# EFEITOS DAS MUDANÇAS CLIMÁTICAS NA FISIOLOGIA, COMPORTAMENTO E DISTRIBUIÇÃO DE CARANGUEJOS CHAMA-MARÉ

**BRUNNA DA SILVA VIANNA**

**SÃO VICENTE - SP**
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# UNIVERSIDADE ESTADUAL PAULISTA
## "Júlio de Mesquita Filho"

## INSTITUTO DE BIOCIÊNCIAS

## CÂMPUS DO LITORAL PAULISTA

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**Nome do candidato: Brunna da Silva Vianna**

**Orientadora: Prof. Dra. Tânia Marcia Costa**

**Coorientadora: Prof. Dra. Alessandra da Silva Augusto**

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## CERTIFICADO DE APROVAÇÃO

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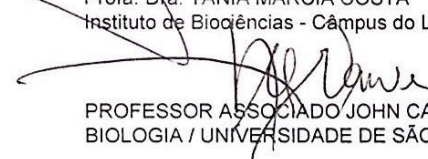
AUTORA: BRUNNA DA SILVA VIANNA
ORIENTADORA: TÂNIA MARCIA COSTA
COORIENTADORA: ALESSANDRA DA SILVA AUGUSTO

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Profa. Dra. TÂNIA MARCIA COSTA
Instituto de Biociências - Câmpus do Litoral Paulista / UNESP
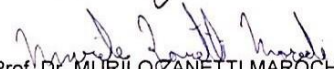
PROFESSOR ASSOCIADO JOHN CAMPBELL MCNAMARA
BIOLOGIA / UNIVERSIDADE DE SÃO PAULO

(PARTICIPAÇÃO VIA SKYPE)
Prof. Dr. CARLOS ARTURO NAVAS IANNINI
Departamento de Fisiologia / Instituto de Biociências - Universidade de São Paulo

Prof. Dr. LEONARDO QUEROBIM YOKOYAMA
Departamento de Ciências do Mar - Campus Baixada Santista / Universidade Federal de São Paulo - UNIFESP

Prof. Dr. MURILO ZANETTI MAROCHI
Instituto de Biociências / Campus do Litoral Paulista - UNESP

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"Não queira se tornar melhor que os outros.

Torne-se melhor para os outros!"

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**Sumário**

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# Resumo

Organismos do entremarés são frequentemente expostos a temperaturas extremas e as alterações climáticas podem trazer consequências negativas para estes organismos. Neste trabalho, avaliamos os efeitos do aumento da temperatura e redução do pH nas respostas fisiológicas e comportamentais de caranguejos chama-maré. *Leptuca uruguayensis* e *Leptuca leptodactyla* ocorrem tipicamente em habitat vegetado e não-vegetado de regiões estuarinas, apesar de coabitarem diversas áreas dos ecossistemas do entremarés. Realizamos experimentos para obtenção das respostas fisiológicas e comportamentais de ambas espécies ao aumento da temperatura. *Minuca mordax* foi exposta ao aquecimento e redução do pH da água para avaliação das respostas fisiológicas. Também estimamos a futura distribuição de *M. mordax* em cenários de mudanças climáticas. As duas espécies de *Leptuca* apresentaram diferenças nas adaptações fisiológicas (em relação ao consumo de oxigênio) e nos mecanismos de termorregulação, em consonância com a seleção de habitat e distribuição geográfica de cada espécie. *Leptuca uruguayensis* selecionou micro-habitat com temperatura inferior enquanto *L. leptodactyla* estava em um substrato com maior temperatura, mas apresentou características conhecidas por promoverem a dissipação de calor e reduzirem a taxa de desidratação, como tamanho e quelípodo maiores e coloração clara. As atividades comportamentais na superfície também foram afetadas pela elevação da temperatura, aumentando o tempo dentro da toca e reduzindo a taxa de alimentação, o que possivelmente alterou a excreção da amônia e índice hepatossomático. O comportamento de "waving display" não foi afetado pela temperatura, sugerindo relação com outros fatores como presença de fêmeas e competidores. O aumento da temperatura e redução do pH causaram alterações nas respostas fisiológicas (consumo de oxigênio, excreção de amônia, índice hepatossomático e osmorregulação) de *Minuca mordax*, efeitos agravados pela exposição combinada destes dois fatores. A distribuição dos caranguejos chama-maré poderá se expandir para maiores latitudes enquanto que temperaturas extremas poderão comprometer a sobrevivência dos organismos do entremarés em habitats nas baixas latitudes. A longo prazo, a exposição às altas temperaturas poderá resultar num suprimento energético ineficiente e comprometer funções fisiológicas, comportamentais, bem como as interações sociais. Por fim, as mudanças climáticas podem impactar negativamente a fisiologia, o comportamento e a distribuição dos caranguejos chama-maré em ambientes do entremarés.

**Palavras-chave:** consumo de oxigênio, Ecofisiologia, expansão na distribuição geográfica, mudanças climáticas, termorregulação comportamental.

# Abstract

Intertidal organisms are often exposed to extremes temperatures and may be harmed by climate change. The present study aimed to evaluate the effects of warming and pH decrease on physiology and behavior of fiddler crabs. *Leptuca uruguayensis* and *Leptuca leptodactyla* inhabit vegetated and unvegetated habitats from estuarine regions, respectively, despite cohabit some areas in intertidal ecosystems. Both species were exposed to warm to analyze the physiological and behavioral effects. *Minuca mordax* was exposed to warm and lower pH water. Also, we estimated the future distribution of *M. mordax* in climate change scenarios. Both species showed different adaptation (regarding oxygen consumption) and thermoregulatory mechanism, which reflect their habitat selection and range distribution. *Leptuca uruguayensis* selected less heated micro-habitat while *L. leptodactyla* was in a warmer substrate beside possessed larger size and major claw and whiten display that enhanced dissipation of heat and lowered desiccation rate. Higher temperature constraint surface activities, increasing refuge use and decreasing feeding, which might have impacted some physiological response (ammonia excretion and hepatosomatic index). Waving display was not affected by temperature, suggesting influence by other factors such as female and competitor presence. Warming and reduced pH altered physiological response (oxygen consumption, ammonia excretion, hepatosomatic index, and osmoregulation) of *M. mordax*, effects enhanced by combined factors. Climate change may cause expansion poleward of crabs' distribution although extreme temperatures may compromise the survival at lower latitudes. In long term, warm may harm energy supply and compromise physiological processes, behavior and range distribution of fiddler crabs in intertidal environments.

**Key-words:** behavioral thermoregulation, climate change, Ecophysiology, oxygen consumption, range expansion

# Introdução

Os efeitos das mudanças climáticas podem ser percebidos nos tempos atuais. O aumento da emissão antropogênica de gases de efeito estufa tem causado aumento de cerca de 1 °C em relação aos valores registrados anteriormente à Revolução Industrial. De fato, algumas regiões do planeta têm presenciado aumento de 1,5 °C em pelo menos uma estação do ano (IPCC, 2018). No entanto, é esperado que a temperatura aumente cerca de 3,7 °C até o final deste século (IPCC, 2014).

A queima de combustíveis fósseis aumenta a concentração atmosférica dos gases de efeito estufa, como o gás carbônico ($CO_2$), sendo uma fração absorvida pelos oceanos, o que causa a redução do pH dos mares (Dupont & Portner, 2013). Desde 1850, o pH da superfície oceânica reduziu cerca de 30% (Dupont & Portner, 2013) e até 2300 é prevista uma redução do pH na superfície do oceano de no máximo 0.77 (Caldeira & Wickett, 2003).

Mudanças nas condições ambientais podem ter impacto nos organismos, especialmente nos ectotérmicos do entremarés. Estes organismos apesar da possível plasticidade adaptativa, já vivem perto do seu limite térmico, sendo frequentemente expostos às temperaturas letais (Kearney et al., 2009). Por este motivo, os ectotérmicos do entremarés podem não ser capazes de se adaptarem a novos limites térmicos, ficando mais vulneráveis aos efeitos das mudanças climáticas (Stillman & Somero, 2000).

O aquecimento e redução do pH oceânico podem alterar as taxas metabólicas e a demanda energética com consequências negativas para a performance fisiológica dos organismos ectotérmicos (Gestoso et al., 2016; Paganini et al., 2014). Um suprimento ineficiente de energia compromete diversas funções fisiológicas como a manutenção da homeostase, crescimento, reprodução e sobrevivência desses organismos (Pörtner et al., 2017).

As mudanças climáticas também podem alterar o comportamento dos organismos do entremarés. O aumento da temperatura, por exemplo, pode alterar a taxa de alimentação (Wu et al., 2017) ou ainda restringir as atividades na superfície (Munguia et al., 2017; Payette & McGaw, 2003). Deste modo, os organismos podem passar mais tempo em refúgios térmicos comprometendo a obtenção de recursos alimentares e as interações sociais (Allen & Levinton, 2014; Reaney, 2007).

Mudanças nas variáveis ambientais podem alterar a distribuição dos organismos. Por exemplo, o aumento da temperatura nas últimas décadas resultou em expansão na distribuição de espécies vegetais de manguezais (Cavanaugh et al., 2014; Devaney et al., 2017) bem como

de macroinvertebrados, como o caranguejo chama-maré *Minuca pugnax* (Johnson, 2014), espécies tropicais que supostamente tem sua distribuição limitada pelas temperaturas baixas. Deste modo, o aumento da temperatura poderá causar expansão na distribuição em direção às altas latitudes e espécies que antes não conviviam poderão estabelecer novas relações interespecíficas.

Para avaliar os efeitos das mudanças climáticas na fisiologia, comportamento e distribuição dos caranguejos chama-maré, a presente tese foi dividida em três capítulos. No capítulo 1 apresentamos as respostas fisiológicas e comportamentais ao aumento da temperatura. Para tal, selecionamos como modelos o caranguejo chama-maré *Leptuca uruguayensis*, que geralmente habita áreas vegetadas, e *Leptuca leptodactyla*, que ocorre em habitat não-vegetado de regiões estuarinas. Apesar da preferência por diferentes habitats, ambas espécies podem coabitar em diversas áreas de ambiente do entremarés e nossa hipótese foi que elas responderiam diferentemente ao aumento da temperatura. No capítulo 2, nosso objetivo foi avaliar os efeitos do aumento da temperatura e redução do pH da água nas respostas fisiológicas do caranguejo chama-maré *Minuca mordax*. Nossa hipótese foi que a alteração e a interação destas variáveis resultariam em desafios à manutenção da homeostase. Já o estudo do capítulo 3 foi realizado durante o doutorado sanduíche na Ohio State University (Columbus, USA), onde avaliamos os efeitos do aumento da temperatura na futura distribuição da espécie *M. mordax* em diferentes cenários de mudanças climáticas. Na presente tese, obtivemos um panorama dos efeitos das mudanças climáticas em importante macroinvertebrados do entremarés. Considerando o papel destes organismos na estrutura das comunidades de manguezais e ecossistemas associados, e em processos ecológicos como a bioturbação, os impactos das mudanças climáticas nesses organismos poderá repercutir nestes ecossistemas.

**Capítulo 1**

**Effects of temperature increase on the physiology and behavior of fiddler crabs**

Vianna, Brunna Silva [a],*; Miyai, Caio Akira [a,b]; Augusto, Alessandra [a,b]; Costa, Tânia Marcia[a,b]

[a] Biosciences Institute, São Paulo State University (UNESP), Coastal Campus, Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, SP, Brazil.

[b] Aquaculture Center (CAUNESP), São Paulo State University (UNESP), Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil.

*Corresponding author. E-mail address: brunnavianna@live.com

**Abstract**

Intertidal organisms rely on physiological and behavioral adjustment to maintain the homeostasis under warm exposure. We evaluated experimentally the effects of the temperature increase due to climate warming on physiology and behavior of two fiddler crab species, *Leptuca uruguayensis*, which inhabit mostly vegetated areas and *Leptuca leptodactyla* that inhabit unvegetated areas. Our hypothesis was that *L. uruguayensis* would be more sensitive to warming than *L. leptodactyla*. Crabs were exposed to different temperatures: 27 °C (control), 31 and 33 °C (+4 and +6 °C, respectively) for 15 days to evaluate the physiological response (oxygen consumption, ammonia excretion, hepatosomatic index, and $Q_{10}$) and for 2 days to observe the behavioral response (feeding rate and duration of burrow retreat). We also tested in situ the effect of temperature on claw waving display of both species. We found that both responded differently to temperature rise, in which *L. uruguayensis* showed adaptation limit, while *L. leptodactyla* were able to adjust their metabolic rate to temperature rise. Despite these differences, temperature increase impacted the physiological response and surface activities of both species. Ammonia excretion was reduced in both, indicating changes on energetic substrate, amino acid catabolism or feeding. Higher temperatures reduced the hepatosomatic index, which might also be related to decreased feeding and in long term may occur insufficient energy levels. Further, temperature increased the duration of burrow retreat in both specie, potentially impacting social interactions, as matting. Temperature increase did not affect claw waving display frequency, suggesting that other factors may affect this behavior, e.g. female and competitor presence, accordingly to its primary role on matting attraction and territory defense. Behavioral thermoregulation (microhabitat selection) and morphological attributes might benefit thermoregulation of each specie, since were found no differences on body temperature between them. Therefore, climate warming will pose a physiological challenge and constrain surface activities of fiddler crabs, potentially altering energy supply and social interaction of crabs.

