 145. The latency in seconds in the control treatments in which the crabs were exposed to distilled water averaged 426 ± 234. The proximity to chemical cue source in seconds in the treatments where the crabs were exposed to the chemical cue of the prey was similar to the treatments in which the crabs were not exposed to the stimulus (Table 1; Fig 1, b). The mean time in seconds that the crabs in the control treatments moved during 5 min of exposure to distilled water was 193.2 ± 143. The crabs in the treatment with chemical cue exposure approached to chemical cue source in 181 ± 153 seconds during 5 min of exposure to *M. edulis*.

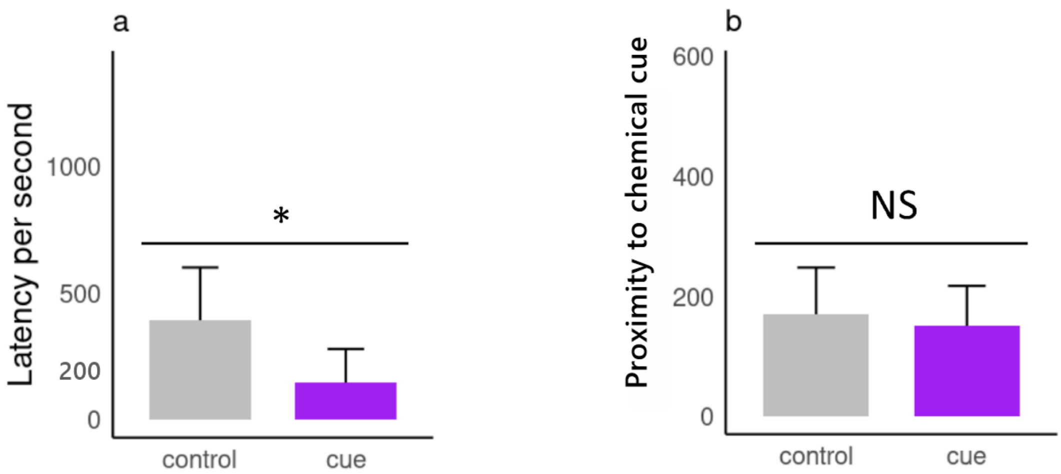

Figure 1. Behaviors observed in *Carcinus maenas* in treatments with the presence and absence (control) of the chemical cue of *Mytilus edulis*. **a)** Time in seconds of the latency of the crab *C. maenas* after insertion of the chemical cue of *M. edulis* (***p* = 0.0245***), the asterisk and horizontal bars indicate statistical significance. **b)** Time in seconds of the proximity to chemical cue source of the crab *C. maenas* after insertion of the chemical cue of *M. edulis* (*p* = 0.6346), the abbreviation NS and the horizontal lines indicate that there was no statistical significance

Table 1. Comparisons of the Generalized Linear Model (GLM) for the response variables of the chemical perception of *Carcinus maenas* when exposed to the chemical cue of the prey *Mytilus*

edulis. Adopted significance level: p<0.05. (* indicates statistical significance).

Response variables	***df***	**Deviance**	***df* Resid**	**Dev**	***p***
Latency per second	1	13.498	38	44.756	**0.0245***
Proximity to chemical cue source	1	0.1789	37	43.406	0.6346

4.2 The effect of temperature and hypoxia on the chemical perception of *Carcinus maenas* crab towards the chemical cue of prey

The latency in seconds in the treatments in which the crabs were exposed to the chemical cue of the prey at an average temperature of 14 °C and 18 °C with hypoxia induction at 2.0 mg/l (20%) was similar to the latency in the treatment in which the crabs were exposed to an average temperature of 14 °C and 18 °C with 10mg/l (100%) of dissolved oxygen (Table 2; Fig 2a). In both treatments, the crabs had an average latency of 39.5 seconds during exposure to the chemical cue of *M. edulis.* The time in seconds for the crab to reach the approach of the prey chemical cue in the treatment where the crabs were exposed at an average temperature of 14°C and 18 °C with hypoxia induction at 2.0 mg/l (20%) was similar or time in which the crabs were exposed to an average temperature of 14 °C and 18 °C with 10mg/l (100%) of dissolved oxygen (Table 2; Fig 2b). The crabs had an average time in seconds to the odor source of 112,5 seconds during exposure to the *M. edulis* chemical cue.