**Keywords:** climate warming, ecophysiology, *Leptuca* sp., microhabitat selection, thermoregulatory behavior, waving display.

# 1. Introduction

Intertidal environments present a wide thermal heterogeneity and organisms in these ecosystems are constantly exposed to thermal stress [1–3] desiccation risk [4–6], and frequently have the body temperature close to the lethal temperature [4,7]. These organisms that live close to maximum thermal limits might be more limited to adapt to new thermal limits when exposed to warmer temperatures [4,7]. Further, thermal stress imposed by intertidal environments, constrains activities and alters habitat use by ectotherms [1,8]. In this way, intertidal ectotherms must be harshly affected by climate warming.

Global warming reached approximately 1 °C above pre-industrial levels in 2017 and some regions has already been 1.5 °C warmer in at least one season in the last decade [9]. Warming may increase metabolic rate and energy can be mobilized from hepatopancreas [10] to physiological processes and activities. However, exposure to higher temperature could reduce metabolic rate which reduces the physiological energy costs and confers a short-term tolerance above the critical temperature [11–13]. So, the metabolic adjustment to changing temperature suggests if the condition comprise the natural range of the species or a limitation of the species to adjust to that environmental condition [14].

Moreover, temperature rise can alter the foraging behavior (e.g. stone crab) by reducing feeding and handling time of preys [15]. Behavioral response to warming is related to physiological sensitivity to thermal stress. Indeed, fiddler crabs can adjust behaviorly body temperature according to their tolerance [16], e.g. selecting a microhabitat with favorable condition or retreating into the burrow, which leads crabs to reduced body temperature, especially in the burrows located in open unshaded area [1]. However, resource availability like food supply would increase activities as waving display played by the fiddler crabs, e.g. *Leptuca pugilator*, leading to reduced refuge use and increased exposure to thermal stress [3].

Claw-waving displays of fiddler crabs have a primary function on female mating attraction and to territory defense [17]. While waving, the crabs stay longer outside the burrow, being exposed to heat stress [18]. However, the major claw of male fiddler crab appearsto have a thermoregulatory function, by dissipating heat to the air around the claw [5,19]. Accordingly, the thermal condition experienced by the claw can dissipate heat by conduction and rapidly influence the body temperature of crabs [19].

Warming can affect physiological performance [20] and fundamental behavior like foraging, that could compromise the persistence of the species and address farther ecological

impacts [21]. This way, the present study aimed to investigate the effect of temperature increase on physiology and behavior of two fiddler crab species, *Leptuca uruguayensis* (previously *Uca uruguayensis*) and *Leptuca leptodactyla* (previously *Uca leptodactyla*) [22]. Both species have similar size and a close sympatric association [23], although *L. uruguayensis* inhabit mostly vegetated area while *L. leptodactyla* inhabit unvegetated area [24,25]. Vegetation presence reduces the luminous intensity, the soil and burrow temperature of fiddler crabs [25], and keep the soil moisture [26]. Thus, crabs would be less exposed to heat and desiccation stress under vegetation cover. Therefore, we estimated that *L. uruguayensis* would be more sensitive to warmer temperatures than *L. leptodactyla*, assuming that these species would show different thermal tolerance in accordance with their distribution.

## 2. Methods

### 2.1 Study area and animal collection

Crabs were collected in a muddy-sandy bank at Itaguare River, Bertioga, Sao Paulo state, Brazil (23°77'S; 45°96'W) to proceed the experiments of temperature increase on physiology and behavior of both species. Animals were transported and acclimated in plastic container with sediment from collection site. Soil moisture were kept with water (15 psu) and crabs were maintained under a 12/12 h light/dark cycle. After two days of acclimation (25 °C) to reduce stress from transportation, animals were exposed to the treatment conditions as described below. Field observations of temperature effects on waving display of *L. uruguayensis* and *L. leptodactyla* were also conducted in a muddy-sandy bank at Itaguare River.

### 2.2 Experimental design

We tested the effect of temperature increase on physiology and behavior of two sympatric fiddler crabs. Our hypothesis was that temperature increase would alter metabolic rate, increase energetic demand, constrain surface activity and feeding. Two laboratorial experiments were conductedwith 3 x 2 factorial design (temperature: 27, 31, and 33 °C x species: *L. uruguayensis* and *L. leptodactyla*). Temperature range was set considering the summer air temperature mean in São Paulo coast (27 °C) from SIMCOSTA database (Brazilian

Coast Monitoring System) [27] and the predicted temperature increase of +4 °C (31 °C) and +6 °C (33 °C) in the end of 21st century and beyond, respectively [28,29]. Physiological response was evaluated measuring the variables: oxygen consumption, ammonia excretion, hepatosomatic index and temperature coefficient ($Q_{10}$) (N = 12). We also evaluated the behavioral response analyzing the feeding rate and time spent inside the burrow (N = 8). These variables indicate the metabolic rate (oxygen consumption and ammonia excretion), energy demand and acquisition (hepatosomatic index and feeding rate), and the ability to deal with heat stress ($Q_{10}$ and burrow retreat). We selected only males to reduce variability, since males shows one major claw and one small feeding claw while females have two small feeding claws. Also, males engage waving displays with major claw, behavior analyzed in the field experiment.

We proceeded an additional experiment in the field considering the importance of social interactions to claw waving display behavior [17] and the thermoregulatory role of major claw of male fiddler crabs [5]. Thus, we evaluated the effect of temperature (ranging from 21 to 33 °C) on claw waving display frequency of both *L. uruguayensis* and *L. leptodactyla* (N = 30). Waving display could increase the air flow around major claw and enhance heat dissipation. So, this behavioral mechanism could potentially improve the thermoregulatory benefit of major claw [5]. Therefore, we supposed that waving display would be more frequent under higher temperature.

## 2.3 Physiological response

To evaluate the physiological response of temperature increase, male crabs were exposed to 3 temperatures, as mentioned above (section 2.2). As male *L. uruguayensis* reaches sexual maturity at ~7.0 mm [30] and *L. leptodactyla* at 8.3 mm of carapace width (CW) [31], only adults were selected (*L. uruguayensis*: CW = 9.7 ± 0.4 mm, major claw length (CL) = 17.4 ± 1.7 mm; *L. leptodactyla*: CW = 10.2 ± 0.5 mm, CL = 19.7 ± 1.8 mm). Microcosms (15 x 10 x 12 cm) were filled with a sediment column of 10 cm, the maximum burrow depth of both species [32]. Crabs were kept 16 days in a germination chamber (FANEM Mod. 347 CDG) under a 12/12 h light/dark cycle. Initial air temperature was set 27 °C for 24 h of acclimation then temperature was maintained at the control treatment or increased in a rate of 1 °C h$^{-1}$ [33,34] until the predicted climate treatments (31 and 33 °C). Air temperature at 2 cm from the ground level were measured daily using digital thermometer. To maintain the soil moisture,

each microcosm received 100 mL of water with salinity 15 ‰, the mean salinity in an area these species inhabit [24].

Hence, crabs were transferred to individual respirometric chambers containing ~600 mL of water at the same salinity and temperature of the treatment. Aeration were kept for 30 minutes aiming to reduce stress from manipulation [35–37]. Then aeration was removed, oxygen concentration was measured using an oximeter (monitor and probe YSI, Models 53 and 5905, respectively) and chamber were sealed. After 4.5 h, oxygen concentration was measured again. Oxygen consumption were corrected against control chamber (without animals) and expressed as specific dry weight ($\mu g\ mg^{-1}$ dry weight $h^{-1}$) [36]. Water samples (50 mL) were collected and frozen to posterior analyses of ammonia excretion by colorimetry method [38]. Ammonia excretion were also expressed as specific dry weight ($\mu g\ mg^{-1}$ dry weight $h^{-1}$). Crabs were killed by freezing, dorsal carapace were removed to separate the hepatopancreas that were dried in air circulation oven at 60 °C for 48h to obtain the dry weight. Hepatosomatic index was calculated as a proportion of hepatopancreas dry weight to the body dry weight [37]. $Q_{10}$ correspond to the differences on metabolic rate between two temperatures and could indicate a thermal sensitivity of animals to variability of climate conditions [39]. $Q_{10}$ were calculated based in Parker and collaborators [40].

## 2.4 Behavioral response

### 2.4.1 Feeding rate and burrow retreat

Feeding rate and duration of burrow retreat were measured in male fiddler crabs exposed to different temperatures (see section 2.2). Social context, such as female presence and male density, influences the behavior of fiddler crabs, e.g. courtship signal [41] and refuge use [42]. To create a social context and stimulate activity of crabs, 9 individuals were placed in the microcosms: three males of *L. uruguayensis* and *L. leptodactyla*, and 3 females randomly selected from both species, i.e. 2 females from one species and 1 female from another. We randomly selected females from both species, considering that male fiddler crabs may court females of their own species as well as of heterospecific species [42]. Thus, we used female from both species in each microcosm. Only adults were used (at ~6 mm [30]. Carapace width of both sexes and major claw length of males were measured using calipers (*L. uruguayensis*: male, CW = 8.5 ± 1.0 mm, CL = 14.6 ± 2.1 mm, female CW = 8.7 ± 0.9 mm; *L. leptodactyla*:

CW = 9.5 ± 0.7 mm, CL = 17.5 ± 2.4 mm, female CW = 9.2 ± 0.9 mm). Sediment were collected from the same site of animal collection and organic fragments were manually removed. Microcosms had a sediment column of 10 cm and 20 cm of diameter. Crabs were kept in plastic containers inside the microcosms for five minutes and released at the same time.

Temperature was set with daylight natural UVA heat lamps (100 W; Model Repti Zoo) placed at different distances from substratum to regulate different temperatures, 12h light/dark cycle, and the laboratory temperature was maintained at 25 °C. Air and surface temperature were measured daily in 4 aleatory spots of each microcosms. After 2 days of exposure, microcosms were video recorded for 40 minutes, but the initial 10 minutes were not used in the analyses. Two focal animals were randomly selected in each mesocosm to observe both behavioral responses. Feeding rate were measured by the total of the minor claw lifted to buccal cavity in 5 minutes, divided by 5 (feeding rate/minute) [43]. Other 10 min of video were randomly selected to measure the amount of the time the second focal crab spent inside the burrow, expressed graphically as percentage of the time [44].

## 2.4.2 Waving display

To evaluate the effect of temperature on major claw waving displays, males were observed at diurnal low tides with different air temperatures during the reproduction season. The waving display has a primary function to mating attraction [17]. Thus, we selected as focal animal the males in reproductive maturity.

The luminous intensity is related to solar radiation incidence and heat condition. Here, we measured the luminous intensity to characterize the microhabitat of each species (5 replicates per species) prior and at the end of the observation. Then, adult intact crab with all pereopods were observed for 10 minutes and claw-waving display frequency were quantified. In the end of the period, the internal major claw and body temperature of the crab was measured, inserting the thermo-sensor of the digital thermometer into the major claw and body cavity, through the posterior margin of carapace [7]. We measured CW and CL using calipers (*L. uruguayensis*: CW = 9.0 ± 0.8 mm, CL = 15.9 ± 2.2 mm; *L. leptodactyla*: CW = 9.6 ± 1.2 mm, CL = 19.0 ± 3.0 mm). Then, the temperature was measured at the surface of the sediment and at the air 2 cm above the ground level (Table 1).

Table 1: Mean ± SD of air and surface temperature and luminous intensity of microhabitat of Leptuca uruguayensis and L. leptodactyla in Itaguare River.

| Species | Air temperature (°C) | Surface temperature (°C) | Luminous intensity (lux) |
|---|---|---|---|
| *L. uruguayensis* | 27.3 ± 3.2 a | 29.7 ± 3.9 a | 26076.38 ± 13353.34 a |
| *L. leptodactyla* | 27.9 ± 3.4 a | 32.4 ± 4.8 b | 65058.28 ± 30129.87 b |

Different letters indicate differences between species for the same variable (column).

## 2.5 Data analysis

Variables from the physiological response (oxygen consumption, ammonia excretion, hepatosomatic index), and behavioral response (feeding rate) were analyzed with a Generalized Linear Model (GLM), followed by Fisher's least significant difference (LSD) test, with physiological response as dependent variable, species and temperature as fixed factors. Data from duration of burrow retreat were homoscedastic and were analyzed by a two-way ANOVA followed by LSD pos hoc test. Number of waving display were analyzed using a Generalized Linear Mixed Model (GLMM), with number of displays as dependent variable, species as fixed factors, temperature of crab (body and major claw), temperature of sediment surface and air as random effects. Plot of predicted values against residuals had a shotgun pattern. Environment variables of both *L. uruguayensis* and *L. leptodactyla* were compared by Multivariate General Linear Model (MGLM), thus analyzed its univariate components. Data of crabs from both species (temperature of body and major claw, CW, and CL) were compared by a MGLM and its univariate components.

## 3. Results

## 3.1 Physiological response

Temperature exerted a physiological effect in both fiddler crab species. Comparing the oxygen consumption from both species at control (27 °C), *L. uruguayensis* showed higher consumption than *L. leptodactyla* (53%). However, temperature increase to 33 °C reduced 47% the oxygen consumption of *L uruguayensis*, while in *L. leptodactyla* the oxygen consumption increased 50% with temperature rise, comparing with control (GLM, Wald = 21.3871, $p \leq$

0.001) (Figure 1A). Temperature rise to 31 °C reduced the ammonia excretion rate of both *L. uruguayensis* and *L. leptodactyla* (Figure 1B) (GLM, Wald = 6.008, $p$ = 0.049594), although at 33 °C the excretion rate of *L. uruguayensis* increased, being similar to the control while the excretion rate of *L. leptodactyla* remained low. Hepatosomatic index (Figure 1C) was higher in *L. leptodactyla* (GLM, Wald = 6.05261, $p$ = 0.013886). However, temperature rise to 31 and 33 °C reduced this index in both species (GLM, Wald = 28.13991, $p \leq$ 0.001). $Q_{10}$ value between control temperature and 31 °C of *L. uruguayensis* were ~1, while of *L. leptodactyla* were ~3. However, with temperature rise to 33 °C, $Q_{10}$ of *L. uruguayensis* were lower than 1, reflecting the lower oxygen consumption, but $Q_{10}$ of *L. leptodactyla* were still ~3 (Table 2).
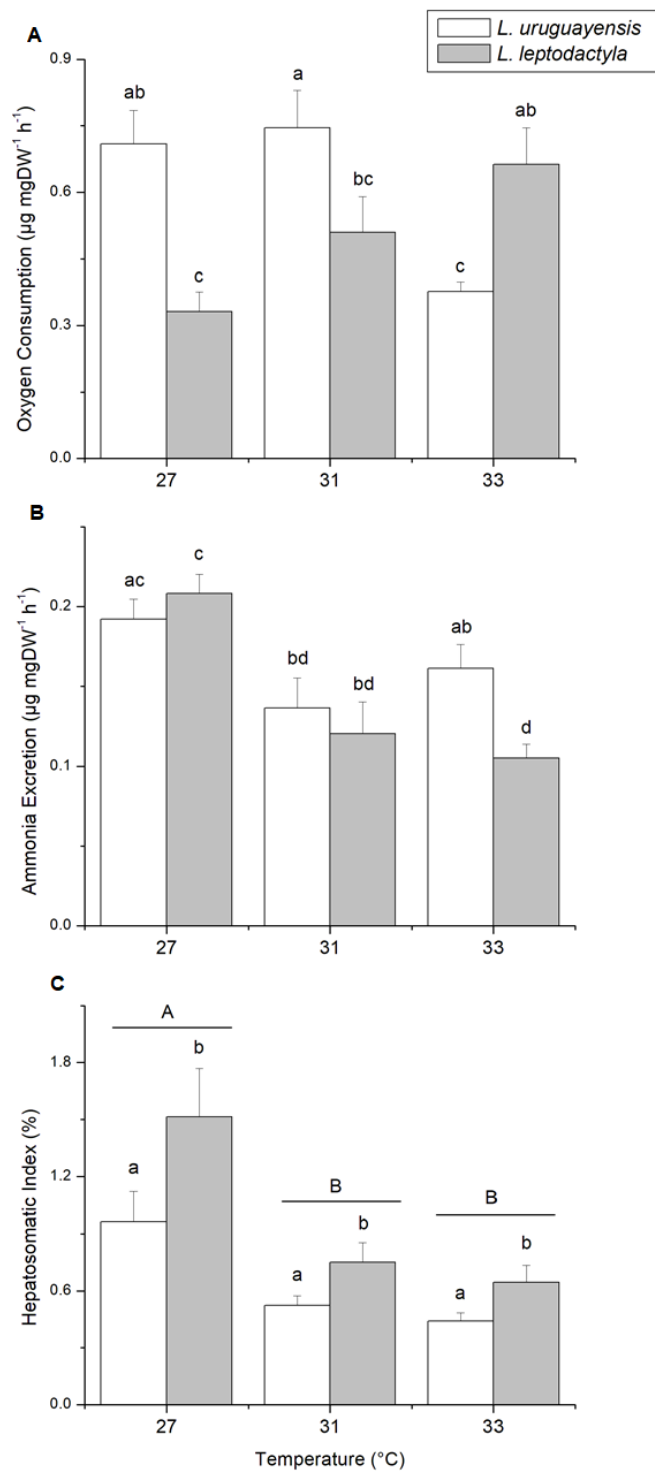
Figure 1: Oxygen consumption (A), ammonia excretion (B), and hepatosomatic index (C) of *Leptuca uruguayensis* and *L. leptodactyla* exposed to different temperatures. Mean ± SE. Different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments (species and temperatures). Capital letters above horizontal bars indicate significant differences between temperature treatments, irrespectively of the species.

Table 2: Thermal coefficient ($Q_{10}$) in *Leptuca uruguayensis* and *L. leptodactyla* for different temperature ranges

| Temperature intervals | *L. uruguayensis* | *L. leptodactyla* |
|---|---|---|
| 27 - 31 °C | 1.13 | 2.92 |
| 31 - 33 °C | 0.35 | 3.17 |
| 27 - 33 °C | 0.03 | 3.73 |

## 3.2 Behavioral response

### 3.2.1 Feeding rate and burrow retreat

Temperature had effect on behavioral response of fiddler crab. Feeding rate of *L. uruguayensis* was reduced under warmer temperature (31 and 33 °C) (Figure 2A). *Leptuca leptodactyla* instead, had a lower feeding rate at control temperature (27 °C) and no differences in this response with temperature increase (GLM, Wald = 12.21726, $p = 0.002$). Temperature affected time of burrow retreat that increased in both species exposed to higher temperatures (two-way ANOVA, $F_{2,42} = 21.1234$, $p \leq 0.001$), and no differences were found between both species (Figure 2B).
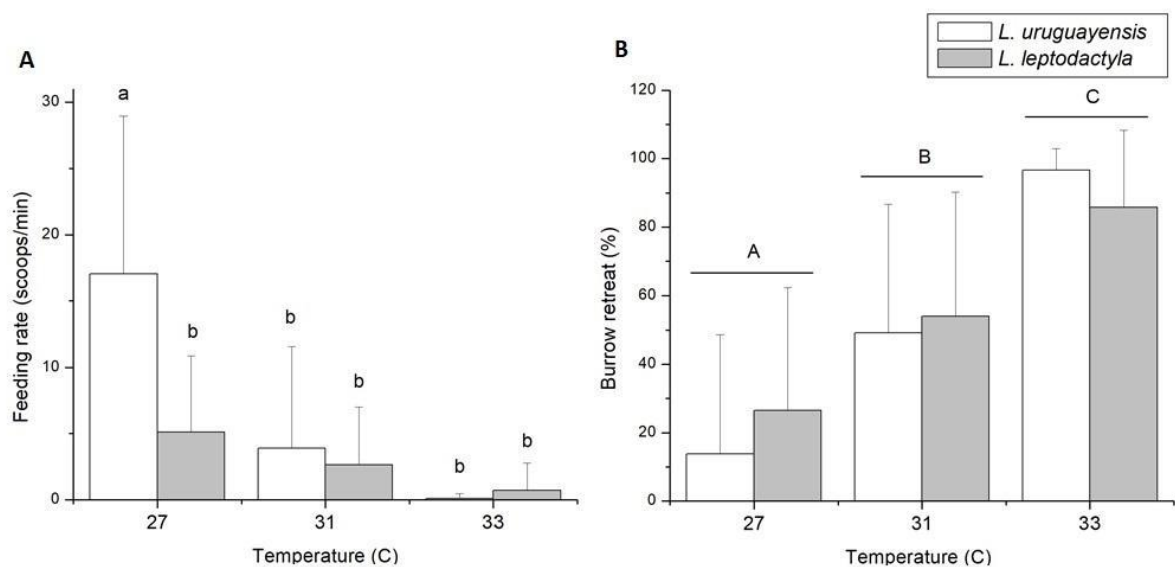


Figure 2: Feeding rate (A) and proportion of time inside the burrow (B) of *Leptuca uruguayensis* and *L. leptodactyla* exposed to different temperatures. Mean ± SD. Different

lower-case letters indicate significant differences ($p \leq 0.05$) among treatments (species and temperatures). Capital letters above horizontal bars indicate significant differences between temperature treatments.

### 3.2.2 Waving display and temperature

We found that claw waving display was not affected by temperature of crabs and environment for both *L. uruguayensis* and *L. leptodactyla* (GLMM, Wald = 0.666084, $p$ = 0.414420). Environmental variables showed differences where *L. uruguayensis* and *L. leptodactyla* were waving (MGLM, Wilks' Lambda = 0.154808, $F_{3,56}$ = 101.91, $p < 0.001$). Surface temperature and luminous intensity were higher in *L. leptodactyla* than in *L. uruguayensis*. No differences were found in air temperature which was also similar to the summer mean from São Paulo coast, value used as control on physiological and behavioral experiment at laboratory (Table 1). Temperature of body and claw were similar, but carapace width and major claw length differed between these species, higher in *L. leptodactyla* (MGLM, Wilks' Lambda = 0.154808, $F_{3,56}$ = 101.91, $p < 0.001$).

## 4. Discussion

*Leptuca uruguayensis* and *L. leptodactyla* showed different physiological response to warmer temperatures, oxygen consumption decreased in *L. uruguayensis* and increased in *L. leptodactyla*. Warmer temperature decreased the energy reserve from hepatopancreas and constraint the surface activity of both fiddler crab species. In contrast, the behavior of waving display was not affected by temperature change.

Rise temperature beyond *pejus* temperature (the onset of capacity limitation and hypoxemia) causes a decrease on oxygen supply due to failure on ventilatory and circulatory system to attend the oxygen demand. Metabolic depression and anaerobic metabolism are mechanisms that reduce the energy requirement and extend the thermal tolerance for a short term. However, in long term may compromise energy supply, growth, and survival of animals [11]. Decreased metabolic rates with temperature increase ($Q_{10} < 1$) may reflect a thermal limitation of the species [14,39], like observed in *L. uruguayensis* at 33 °C. Warming of +6 °C also decreased metabolic rates of the sea urchin *Heliocidaris erythrogramma*, which might suggest a suppression of physiological processes to maintain fitness [29]. Further, *L.*

*uruguayensis* showed elevated oxygen consumption when exposed to control temperature (27 °C). Although this might be due to seasonal variation, higher oxygen consumption might also reflect the physiological challenge impose by warmer temperatures, since this species normally inhabits vegetated areas. In São Paulo coast, this species is living close to its thermal and geographic range limits [45]. Meanwhile, *L. leptodactyla* were able to adjust the metabolic rate to temperature rise ($Q_{10} > 3$), increasing the metabolic rate under a higher energy demand condition which suggest that *L. leptodactyla* have a higher thermal limit than *L. uruguayensis*.

Temperature increase to 31 °C reduced ammonia excretion of both *L. uruguayensis* and *L. leptodactyla*. This result might be related to reduction of feeding or change on nitrogenous end-product excretion, e.g. urea [46], that was not identified by the ammonia excretion analyses. Moreover, energetic substrate selection would produce different end-products. Per example, if amino acids are the main substrate, it would produce larger amount of nitrogenous waste [47] compared to catabolism of mainly non-nitrogen compound [48]. Thus, crabs might had increased the catabolism of non-nitrogen compounds, like lipids, under higher temperature. However, temperature increase to 33 °C increased the ammonia excretion of *L. uruguayensis*, which might indicate a compensatory metabolic response to cope with higher thermal stress that crabs are unable to endure (regarding the decrease on oxygen consumption), contrarily to the response of *L. leptodactyla* that showed lower ammonia excretion and higher oxygen consumption at 33 °C.

Hepatopancreas is a multifunctional organ related to absorption of nutrients, intracellular digestion of food particles [10], and storage the energy source accessed when energy demand increases [49] or feeding reduces [50]. Warmer temperature reduced hepatosomatic index of both species, probably related to reduced feeding rate, then increasing the use of energy reserve from hepatopancreas. Yet, temperature increase might also compromise the function of this organ, likely reducing the production of digestive enzymes.