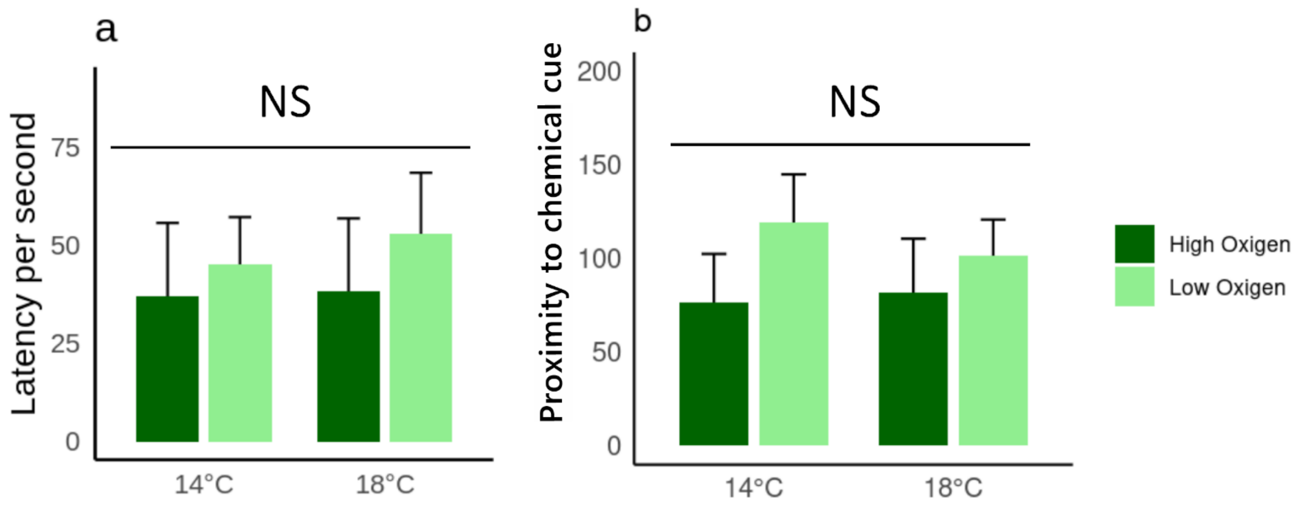

Figure 2. Observed behaviors in *Carcinus maenas* in treatments with different temperatures and the presence and absence (control) of the chemical cue from *Mytilus edulis*. **a)** Latency of the crab *C. maenas* after insertion of the chemical cue; the horizontal bars of the boxplots refer to the median and the vertical lines to the spread of the latency data per seconds after insertion of the *M. edulis* prey chemical cue (p = 0.2453). **b)** Time in seconds that the crab *C. maenas* takes to approach the source of the chemical cue of *M. edulis* (p = 0.6722); the horizontal bars of the boxplots refer to the median, the vertical lines to the data distribution and the black dots to the discrepant values of the time of approach to the chemical cue per seconds after the insertion of the odor of the prey of *M. edulis*. The abbreviation NS and the horizontal lines indicate that there was no statistical significance

Table 2. Comparisons of the Generalized Linear Mixed Model (GLMM) for the response variables of the chemical perception of *Carcinus maenas* at different levels of oxygen and temperatures. Adopted significance level: $p<0.05$.

Response variables	*df*	Deviance	Df Resid	Dev	*p*
Latency per second	1	13.498	38	44.756	0.2453
Proximity to chemical cue source	1	0.1789	37	43.406	0.6722

5 DISCUSSION

Our results indicate that there was no synergistic interaction between the temperature and hypoxia stressors and both alone did not affect the latency performance of the crab *Carcinus maenas* when exposed to the chemical cue of *Mytilus edulis* prey. Increasing temperature and decreasing oxygen also did not produce any noticeable effect on other behaviors evaluated, namely, the time taken to move to the prey chemical cue source. Contrary to expectations, neither of the two behaviors tested in *C. maenas* was affected by the temperature and hypoxia stressors together. Thus, we believe that the species *C. maenas* may have competitive advantages over species more sensitive to these two stressors, which contributes to its expansion as an invasive species in different environments.