Temperature rise constrain surface activities of intertidal invertebrates. At the hottest hours of the day, body temperature of fiddler crabs also increases, and surface activity is reduced, especially in unshaded areas [1]. Thermal refuge, such as burrow retreat, reduces body temperature and alleviates thermal stress [1,8,23]. Temperature rise increased the time inside the burrow in both fiddler crab species. In addition, warmer temperature reduced feeding rate of *L. uruguayensis*. However, *L. leptodactyla* showed lower feeding rate in all temperature treatments. This species is normally distributed in unvegetated areas [24,25], so their activities must be dependent to sun heat exposure, which could explain the lower feeding rate of *L. leptodactyla* at laboratorial experiments. Further, fiddler crabs are deposit feeder sorting the

inorganic from the organic particles in the mount [23,51], that could be microalgae, vascular macrophytes, macroalgae, detritus and fish carcasses [52]. *L. leptodactyla* might select high quality food type, although not yet determined, requiring lower frequency of scoops to satisfy their feeding demand. Also, the number of spoon-tipped setae on the second maxillipeds results in different abilities to remove organic matter from the sediment [53]. *L. uruguayensis* has around 150 spoon-tipped setae [53] while *L. leptodactyla* has 222, approximately [51]. Therefore, *L. leptodactyla* might have greater ability to sort organic matter from the sediment, attending its feeding demand primarily.

Waving display has a primary function to mating attraction and territory defense. Therefore, waving display is stimulated by the presence of females and competitors. We found that temperature did not affect the frequency of claw waving display, refuting our hypothesis that the waving display would be affected by warmer temperature. Heat is rapidly transfer between major claw and body of fiddler crabs [5]. In fact, males with larger major claw experience lower body temperature [1]. However, the thermoregulatory function of major claw must be related solely to heat dissipation promoted by the presence of the enlarged structure hence temperature does not affect the waving display frequency.

Despite both *L. uruguayensis* and *L. leptodactyla* are described as sympatric species, Checon and Costa [54] observed a spatial segregation at a very small scale, regarding tide influence. *L. uruguayensis* occupied areas closer to tide line that is supposed to keep soil moisture and lower thermal variability. By contrast, *L. leptodactyla* were more frequent at upper level, submerged only at spring tides [54]. We observed that *L. uruguayensis* selected a less heated microhabitat with lower sediment surface temperature and luminous intensity. Microhabitat selection can benefit reducing body temperature when environmental temperature approach lethal temperature [1,2]. Although *L. leptodactyla* selected warmer substrate, body temperature was similar to *L. uruguayensis*, thus crabs were able to thermoregulate despite the warmer microhabitat. Males of *Astruca mjoebergi* with larger major claw shows lower body temperature than males with smaller major claw [1]. *Leptuca leptodactyla* had a larger major claw, attribute that benefit thermoregulatory capability. Regarding the body size, fiddler crabs with wider carapace show a slower desiccation rate [6], which might alleviate dehydration in *L. leptodactyla* at unvegetated habitat. Also, this species usually displays whitening carapace, chelipeds and pereopods [23], that benefits cooling the crabs [18,55]. Both species were able to thermoregulate regarding intrinsic physiological tolerance.

## 5. Conclusions

We observed that both species showed different response to warmer temperature. *Leptuca uruguayensis* decreased the metabolic rate with temperature rise and selected less heated microhabitats, concerning the physiological challenges imposed by higher temperatures to this species. However, *L. leptodactyla* were able to adjust the oxygen consumption to temperature increase. Also, this species has larger major claw and whitening ability that play a role on thermoregulation. Despite these differences, temperature increase impacted the physiological response and surface activities of both species. Temperature rise decreased the time and frequency of fundamental surface activities, such as feeding. In long term, crabs could use completely the energy reserves from hepatopancreas and deal with insufficient energy supply, harming others physiological functions like growth and reproduction. Warming temperature increased the time inside the burrow, which potentially compromise social interactions, such as matting. In conclusion, we can estimate that climate warming will impact negatively the physiological and behavioral performance of intertidal fiddler crabs.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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**Capítulo 2**

# Short-term exposure to ocean warming and acidification impose physiological challenges to the fiddler crab *Minuca mordax*

Vianna, Brunna Silva [a],*; Costa, Tânia Marcia [a,b]; Augusto, Alessandra [a,b];

[a] *Biosciences Institute, São Paulo State University (UNESP), Coastal Campus, Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, SP, Brazil.*

[b] *Aquaculture Center (CAUNESP), São Paulo State University (UNESP), Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil.*

*\*Corresponding author. E-mail address: brunnavianna@live.com*

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**Abstract**

Climate change is predicted to alter oceanicparameters, such as temperature, pH, salinity, and in consequence affect the physiology of organisms. Here, we used an experimental approach to evaluate the effects of ocean warming and acidification on physiological status of intertidal fiddler crab *Minuca mordax*. Animals were exposed to different temperatures (20, 25, 30 e 40 °C) and pH (environmental: 6.5; reduced: 5.8). After three days of exposure, we analyzed the oxygen consumption, total ammonia excretion, thermal coefficient, Oxygen:Nitrogen atomic ratio, hepatosomatic index, and hemolymph osmolality. We found that ocean warming and acidification poses an important threat to fiddler crabs. Warming caused an increase in oxygen consumption and temperatures of 40 °C altered osmoregulation in the crabs. Acidification elevated oxygen consumption and ammonia excretion rate of *M. mordax*, indicating increased catabolism of amino acids and raised excretion of nitrogen ionic form to promote net acid excretion. In addition, lower pH impacted the use of energy reserve. However, combined effect of warming and acidification impaired the adaptation of metabolic rate to rise temperature and the excretion of nitrogenous waste. Oxygen:Nytrogen ratio increased and lipids become more important fueling energetic substrate under warming, indicating rise energy demand. Lower pH did not change the energetic substrate, and crabs used protein to fuel the metabolism, although crabs used lipids as energetic substrate under combined warm and acidification (at 40 °C). Both factors synergistically affected physiological response elevating energy demand, altering the excretion of nitrogenous waste and compromising metabolic response to temperature rise. Therefore, ocean warming and acidification impose physiological challenge to performance and survival of fiddler crabs.

**Introduction**

Nowadays, the climate change effects on  atmosphere and ocean temperatures are noticeable. The global average temperature in the last 30 years has been successively increase the Earth surface temperature than any preceding period since 1850. Combined land and ocean temperature show a warming of 0.85 °C, over the period 1880 to 2012 (IPCC 2014). Also, the amounts of snow and ice have diminished, and sea level has risen (Cronin 2012; IPCC 2014) bringing consequences to coastal wetlands (Ross & Adam 2013).

Ocean global mean surface temperature is likely to be 3.7 to 4.8 °C warmer until the end of the 21st century (IPCC 2014). It is known that high temperature can affect the development, reproduction and survival of ectothermal organisms (Harianto et al. 2018; Tagliarolo et al. 2018; Wilkens & Fingerman 1965). Temperature change can enhance lethal effects and compromise thermal sensitiveness of intertidal invertebrates (Edney 1961; Fusi et al. 2017) and vertebrates (Madeira et al. 2017). These lethal effects are due to the inability of respiratory and circulatory activity to follow up the increased demand for oxygen and these tissues may collapse due to the reduced supply of oxygen (hypoxia) and energy (Pörtner 2002). Also, denaturation of enzymes and other protein molecules in high temperatures may harm survival of the organisms (El-Shesheny et al. 2016).

Another factor associated with climate changes is the ocean acidification. The oceans have been absorbing the excess of carbon dioxide ($CO_2$) emitted to the atmosphere mainly by the burning of fossil fuels, leading to a reduction on oceanic pH (Waldbusser & Salisbury 2014). The maximum pH reduction predicted to ocean surface is 0.77 unit around the year 2300 (Caldeira & Wickett 2003). Decreased pH would lead to a drop in pH of body fluids (Fehsenfeld & Weihrauch 2013) and increase the metabolic rate of organisms (Zittier et al. 2013). Physiological costs involved with osmotic balance and acid-base regulation (Fehsenfeld & Weihrauch 2013) in long-term exposure can result in a mismatch between energy supply and

demand harming physiological state and survival of marine organisms of different taxa and life stages (Basallote et al. 2012; Gravinese 2018).

Environmental factors can alter the physiological state of marine invertebrates possible affecting the fitness and the functional capacity at ecosystem-level process (Pörtner 2010). Ocean warming and acidification acting combined might cause an impact even more negative on metabolism (Gestoso et al., 2016; Paganini et al. 2014), reducing metabolic scope (Dissanayake & Ishimatsu, 2011; Zittier et al. 2013) of intertidal organisms. In addition, warming and acidification combined affect the larval offspring quality of a reef fish (Miller et al., 2015) and reduces larval survivorship of crabs (Gravinese et al., 2018).

Fiddler crabs are key species in estuarine environments and alteration on their abundance or bioturbation potential may affect the ecosystem dynamics (Citadin et al., 2018). We selected the fiddler crab *Minuca mordax* as experimental model organism to evaluate the physiological responses to the exposure to combined ocean warming and acidification. We hypothesized that alteration and interaction of these environmental variables would lead crabs to physiological challenges, altering metabolic rates, ammonia excretion, osmotic regulation, and increasing energetic needs to physiological compensatory mechanism due to ocean warming and acidification.

## Materials & Methods

### *Study organism and animal collection*

The intertidal fiddler crab *M. mordax* (Smith 1970) – previously recognized as *Uca (Minuca) mordax* (Shih et al. 2016) – were manually collected in Jaguareguava River, Bertioga, São Paulo State, Brazil (23°49'S 46°09'W), by digging the burrows during low spring tides. Only adult males (>11.9 mm carapace width, Fransozo et al. 2009) in intermolt period were used in this experiment. They were transported in container with sediment from the surface of

collection site. Animals were distributed in aquaria (25L; 3 animals per aquarium) containing soil from collection area. Acclimation experiments were performed simulating semidiurnal tidal cycle during 3 days in control condition: 25 °C, environmental pH (pH 6.5), and photoperiod was set up at 12h light:12h dark.

*Experimental design and set up*

*Minuca mordax* has a geographic distribution ranging from Belize through Torres, Rio Grande do Sul, Brazil (Thurman et al. 2013). Temperature at the species range were identified based on data from World Ocean Atlas (WOA) 2013 (https://www.nodc.noaa.gov/OC5/woa13/). Crabs were exposed to control temperature (summer mean temperature) and climate change scenarios. The summer mean temperature of seawater surface in the range of the specie's distribution was 25.8 °C. Here, we considered 25 °C as control temperature and 30 °C the warmer scenario considering the increase of 3.7 to 4.8 °C until the end of the 21st century (IPCC 2014). The warmer temperature (40 °C) was above the highest predictions of worst scenario for 2100 to ensure strong response considering the short-term exposure of the present experiment (Gestoso et al. 2016; Pörtner 2008), and considering that the frequency of extreme weather events are predicted to increase (Jennerjahn et al., 2017). Thus, crabs may be more often exposed to lethal temperatures. In the southern limit of the specie, the annual seawater temperature reaches 20 °C and this temperature of exposure were also tested. Environmental pH of collection site (pH 6.5 ± 0.1) was used as control. Considering environmental pH mean, hypercapnic water was determined with a reduction of 0.7 unit (pH 5.8), according to predictions made by Caldeira & Wickett (2003) beyond 2100.

To evaluate the effects of ocean warming and acidification on crab's physiology, the organisms were exposed to different scenarios. Crabs (n = 6; 3 crabs by aquarium; 2 aquaria per treatment) were exposed for three days to simulated semidiurnal tidal cycle in one of the 8

treatments: 4 water temperatures (20, 25, 30 and 40 °C), 2 pH condition (6.5 and 5.8).

Reduced pH was obtained by bubbling $CO_2$ in water using a cylinder equipped with manometer, diffuser, and solenoid valve (Dissanayake & Ishimatsu 2011; Moreira et al. 2018; Ramaglia et al. 2018). Water temperature was adjusted with air conditioning and immersion heater and maintained with constant aeration. Salinity was adjusted to 25‰. Two tidal cycles were simulated per day and the depth of water column were ~10 cm. Crabs were submerged for 4 h, the common duration that their burrows are submerged in the field. While animals were submerged, temperature and pH were measured hourly. Temperature was measured in the middle of water column using a mercury thermometer. The pH measurements were carried out on samples of water took from aquaria (40 x 23 x 25.5 cm) using a pH meter. Water samples were also collected once per day during experimental exposure to measure the total alkalinity (TA) determined by titration (Pires et al. 2015). Mean total alkalinity, pH, temperature, and salinity were inputted into CO2SYS software (Robbins et al. 2010) to calculate the $CO_2$ partial pressure ($pCO_2$), the saturation state of calcite ($\Omega$Cal) and aragonite ($\Omega$Ar) (table 1). The carbonate dissociation constants K1 and K2 selected were from Mehrbach et al. (1973) reffited by Dickson and Millero (1987), and KSO4 from Dickson (1990).

Table 1: Carbonate chemistry of water during *Minuca mordax* exposure to different scenarios. Salinity 25 ‰. Data are expressed by mean ± SD. N = 6 (2 aquariums per treatment x 3 days).

| T (°C) | $pH_{NBS}$ | TA ($\mu mol.Kg^{-1}$) | $pCO_2$ ($\mu atm$) | $\Omega$Cal | $\Omega$Ar |
|---|---|---|---|---|---|
| 20 | 6.5 | 1507 ± 130 | 16557 ± 1425 | 0.062 ± 0.005 | 0.039 ± 0.004 |
|  | 5.8 | 1397 ± 168 | 77282 ± 9304 | 0.011 ± 0.002 | 0.008 ± 0.001 |
| 25 | 6.5 | 1555 ± 126 | 17998 ± 1460 | 0.076 ± 0.006 | 0.049 ± 0.004 |
|  | 5.8 | 1391 ± 176 | 81074 ± 10235 | 0.014 ± 0.002 | 0.009 ± 0.001 |
| 30 | 6.5 | 1589 ± 334 | 19257 ± 4169 | 0.086 ± 0.018 | 0.067 ± 0.024 |
|  | 5.8 | 800 ± 174 | 49094 ± 10689 | 0.009 ± 0.002 | 0.006 ± 0.001 |
| 40 | 6.5 | 1647 ± 208 | 22087 ± 2797 | 0.136 ± 0.017 | 0.091 ± 0.012 |
|  | 5.8 | 1343 ± 284 | 90879 ± 19188 | 0.022 ± 0.005 | 0.015 ± 0.003 |

*Physiological response*

*Oxygen consumption, total ammonia excretion, O:N ratio, and thermal coefficient*

Oxygen consumption (OC) and total ammonia excretion (AE) were measured after 3 days of exposure. The animals were individually placed in respirometric chambers (580 mL) containing water in the same condition (salinity, temperature and pH) of experimental treatments. Individuals were maintaining during 30 minutes with aeration (open chamber) to reduce the stress of manipulation (Augusto & Masui 2014; Dissanayake & Ishimatsu 2011). Then, the aeration was removed, the oxygen concentration was measured with oximeter at the start and after 90 minutes with non-circulation (closed chamber). Control chambers containing water with the same experimental condition, but without animals, were analyzed (Xu et al. 2016). OC was standardized against individual weight and expressed as specific dry mass ($\mu$g mg$^{-1}$ dry weight h$^{-1}$) (Augusto & Masui 2014; Dissanayake & Ishimatsu 2011). Water samples (50 mL) were collected from respirometric chambers to proceed AE analysis. Total ammonia concentration of water (non-ionic $NH_3$ and ionic form $NH_4^+$) was measured in triplicate (10 mL each sample) by colorimetry (Koroleff 1976). AE were also expressed as specific dry mass ($\mu$g mg$^{-1}$ dry weight h$^{-1}$).

The Oxygen:Nitrogen (O:N) atomic ratio was calculated dividing the oxygen consumed (mol) by the ammonia excreted (mol) (Mayzaud & Conover 1988). O:N ratio indicate the metabolic substrate. Values in the range of 3 to 16 show pure protein catabolism while above 16, lipid start to play a role as energy substrate. Equal amounts of protein and lipid catabolism correspond to values between 50 and 60 (Mayzaud & Conover 1988).

Temperature coefficient ($Q_{10}$) can be estimated to analyze the variation in metabolic rates between two temperature (Fusi et al. 2016). $Q_{10}$ of oxygen consumption was calculated by the equation (1),

$$Q_{10} = (OC_2/OC_1)^{[10/(t2-t1)]} \tag{1}$$

Where $OC_1$ is the oxygen consumption measured at temperature 1 ($t_1$), $OC_2$ is the oxygen consumption measured at temperature 2 ($t_2$), ($t_1 < t_2$). $Q_{10}$ was calculated from mean data (Dissanayake & Ishimatsu 2011; Parker et al. 2017). The thermal coefficient will be equal 1 if the OC is independent of temperature and > 1 if OC increases with temperature (Parker et al., 2017).

*Hemolymph osmolality*

Crabs were killed by freezing and hemolymph samples (20 µL) were obtained inserting a needle attached to a syringe (1 mL) through the base of the fifth walking leg and immediately frozen. Hemolymph osmolality (HO) was estimated by a vapour pressure microsmometer (Augusto et al. 2009; Simonik & Henry 2014).

*Hepatosomatic Index*

The animals were weighed with an analytical scale (0.001 g) (Shimadzu, AY220), the dorsal carapace was removed and hepatopancreas (also called midintestinal gland, Rőszer 2014) was separated. Both animal and hepatopancreas tissue were dried at 60 °C for 48h and weighed (dry weight). Hepatosomatic index (HSI) were determined as the percentage of hepatopancreas's dry weight to the dry weight of the crabs' body (Bui & Lee 2014; Gerotto et al. 2015).

*Statistical analysis*

Effects of temperature and pH on physiological parameters (OC, AE, HO, ON ratio and HSI) were analyzed by two-way ANOVA. Before analysis, data was tested for normality of distribution and homogeneity of variance and log-transformed if not attending ANOVA's

assumption. LSD post hoc tests were made to identify significant differences ($p < 0.05$) between groups. Data were expressed by mean ± standard error of the mean.

## *Ethical notes*

Animal collection were authorized (protocol number 44884-3) by the System of Authorization and Information on Biodiversity (Sistema de Autorização e Informação em Biodiversidade – SISBIO, Brazil) from the Ministry of Environment of Brazilian government (Ministério do Meio Ambiente – MMA, Brazil).

## Results

### *Mortality*

No mortality was observed at control condition and with temperature change to 20 and 30 °C under environmental pH. Temperature increase to 40 °C (under control pH) caused a mortality of 16% of crabs. However, exposure to both warming and lower pH caused further mortality of 16 and 33% of crabs at 30 and 40 °C, respectively.

### *Oxygen consumption, thermal coefficient and ammonia excretion*

Higher temperature and lower pH increased OC of *M. mordax* (Figure 1A). Comparing with control temperature (25 °C), the OC under normocapnia increased 59% at 20 and 30°C and 79% at 40 °C. Under hypercapnia, the OC decreased 55% at 20 °C comparing with 25 °C and no significant difference was found between the control and 30 or 40 °C. Comparing normo- and hypercapnia, crabs under hypercapnia increased the OC 56% at control temperature. But, the combined effect of temperature change and pH decrease caused reduction on OC of 58% and 39% at 20 and 40 °C, respectively, comparing with normocapnia at same temperature. No

significant differences between OC in normo- and hypercapnia at 30 °C (ANOVA, $F_{(3,33)}$ = 11.2160, $p < 0.001$). $Q_{10}$ values (table 2) were higher than 2 with the temperature increase from 25 to 30 and 40 °C in normocapnia, also in hypercapnia, between control temperature and 20 and 30 °C. Otherwise, under the synergic effect of temperature rise and pH reduction (at 40 °C, hypercapnia), the thermal coefficient was lower than 1.

Temperature did not affect AE of *M. mordax* under normocapnia (Figure 1B). AE increased 69% at hypercapnia in control temperature, despite no differences was found between normo- and hypercapnia at 20, 30 and 40 °C. Synergistic effect of temperature and pH, decreased AE in approximately 70%, comparing with AE with the exposure to pH reduction at 25 °C (ANOVA, $F_{(3,29)}$ = 9.6181, $p = 0.0001$).
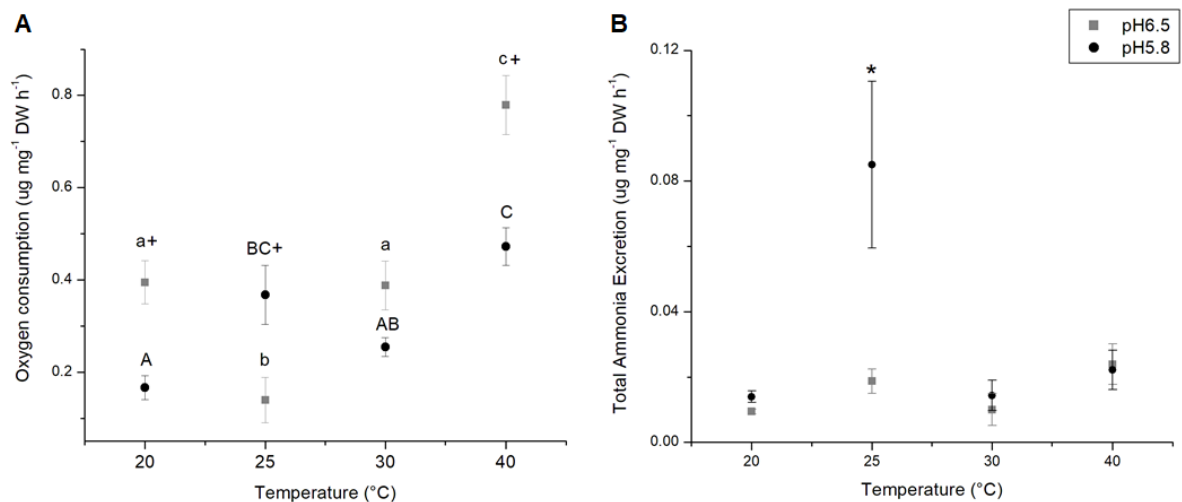


Figure 1: Oxygen consumption (A) and ammonia excretion (B) of *Minuca mordax* exposed to temperature increase and reduction of pH. Data are given in Mean ± SE. White and dark gray bars represent normocapnic (pH 6.5) and hypercapnic (pH 5.8) treatments, respectively (n = 4-6). Lowercase and uppercase letters denote differences between normocapnic or hypercapnic treatments, respectively. Plus symbols (+) represent significant differences between normo- and hypercapnia and asterisk symbol denotes differences among treatments ($p < 0.05$).

Table 2: Thermal coefficient ($Q_{10}$) in *Minuca mordax* for different temperature ranges and pH

| Temperature intervals | pH 6.5 | pH 5.8 |
|:---:|:---:|:---:|
| 20-25 °C | 0.12 | 4.89 |
| 25-30 °C | 8.18 | 2.26 |
| 30-40 °C | 2.00 | 0.11 |
| 25-40 °C | 3.20 | 0.81 |

### *O:N ratio*

In exposure to normocapnia, *M. mordax* consumed protein as energetic substrate at 25 °C (table 3). Change in temperature altered the O:N ratio under normocapnia, although not statistically different, indicating a lipid-protein oriented metabolism at 20, 30 and 40 °C. Hypercapnic acclimated crabs showed low O:N ratio and used protein as energetic substrate at 20, 25 and 30 °C. However, effect of both extreme temperature and lower pH, caused increase on O:N ratio, indicating that lipid is also used as energetic substrate by crabs under hypercapnia at 40 °C. Comparing normo- and hypercapnia, the substrate was the same (protein) at control temperature, but at 20 and 30 °C, O:N decreased in exposure to hypercapnia (despite no differences at 30 °C) and crabs consumed protein. At 40 °C both normo- and hypercapnic acclimated crabs showed protein-lipid oriented metabolism.

Table 3: O:N atomic ratio and energetic substrate of *Minuca mordax* in temperature and pH treatments. Values represent Mean ± SE (n = 4-6). Lowercase letters represent significant differences between normo- and hypercapnia at the same temperature. Uppercase letters represent differences between temperatures at the same pH ($p < 0.05$).

| | Normocapnia | Energetic substrate | Hypercapnia | Energetic substrate |
|:---:|:---:|:---:|:---:|:---:|
| 20°C | 21.53 ± 3.27[aA] | Protein-lipid | 5.92 ± 1.24[bA] | Protein |
| 25°C | 3.92 ± 0.94[aB] | Protein | 4.62 ± 0.96[aA] | Protein |
| 30°C | 29.03 ± 8.96[aA] | Protein-lipid | 12.34 ± 3.42[aA] | Protein |
| 40°C | 22.45 ± 7.77[aA] | Protein-lipid | 16.92 ± 2.63[aA] | Protein-lipid |

*Hemolymph osmolality*

*Minuca mordax* is a hyper-osmoregulator in diluted habitat and control crabs maintained an osmolality of $663 \pm 54$ mOsm kg/$H_2O$ (Figure 2). Temperature affected the HO (ANOVA, $F_{(3,35)} = 5.403$, $p = 0.003$). No significant difference was found between temperatures, although at 40 °C crabs showed rise HO (normocapnia = $787 \pm 49$ mOsm kg/$H_2O$; hypercapnia = $895 \pm 35$ mOsm kg/$H_2O$). Exposure to hypercapnia did not affect osmoregulation and no significant differences were found between normo- and hypercapnia. There were no effects of the interaction between temperature and pH.
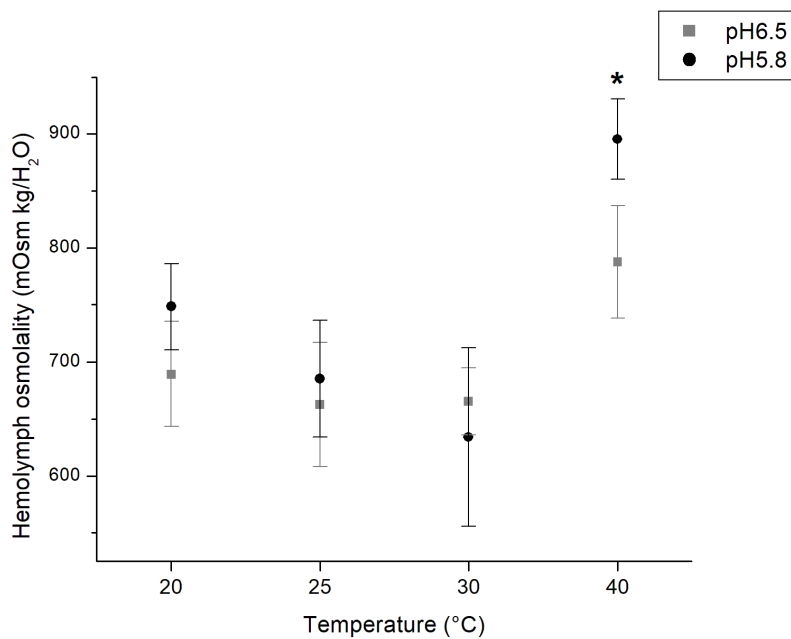


Figure 2: Hemolymph osmolality of *Minuca mordax* exposed to temperature increase and reduction of pH. Data are given in Mean $\pm$ SE. Gray squares and black circles symbols represent normocapnic (pH 6.5) and hypercapnic (pH 5.8) treatments, respectively (n = 4-6). Salinity 25 ‰. Asterisk symbol represents significant differences between temperatures ($p < 0.05$).