The *C. maenas* crab is native to European waters and is considered invasive in other continents, such as the east coast of North America (Romano, 2006) and also had some reports in South America, however, without established populations so far (Darling et al., 2008). It is a species that presents in its life cycle a pattern of migration during the warmer months from June to September towards the coast, where it ends up facing more drastic variations in temperature (Taylor & Butler, 1973). Its invasive capacity may be associated with greater resistance to stressors due to temperature variations faced during their migration, allowing their expansion at different latitudes (Klassen & Locke, 2007). The region of Great Britain in northern Europe, where this crab species is commonly found, has been experiencing large temperature variations from heatwaves, which is accelerating the process of warming coastal waters (Hiscock et al., 2004; Morit, 2022). Forecasts from the United Kingdom's Climate Impact Program show that annual average seawater temperatures could reach more than 2 degrees by the 2050s, i.e. the 4°C increase could be surpassed even before the end of the century (Hiscock et al., 2004). This precipitous increase can be a very important factor in the distribution and abundance of animals, especially in the most sensitive organisms (Leung et al., 2021; Marochi et al., 2022). Our results, contrary to what we expected, demonstrated that the crab *C. maenas* did not change its behavior due to the increase in temperature, not even when it was associated with a decrease in oxygen. This can be elucidated by the fact that this species belongs to the decapod crustaceans with a lethal hypoxia threshold below the general average (1.43 mg/l), equivalent to 10% dissolved oxygen in water (Raquel & Carlos, 2008). In simpler terms, if exposed to the same level of hypoxia, other crustacean species would likely not survive, highlighting *C. maenas* exceptional tolerance. Therefore, *C. maenas* appears to possess greater physiological adaptability than other organisms, enabling its expansion and establishment, even amidst predictions of temperature increase and oxygen decrease by the end of the century.

The study of the synergistic interaction between stressors has been a global challenge, but which could bring interesting results in relation to their possible effects on organisms and the environment. When studying a dynamic environment such as tide pools, it is important to take into account the fact that the main variables related to climate change occur simultaneously (Draper & Weissburg, 2019; Manríquez et al., 2021). Multiple stressors can occur without affecting an organism's behavioral and physiological performance and this may be related to adaptability and how resilient the animal can be to certain stressors (Klassen & Locke, 2007; Tepolt & Palumbi, 2020). In coastal environments, where there is high variation of biotic and abiotic variables, we can observe the occurrence of different organisms, which are exposed to these stressors (Klugh, 1924). This exposure is an important factor for the settlement and migration of species, since in the early stages of life, selective pressure is greater, interfering with future populations. Ectothermic organisms may be able to tolerate combined stresses within a specific range of conditions using their phenotypic plasticity capacity (McBryan et al., 2013). But when stressors exceed the tolerance range, populations are likely to disappear, migrate to environments with more favorable conditions, or remain as a result of conditioned tolerance through natural selection (McBryan et al., 2013). The crab *C. maenas* has been frequently studied due to its expansion potential and also because it is considered an interesting model to explore adaptive dynamics in the marine environment (Tepolt, 2020). Its tolerance to wide variations in temperature and other variables such as salinity and hypoxia has allowed this crab species to spread beyond its main distribution, becoming known as one of the most successful aquatic invaders in the world (Taylor & Butler, 1973; Darling et al., 2008; Tepolt & Palumbi, 2020). Thus, it is possible to understand why the behavioral performance of *C. maenas* when exposed to two stressors, in the present study, was not affected by a window of variation predicted for the end of the century. Although the crab *C. maenas* has demonstrated a common performance under conditions of increased temperature and hypoxia, its larvae have a smaller window of tolerance to these stressors, which may restrict the distribution area of this species depending on the scenario of variation of the stressors (Brave, 2007).

The vulnerability of coastal systems to hypoxia is greater than is currently recognized and the main studies that have evaluated tolerance to low levels of oxygen have used a threshold of 2.0mg/l of dissolved oxygen as the default value to determine that an area is being affected by hypoxia (Raquel, 2008). However, this value has been considered inadequate as a hypoxia threshold for most species, since the tolerance threshold that covers the main taxa is around 4.0 mg/l of dissolved oxygen, which is equivalent to 40 % (Raquel & Carlos, 2008; Jane et al., 2021). The value that has been used is lower than the average of the lethal limit of these species and this shows us that the hypoxic areas are being underestimated. If we consider that places with oxygen levels above 2.0 mg/l can also be considered hypoxic, several species are being affected while these low oxygen zones are not being mapped with due importance. Another limiting factor is that most studies with stressors only evaluate the isolated effect of a stressor, which is not consistent with the more realistic scenarios that organisms face in the environment (Pörtner, 2010). Therefore, evaluating the role of mobility and response of *C. maenas* under conditions with multiple stressors and projections for the end of the century can contribute with results that will serve as support to understand how other organisms may be facing the fluctuations of variables over decades. The results of this study can serve as a warning and also as a precautionary limit for species more sensitive to relevant stressors such as temperature and hypoxia.

6 CONCLUSION

With the present study, we concluded that the crab *Carcinus maenas* did not present significant differences in its chemical perception in relation to the presence of mussel chemical cue under conditions of increased temperature and hypoxia induced by nitrogen. These results reveal that despite the predictions of temperature increase and oxygen decrease for the end of the century being worrying values, this species has the potential to have a competitive advantage over other species, since it is still capable of resisting these stressors, which may also explain its invasive ability to be so successful. Such results should not be generalized to other crustacean species, since this taxon is still considered one of the most vulnerable to multiple stressors. The resilience to multiple stressors of *C. maenas* can serve as an indicator of the effects of stressors associated with climate change, and how such results can affect the life history and tolerance window of species that live in habitats with more drastic abiotics variations.

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10 CONSIDERAÇÕES FINAIS

Nossos achados destacam a complexidade das respostas dos caranguejos às mudanças ambientais, ressaltando a importância de considerar múltiplos fatores ao avaliar os impactos das mudanças climáticas sobre essas espécies marinhas. Os dois estudos apresentados oferecem conclusões importantes sobre os impactos das mudanças climáticas em diferentes espécies de caranguejos. As diferenças nas respostas entre as espécies *P. transversus* e *C. maenas* mostra a necessidade de abordagens que considerem as caracteristicas específicas do habitat da espécie para compreender as adaptações e vulnerabilidades individuais dentro de um ecossistema diversificado. No primeiro estudo, foi revelado que o caranguejo *P. transversus* não reconhece a pista química do predador *B. soporator*, e que o aumento da temperatura reduz significativamente sua atividade de forrageamento. A incapacidade de detectar predadores aumenta a vulnerabilidade dos caranguejos à predação, enquanto a redução na atividade de forrageamento, devido ao estresse térmico, compromete sua capacidade de se alimentar adequadamente. Esses achados indicam que o *P. transversus* já enfrenta condições de estresse térmico em seu habitat, que podem se intensificar com os aumentos de temperatura previstos pelo cenário climático RCP 8.5 do IPCC. Esse cenário resultará em uma diminuição na taxa de sobrevivência dos caranguejos, potencialmente alterando a estrutura e a função dos ecossistemas de poças de maré. A redução no forrageamento levará a uma menor pressão de predação sobre as macroalgas e outros recursos alimentares, modificando a composição do habitat, o que pode afetar significativamente a dinâmica populacional da espécie e a biodiversidade local. No segundo estudo, concluiu-se que o caranguejo *Carcinus maenas* não apresentou diferenças significativas na sua percepção química em relação à presença do odor de mexilhão sob condições de aumento de temperatura e hipóxia induzida por nitrogênio. A resiliência do *C. maenas* pode servir como um indicador dos efeitos dos estressores associados às mudanças climáticas e como esses resultados podem afetar a história de vida e a janela de tolerância das espécies que vivem em habitats com variações abióticas mais drásticas. Esses resultados revelam que, apesar das previsões preocupantes de aumento de temperatura e diminuição de oxigênio para o fim do século, essa espécie tem potencial para ter uma vantagem competitiva sobre outras espécies, pois ainda é capaz de resistir a esses estressores, o que pode explicar seu sucesso invasivo. Tais resultados não devem ser generalizados para outras espécies de crustáceos, uma vez que esta classe é ainda considerada uma das mais vulneráveis a múltiplos estressores. Ambos os estudos destacam a importância de futuras pesquisas explorarem mais profundamente os efeitos isolados de variáveis como temperatura, assim como as interações complexas entre múltiplas variáveis. Recomenda-se também a continuidade de estudos que abordem não apenas as respostas imediatas das espécies as variáveis, mas também suas capacidades adaptativas a longo prazo diante das pressões ambientais crescentes.