### *Hepatosomatic Index*

HSI was not affected by temperature on crabs exposed to normocapnia (Figure 3). Comparing normo- and hypercapnia, crabs showed higher HSI on hypercapnia at 20, 25 e 30 °C (increases of 57%, 63%, 57%, respectively) (ANOVA, $F_{(1,34)} = 17.437$, $p < 0.001$). There were no effects of the interaction between temperature and pH.



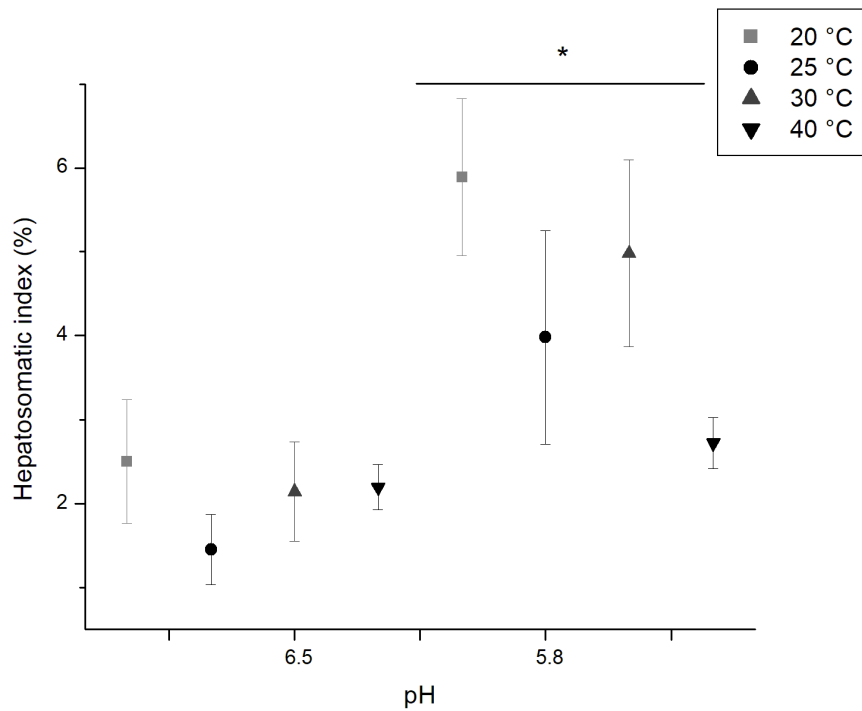Figure 3: Hepatosomatic index of *Minuca mordax* exposed to temperature increase and reduction of pH. Data are given in Mean ± SE. White to dark gray bars represent temperatures treatments (20, 25, 30, and 40 °C) for both normocapnic (pH 6.5) and hypercapnic (pH 5.8) (n = 4-6). Asterisk symbol above horizontal bar represents significant differences between normo- and hypercapnia ($p < 0.05$).

### Discussion

Warming enhanced the metabolic rates and energy demand of *M. mordax*. Besides, osmoregulation was impaired at temperature extreme. Hypercapnia affected the use of energy reserve and enhanced the oxidation of nitrogenous compounds. Combined warm and hypercapnia compromised the adjustment of metabolic rate to temperature rise and altered the excretion of nitrogenous waste.

Rise temperature increases the tissue oxygen demand (Fusi et al. 2015), being expected increases on metabolic rates (Fusi et al. 2017; Pörtner 2002) and energetic demand (Portner 2010). However, when temperature rise to 30 °C, it caused further increase on oxygen consumption ($Q_{10} \sim 8$), suggesting an elevated compensation of metabolic rate to temperature increase. This response must be related to the habitat selected by the species, since *M. mordax* normally inhabit vegetated areas (Masunari 2006; Salmon 1967) and must be less exposed to heat stress than others fiddler crabs which inhabit unshaded habitats. Hypercapnia also increased the oxygen consumption, probably related to energy requirement of active mechanism to acid base regulation. Although, this mechanism seemed to be harmed in crabs exposed to both higher temperature and lower pH. The interaction of temperature increase and lower pH reduces the hemocyanin oxygen affinity (Bridges 2001) and this condition must be limiting to *M. mordax*.

Temperature change to 20 °C also increased the oxygen consumption. This result was unexpected since the metabolic rate usually reduces with temperature decrease (Parker et al. 2017; Pörtner 2002; Verberket al. 2016). Despite the oxygen solubility increases with temperature reduction, the oxygen diffusivity decreases, resulting in a reduced environmental oxygen availability at the respiratory surface in cold water while the water viscosity increase (Verberk et al. 2011). Furthermore, small animals, such as the fiddler crabs are heavily affected by viscosity (Verberk et al. 2016). *Minuca mordax* might be under a reduced oxygen availability and higher energy demand condition, since it used lipids as energetic substrate. Although lipids

provide more energy, they require larger amount of oxygen to be oxidized (Mayzaud & Conover, 1988). Thus, crabs might had compensated increasing the ventilatory rate.

Acidification may reduce pH of hemolymph and two mechanisms to regulate the acid-base state are raised gas exchange (Fehsenfeld & Weihrauch 2013; Zittier et al. 2013) and ammonia excretion to buffer pH disturbance (Hammer et al. 2012; Fehsenfeld and Weihrauch 2016). Non-ionic ammonia ($NH_3$) diffuses into acidified vesicles, is transformed in the ionic form ($NH_4^+$), to be then released (Weihrauch et al. 2004). This mechanism of increasing the catabolism of amino acids to supply body fluids with ammonia to aid in acid excretion, was observed in *Carcinus maenas* exposed to hypercapnia (Hammer et al. 2012). Additionally, amino acids are metabolized as energy source that could be used to ionic regulation (Shinji et al. 2012). Increased total ammonia excretion of *M. mordax* under hypercapnia can be due to increased catabolism of amino acids used as energy source and rise excretion of the ionic form ($NH_4^+$) to buffer a possible acid-base disturbance.

Crabs exposed to both warmer temperatures and lower pH showed alteration on excretion of nitrogenous end-product. Similar results were observed in the Dungeness crab, *Metacarcinus magister*, that decreased ammonia excretion as well as oxygen consumption when acclimated for 10 days to pH decrease of 0.7 units (Hans et al. 2014). However, *M. magister* were able to fully compensate the respiratory acidosis caused by hypercapnia. So, this result might be due to change of nitrogenous end-product excreted, e.g. urea (Hans et al. 2014). *Minuca mordax* might excrete different waste under combined warm and hypercapnia. Further, the reduction on oxygen consumption and ammonia excretion of *M. mordax* exposed to both warm and hypercapnia, might indicate reduction on catabolism of amino acids and metabolic rate. Crabs may have reallocated energy resources to homeostasis control in detriment to others process, like locomotor activity.

Although the challenge imposed by hypercapnia to acid-base and ionic regulation, crabs maintained the osmoregulatory capability under hypercapnia exposure. Fiddler crabs show in general great osmoregulatory ability. The isosmotic point (osmotic concentration at osmolality of habitat and of hemolymph are equal) of *M. mordax* is 615 mOsm/kg $H_2O$ in Brazilian cost (Thurman et al. 2017). This specie can tolerate a wide range of hemolymph osmolality and may possess a celular machinery for hyper-osmoregulation in diluted habitats (Thurman et al. 2017). However, extreme temperatures compromised osmotic balance of *M. mordax* in both normo- and hypercapnia. *Leptuca thayeri* is a fiddler crab that inhabit vegetated area in mid-tide zone (Principe et al. 2018). This specie shows high dehydration and mortality rates when exposed to heat due to greater carapace permeability and lower ability to avoid desiccation. Although, *L. thayeri* is able to maintain hemolymph osmolality constant when exposed to temperature rise, due to a good salt excretion capability (Principe et al. 2018) which reflects its distribution in meso- to eusaline habitats (Thurman et al., 2013). Temperature increase to 40 °C might have enhanced water loss by evaporation in *M. mordax* and this specie must not have a prominent mechanism to salt excretion, regarding that *M. mordax* is a specie from diluted habitat. Thus, crabs under dehydration condition may have the osmoregulatory function compromised. Further, salt uptake or reabsorption can occur through the Na+/H+ antiporter (McNamara & Faria, 2012) and this mechanism might be related to intracellular pH regulation, resulting in increase of solute concentration on hemolymph of *M. mordax*.

Hypercapnia altered the use of energy reserve by *M. mordax*. Hepatopancreas is a multifunctional organ with cells specialized, per example in glycogen and lipid storage, nitrogen metabolism and excretion of nitrogen metabolites and detoxification (Rőszer 2014). Lower intracellular pH alters enzyme function, reduces glycolysis and impair aerobic metabolism (Hazel et al. 1978 apud Heuer and Grosell 2014). Further, intracellular accumulation of bicarbonate under hypercapnia buffers pH decrease (Fehsenfeld & Weihrauch

2016; Knapp et al. 2015), although impairs cellular process and the use of energy reserves (Heuer & Grosell, 2014). The shrimp *Xyphopenaeus kroyeri* showed increase on hepatosomatic index after exposure to a reduction on pH of 0.7 units in summer, which might be related to decrease on storage energy usage or increased of organ function (Augusto et al. 2018). Hypercapnia likely lead *M. mordax* to a reduction on the use of energy reserve from hepatopancreas (e.g. lipids) and combined with warm, insufficient energy supply may occur in long-term.

Warming temperature and hypercapnia altered the energetic substrate used by fiddler crabs. Energetic substrate required different amount of oxygen to be oxidized, per example the oxidation of proteins requires half amount of oxygen than lipids (Mayzaud & Conover 1988). Accordingly, crabs showed reduction on oxygen consumption and used protein as energetic substrate under hypercapnia. *Callinectes danae* and *X. kroyeri* also used proteins as energetic substrate irrespectively to pH decrease, although *X. kroyeri* showed a reduction on oxygen consumption when exposed to different conditions from its natural habitat, regarding salinity and pH levels (Augusto et al. 2018). However, warming increased the energy demand and high-energetic substrate, i.e. lipids, was used for *M. mordax* under normocapnia and combined warm (40 °C) and hypercapnia.

Warming and acidification impact the physiological response of *M. mordax*. Crabs tend to respond more strongly when exposed to warmer temperature combined with hypercapnia, showing alteration of metabolic response and increasing the energetic demand. This study showed the short-term response and in long-term, organism may adapt to the new condition or may have other functions compromised, as behavior, reproduction and growth due to insufficient energy levels. In conclusion, we predict that ocean warming and acidification may lead intertidal crabs to metabolic changes and the interactions of these environmental factors may cause further physiological impacts challenging the survival of fiddler crabs.

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**Disclosure statement**

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**Capítulo 3**

**Climate warming will expand southward the range distribution of fiddler crab**

Vianna, B. da S. [a]*; Costa, T. M [a]; Montenegro, A. [b]

[a] Biosciences Institute, São Paulo State University (UNESP), Coastal Campus, Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, SP, Brazil.

[b] Department of Geography, Ohio State University, 1036, Derby Hall, 43210, Columbus, OH, USA

*Corresponding author. E-mail address: brunnavianna@live.com

## Abstract

Climate plays a role in species distribution. Compared to pre-industrial values, human activities have at this time caused an increase in average global surface temperature of around 1 °C and this warming can reach up to 3.7 °C by 2100. The aim of the present work was to evaluate the effects of climate change on the future distribution of fiddler crabs. We adopted as model species *Minuca mordax*, an intertidal eurytherm presently found from Belize to Southern Brazil. Current annual and summer average temperatures at the northern and southern limits of the specie's present distribution range were obtained from two distinct observational sets. Analysis indicate that the southern range represents the lower bound of temperature tolerance while the location of the northern limit is not a function of temperature and potentially set by other factors such as biotic interaction, oceanic barriers to larval dispersion, and habitat selection (e.g. salinity). Based on previous experiment proceeded to evaluate the physiological response of *M. mordax* to temperature increase (unpub. data) it was observed that temperature above 30 °C is challenging. Potential changes in the southern limit of the present range, as well as areas within the current range where organisms would deal thermal constraint were estimated for three periods (years 2036-2050, 2086-2100 and 2286-2300), based on statistically downscaled global climate model temperature predictions coming from two greenhouse gas emissions scenarios (RCP 4.5 and 8.5). Climate warming will result in a gradual poleward displacement of the range's southern limit, that could move to as far south as Montevideo, Uruguay by 2100 under the higher emission scenario. Climate warming will potentially enhance physiological challenge at several areas at lower latitudes and this can lead to crabs altering surface activities and microhabitat use. Global warming will alter the future distribution of intertidal fiddler crabs, facilitating their establishment in some areas while compromising the survival in others, potentially affecting behavioral responses and biotic interactions in intertidal environments.

# 1. Introduction

Anthropogenic activities had caused global mean temperature warming of approximately 1 °C above pre-industrial levels and some regions have already experienced an increase of 1.5 °C in at least one season (IPCC, 2018). Temperature is predicted to increase up to 3.7 °C by 2100 (IPCC, 2014). Climate warming can lead physiological stress, compromising reproduction, behavior, and survival of marine invertebrates (Pörtner et al., 2017). Furthermore, tropical and intertidal ectotherms would be the most affected by global warming since many of these are already living close to their upper thermal limits (Darnell et al., 2015; Kearney et al., 2009; Stillman & Somero, 2000).

Climate has a direct impact on the distribution of ecosystems, therefore, the distribution of organisms could also shift with climate change. Some species have already changed their range distribution to higher latitudes in the past few years (Thuiller, 2007), e.g. the range distribution of ghost crabs *Ocypode cordimanus* is expanding southward (Schoeman et al., 2015), whereas the northern range of fiddler crabs *Minuca pugnax* is expanding poleward (Johnson, 2014). Another example is the poleward expansion of Florida mangrove canopies in the last 25 years due to reduction in winter freezing conditions (Cavanaugh et al., 2014). This temperature-caused northward expansion can be magnified by a facilitated seeding positive feedback (Devaney et al., 2017) and is also related to changes in macrofaunal, including crab diversity (Smee et al., 2017). Climate warming can also result in reduction of species diversity (Helmuth et al., 2002) and benefit invasive species (Zhang et al., 2014) with potential ecological impacts.

In a climate change scenario, organisms in the coastal regions may be able to find new areas with favorable environmental conditions for their development, per example expanding their range distribution, while in others they may no longer occur. In this way, we aimed to evaluate the effects of climate change on the future distribution of the intertidal fiddler crab *Minuca mordax*. Our hypothesis was that climate change would alter the future distribution of fiddler crabs toward higher latitudes. To test this hypothesis, we analyzed *M. mordax*' range distribution in coastal environments and estimated the future range distribution in a midrange mitigation and high emission scenarios of greenhouse gases in short and long-term predictions.

# 2. Materials and methods

## 2.1 Range distribution and temperature limits

The fiddler crab *Minuca mordax* (Smith 1870), formerly *Uca* (*Minuca*) *mordax* (Shih et al., 2016), shows a range distribution from Belize northern Caribbean (16°49'N 88°16'W) to Torres, RS, southern Brazil (29°19'S 49°44'W) (Thurman et al., 2010; Thurman et al., 2013). Temperature limits of *M. mordax* distributions were identified based on temperature recorded at the northern (17.15˚N) and southern (29.33˚S) limits of the species range. Monthly sea surface temperature (SST) means were obtained from the World Ocean Atlas (WOA) 2013 – National Oceanic and Atmospheric Administration (NOAA) (https://www.ncei.noaa.gov/) and from remotely sensed values provided by the Group for High Resolution Sea Surface Temperature (GHRSST) - NASA EOSDIS Physical Oceanography Distributed Active Archive Center (https://podaac.jpl.nasa.gov/). The WOA data represents an average of the first 10 meters of the water column. It is based on multiple types of observations and has spatial resolution of 0.25° (~25 km at the equator). We used GHRSST's Foundation SST, which provides an estimate at 10 m depth, with horizontal spatial resolution of 0.01° (~ 1 km at the equator). Temperature limits were estimated by averaging observations along a 100 km band centered at the latitudinal limits and extending 25km offshore. We followed this procedure to calculate annual means temperatures at the limits of *M. mordax*' range distribution.

## 2.2 Statistical downscaling

The *Minuca mordax* distribution detailed above is based on adult individuals, which in this species inhabit mostly upstream rivers, but also estuaries close to the open sea (Crane, 1975) . The downscaling goal was to use climate model output to obtain future SST projections in areas that better represent conditions at the adult organisms' habitat. With this in mind, we generated correlations between course (~100 x 125 km bin) modeled SST and sea surface salinities (SSS) and observed SSTs found within 1km from shore. These correlations were then, in conjunction with future model predictions, used to estimate nearshore SST. Prior to downscaling, the ~1km x 1 km observed SSTs were averaged into ~25km alongshore by ~1 km cross shore bins for the whole domain.

Temperature observations for the downscaling effort came from the same GHRSST SST product described above. These SST observations were organized in annual climatological means covering the years 2003 to 2012, the period for which observations and available climate

model output overlap. Modeled SST SSS were comprised of monthly means from historical and future climate simulations conducted with the Goddard Institute for Space Studies (GISS-E2-R) global climate model (GCM) and were obtained at the Coupled Model Inter-comparison Project Phase 5 (CMIP5) data portal (http://pcmdi9.llnl.gov/). CMIP5 is a multi-model intercomparison experiment coordinated by the World Climate Research Programme (WCRP, Meehl & Bony, 2011). GCM SST and SSS had horizontal resolution of 1.0° x 1.25° (Schmidt et al., 2014), and were organized in annual climatological means from the years 2003 to 2012. The study domain was the area from 40˚S to 30˚N and from 30˚ to 100˚ W and areas above land and further than 1km from cost, were masked.

Relationships between modeled and observed values were obtained by two different methods. Both used as input data historical climate simulations and observed SST for the 10-year period between 2003 and 2012. The first method involved fitting the data to a polynomial of the form:

$$SST_{ns\_ic} = C1 + C2\ SST^n_{g\_im} + C3\ SST^{n-1}_{g\_im} + \dots Ci\ SST_{g\_im} \qquad (1)$$

Where $SST_{ns\_ic}$ is the observed, nearshore temperature at the ic coastal bin (predictand); $SST^n_{g\_im}$ is the GCM temperature at the im model bin to the $n^{th}$ power (predictor); and C1, C2, C3 and Ci constant coefficients determine by the fitting exercise. After preliminary tests, a polynomial of order 3 was selected. Note that the im model bin always contains the ic coastal bin.

The second method incorporated SSS as a second predictor and consisted of fitting the data to a multivariate linear equation in the form:

$$SST_{ns\_ic} = C0 + C1\ SST_{g\_im} + C2\ SSS_{g\_im} \qquad (2)$$

Where $SSS_{g\_im}$ is the GCM sea surface salinity at the im model bin and C0, C1 and C2 constant coefficients determined by the fitting exercise (Aksornsingchai & Srinilta, 2011).

Observed and GCM data from 2003 to 2010 were used to estimate the coefficients for equations 1 and 2. A set of coefficients was obtained for each 25x1km coastal bin within the domain. Downscaling skill was determined by comparing estimated to observed nearshore temperatures for the period between 2011 and 2012. The multivariate method (eq. 2) resulted in overall smaller root mean square errors ($RMS_{err} = 0.6976$) and was chosen over the polynomial based reconstructions ($RMS_{err} = 0.7095$) for the analysis described below. Errors

were significantly smaller at lower latitudes in both polynomial and multivariate methods, although the error at the present southern limit of specie distribution were about 1 °C (Fig. SM1), which means that the estimated SST were ~1 °C warmer or colder than observed SST in that area. Both downscaled estimates offered a better representation of observed nearshore temperature than the original GCM output ($RMS_{err} = 0.9627$) (Fig. SM1).

## 2.3 Future distribution of *Minuca mordax* based on SST change

Future nearshore temperatures were calculated for different periods and climate change scenarios by applying equation 2 to GCM predictions of SST and SSS. Future SST and SSS values come from simulations of the same GCM (GISS-E2-R) used for the downscaling and were also obtained at the CMIP5 data portal. We generated distributions using numerical experiments forced by Representative Concentration Pathways 4.5 and 8.5 (RCP 4.5 and RCP 8.5) scenarios and did this for near-term (years 2036-2050) and long-term predictions (years 2086-2100 and 2286-2300). RCP 4.5 corresponds to a midrange mitigation emission scenario where radiative forcing increases during the 21st century and stabilizes at 4.5 $W/m^2$ after year 2100. RCP 8.5 is a high emission scenario where the radiative forcing reaches about 8.5 $W/m^2$ by the end of 21st century (Moss et al., 2010; Taylor et al., 2012).

The downscaled nearshore temperatures, in conjunction with the temperature-based range limits detailed above, were used to estimate changes to the species' spatial distribution under different time periods and warming projections. Downscaled SSTs were also used to identify, based on the physiological response of *M. mordax* to warmer water (unplub. data) and crustacean sensibility to heating, areas that can become physiologically stressful to the species in the future.

## 3. Results

## 3.1 Present range distribution and temperature

*Minuca mordax* is distributed from Belize to Torres, Brazil (Thurman et al., 2013). The annual means of SST in the northern limit of the specie's distribution were 27.7 and 28 °C (WOA and GHRSST's Foundation SST, respectively). In the southern limit, the SST means

were 20.5 and 20.6 °C (WOA and GHRSST's, respectively).

## 3.2 Future range distribution under climate change scenario

Southern limit of *M. mordax* distribution seemed to be controlled by thermal constraint due to colder temperatures (~20 °C). Consequently, the species may occupy new areas further to the south under the warmer temperatures predicted for emission scenarios RCP 4.5 and 8.5. Figure 1 describes this possible poleward range expansion of *M. mordax* for three future periods up to year 2300 for both emission scenarios, with a more accentuated expansion under high emission scenario (RCP 8.5). The species distribution might expand to Southern Brazil by year 2050 and 2100 (30°40'S 50°30'W and 33°03'S 52°44'W, respectively), and to Uruguay (34°11'S 58°03'W) in 2300 under RCP 4.5 scenario. Whereas under RCP 8.5, crabs may occur as south as at southern Brazil (31°32'S 51°14'W) by 2050, Uruguay (34°29'S 57°05'W) by the end of this century, and Argentina (37°36'S 57°16'W) by 2300.

The northern limit of species distribution seemed not be set by temperature. Crabs occupied warmer areas in equatorial mangroves, as well as colder areas in Southern Brazil, but were not found north of Belize. The northern distribution of *M. mordax* must be delimited by others factor such as ecological interactions (e.g. competition) or dispersal barriers and a temperature based northward range expansion was not predicted here.

As with the southward range expansion, areas that would potentially compromise physiological fitness (temperature over 30 °C) were identified at different periods and climate change scenarios (Fig 1). Under the RCP 8.5 scenario, for the period between 2100 and 2300, several areas along the coast will show SSTs above the 30 °C. The challenging condition threshold temperature may not be reached at any of the analyzed periods under RCP 4.5 conditions.
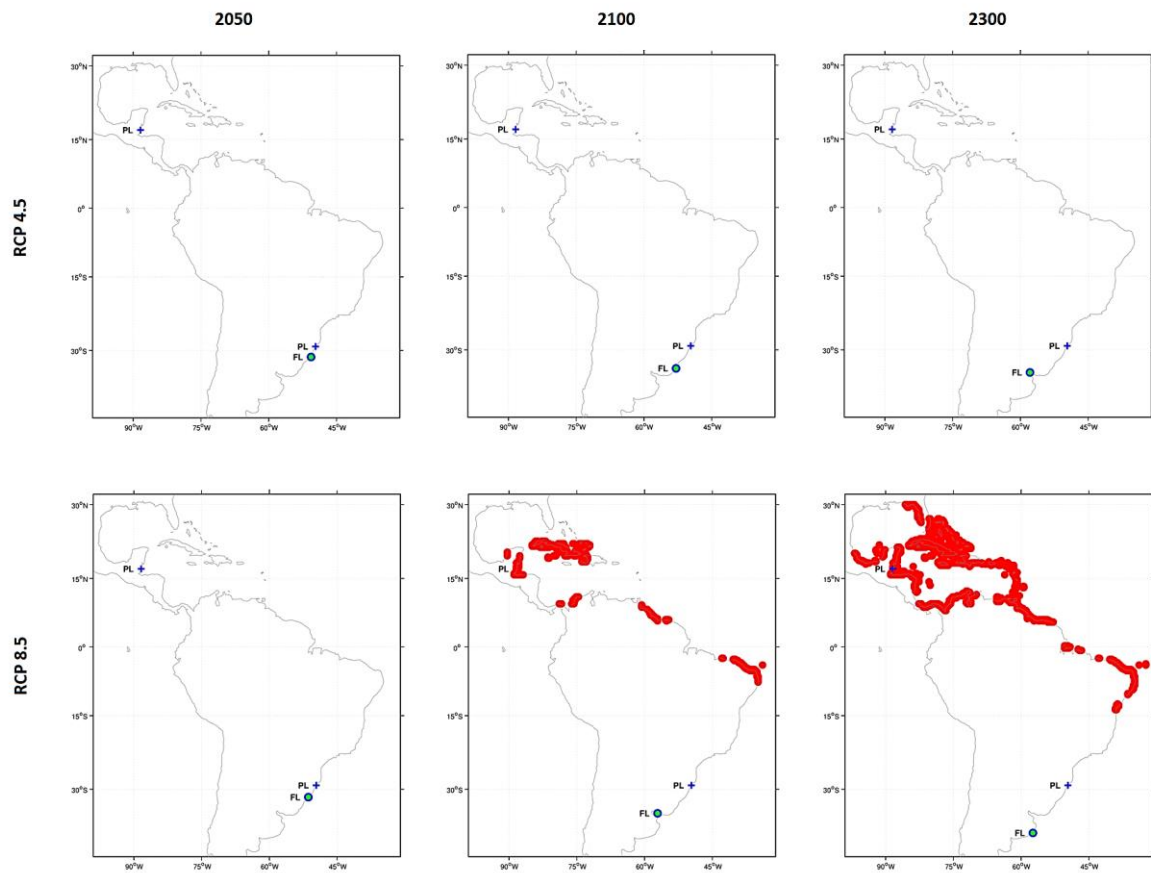
Figure 1. Present (PL) and future (FL) distribution of *Minuca mordax* under emission scenario RCP 4.5 and 8.5 in the years 2050, 2100 and 2300. Red spots indicate physiological challenging areas.

## 4. Discussion

Geographic range distribution of *M. mordax* is not limited by mangrove presence and crabs may occur in a wild biotopes type (Crane, 1975; Masunari, 2006). Northern limit seems to be set by other factors then temperature, such as interspecific competition, physical barriers to larval dispersal and habitat selection, whereas southern limit of range distribution seems to be imposed by sensitive to colder temperature. Thus, climate warming may expand southward the range of *M. mordax*. Warming temperature may be limiting at lower latitudes sites, harming physiological performance of fiddler crabs. Our estimations were made based on superficial oceanic temperature (until 1 km from shore) and a small variation may occur between these areas and the adult location at estuarine areas.

Species distribution may not reflect the potential niche and many factors can constraint

the range distribution of fiddler crabs (Nabout et al., 2009). Present range distribution of *M. mordax* was not limited by mangrove presence, since mangroves ecosystems have a range distribution from Florida, USA (Cavanaugh et al., 2014; Ward et al., 2016), through Santa Catarina, Brasil (Soares et al., 2012), while *M. mordax* has a geographic distribution ranging from Belize to Torres, Brasil (Thurman et al., 2013), to the south of the mangrove southern range limit. However, we suppose that northern limit of *M. mordax's* range distribution is set by other factors then temperature. *Minuca mordax* inhabit mudbanks in oligosaline habitats while others fiddler crabs species inhabit the region of Caribbean islands in a variety of osmotic condition (Thurman et al., 2010). Another species occurring in oligosaline environment of North Caribbean (as well as through Brazilian coast) is *Minuca vocator* and *Minuca burgersi*. The former, like *M. mordax*, is found in a few Caribbean locations, while *M. burgersi* also inhabits oligosaline habitats as well as mesosaline in a wide range of habitats from mudbank to salt flat, being one of the most common specie in North Caribbean (Thurman et al., 2010). It is possible that *M. mordax* is not able to find suitable areas to inhabit, regarding the salinity and type of habitat usually selected. Abiotic variables are determinant for fiddler crabs' abundance. However, biotic variables are the main factors affecting the presence or absence of fiddler crabs, e.g. the presence of a potentially interspecific competitor (Peer et al., 2018). Furthermore, physical barriers can play a role on larval dispersal and development of marine and intertidal organism (Gaylord & Gaines, 2000). Spatial heterogeneity in climate regimes can also represent a barrier to dispersion and gene flow, thus dealing to local extinction (Senner et al., 2018). Therefore, northern limit of *M. mordax'* range distribution might be set by interspecific competition, physical barriers to larval dispersal, and habitat selection.

On the other hand, lower temperatures seemed to be the main factor controlling *M. mordax* distribution at the southern limits where the annual mean SST ranged at about 20 °C. Colder temperature compromise aerobic scope, celular energy levels (Fusi et al., 2016; Pörtner, 2002) and larval fitness (Crickenberger et al., 2017). However, warming temperature is changing the range distribution of many species, such as fishes, zooplankton, and benthic invertebrates (Poloczanska et al., 2016). For instance, the fiddler crab *Minuca pugnax* is expanding northward the range distribution at Atlantic Cost of USA due to warmer temperature of water in the past few years (Johnson, 2014). Range limits of *M. pugnax* are related to the tolerance of larvae to cooler waters (Sanford et al, 2006). Therefore, colder temperature at southern limit may limit the distribution of *M. mordax* toward higher latitudes.

Climate warming may expand southward the geographic range distribution of *M. mordax*. Most remarkable expansion may occur until 2300 and at higher emission scenario

(RCP 8.5). Species that previously do not cohabit, may coexist if the range shifts under climate change and new interspecific interaction, like competition, may be developed (Usinowicz & Levine, 2018). If *M. mordax* find suitable areas further the southern limit in the future, crabs may lead to enhanced competition with others crabs and macroinvertebrates that already inhabit the local.

*Leptuca uruguayensis* is the fiddler crab species that inhabit the Brazilian coast until higher latitudes, in Argentina (Thurman et al., 2013). Fiddler crabs are important bioturbators altering composition of organic matter (Fanjul et al., 2015; Martinetto et al., 2016), water content (Kristensen et al., 2012), and contribute to carbon storage in intertidal ecosystems (Martinetto et al., 2016). Moreover, the abundance of bioturbators affects the meiobenthic community of intertidal environments (Citadin et al., 2018). Considering the southward expansion of *M. mordax* range distribution with climate warming, both *M. mordax* and *L. uruguayensis* may inhabit same areas at higher latitudes in the future, thus potentially altering meiobenthic community and ecosystems dynamics.

Crabs may deal with thermal constraint around lower latitudes at the end of this century up to the year 2300, under higher emission scenario. Upper to 30 °C, metabolic rate of *M. mordax* further increased (observed on physiological experiments). Metabolic rise observed suggest that crabs are in a high-energy demand condition, and lipids from energy reserve were used as metabolic substrate. In long-term of exposure, may cause low energy levels harming many functions, like growth (Pörtner et al., 2017). Thermal stress alters microhabitat usage by fiddler crabs (Darnell et al., 2013; Munguia et al., 2017), social interaction such as matting (Allen & Levinton, 2014) and foraging behavior (Vianna et al., unpubl.; Wu et al., 2017). In addition, warmer temperature compromises reproduction and survival of intertidal ectotherms (Pörtner et al., 2017). Upper thermal tolerance limits are related to maximum microhabitat' temperature (Stillman & Somero, 2000) and some tropical ectotherms can show physiological adaptation to warming temperature being more tolerant than temperate individuals (Fusi et al., 2017). Rather, some tropical intertidal ectotherms can be often exposed to habitat temperature close to or even exceeding the upper thermal limit (Darnell et al., 2015; Stillman & Somero, 2000), thereby being more restricted to adapt to new upper thermal limits with raising temperature (Stillman & Somero, 2000). In this way, global warming may impose physiological challenge and constrain *M. mordax* range in lower latitudes sites.

Furthermore, behavioral adaptation, e.g. burrow retreat could reduce thermal stress of fiddler crabs although would change the duration of surface activities and biotic interaction (Allen & Levinton, 2014; Munguia et al., 2017). Spatial competition intensified by range shifts

in response to warming climate could affect species' persistence in a future scenario (Usinowicz & Levine, 2018). Therefore, climate change is expected to expand southward the range distribution of *M. mordax* but also to compromise fitness and survival of crabs in lower latitudes sites. These results highlight critical areas to monitor climate change impacts on fiddler crab's species.

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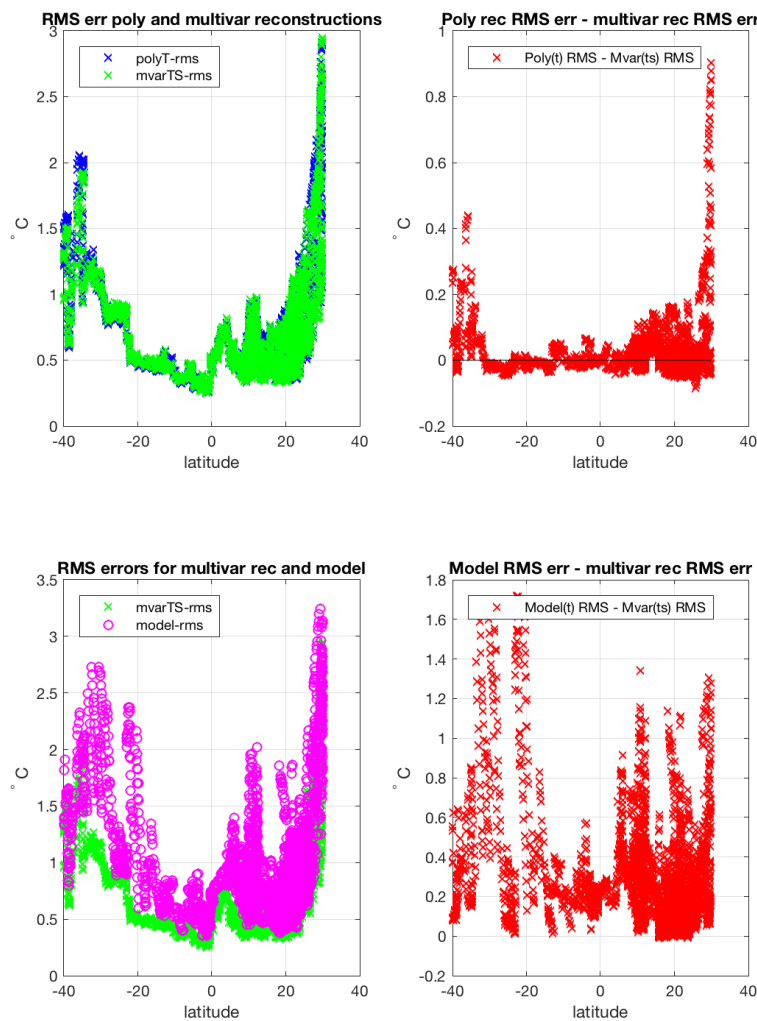
## Supplementary material



Fig SM1. Top right: RMS errors of the polynomial (green) and multivariate (blue) downscaled annual mean temperature as a function of latitude. Top left: Difference between polynomial and multivariate RMS errors (positive values indicate bins where polynomial error is larger than multivariate error). Bottom right: same as top right, but for multivariate downscaled (green) and original GCM (magenta) temperature RMS error. Bottom left: Same as top left but for the difference between multivariate downscaled and GCM RMS error.

## Conclusões

O aumento da temperatura e redução do pH causam alterações nas respostas fisiológicas e os efeitos são agravados pela exposição combinada destes dois fatores. As atividades na superfície, como por exemplo alimentação, são afetadas pela elevação da temperatura, o que pode ocasionar depleção da reserva energética e déficit no suprimento energético. Inferimos que outros comportamentos e interações sociais também serão afetados, reduzindo o fitness da espécie. Observamos ainda que o comportamento de "waving display" realizado pelos machos não é afetado pela temperatura.

Espécies de habitat vegetado e não vegetado apresentaram diferenças nos padrões fisiológicos (em relação ao consumo de oxigênio) e nos mecanismos de termorregulação, como a seleção de micro-habitat e características morfológicas que auxiliam na dissipação do calor. Estas características estão em consonância com a seleção de habitat e distribuição geográfica de cada espécie.

O aquecimento global também poderá causar alteração na distribuição geográfica dos caranguejos chama-maré, com expansão em direção aos pólos enquanto temperaturas extremas poderão comprometer a sobrevivência dos organismos do entremarés em habitats nas baixas latitudes.

As respostas obtidas por diferentes abordagens neste estudo foram complementares e possibilitaram avaliar as possíveis consequências das alterações do clima nos caranguejos chama-maré. Assim, podemos concluir que as mudanças climáticas podem impactar negativamente a fisiologia, o comportamento e a distribuição dos caranguejos, com possíveis efeitos ecológicos nos ambientes do entremarés.

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