



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



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ABSTRACT

Intertidal organisms rely on physiological and behavioral adjustments to maintain homeostasis under warm exposure. We examined the effects of the temperature increase related to climate warming on the physiology and behavior of two fiddler crab species: *Leptuca uruguayensis*, which inhabits mostly vegetated areas, and *Leptuca leptodactyla*, that inhabits unvegetated areas. We hypothesized 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 their physiological responses (oxygen consumption, Q_{10} , ammonia excretion and hepatosomatic index) and for 2 days to observe their behavioral responses (feeding rate and duration of burrow retreat). We also tested *in situ* the effect of air, surface, and body temperatures on the claw-waving display of both species. We found that species were affected differently by increasing temperature. *Leptuca uruguayensis* showed adaptation limit ($Q_{10} < 1$), increasing oxygen consumption. Also, in comparison with control, *L. uruguayensis* decreased the ammonia excretion at 31 °C, but not at 33 °C, indicating a compensatory mechanism to cope with thermal stress. In contrast, *L. leptodactyla* was able to adjust its metabolic rate to temperature rise ($Q_{10} \sim 3$) and reduce ammonia excretion, suggesting changes in the energetic substrate and amino acid catabolism. Higher temperatures reduce the hepatosomatic index of both species, indicating increased use of energy reserve (although only the *L. uruguayensis* feeding rate was reduced). Furthermore, warmer temperatures increase the duration of burrow retreat in both species, potentially impacting social interactions, such as mating. Temperature increase did not affect the claw-waving display frequency, suggesting that other factors may affect this behavior, e.g., the presence of females and competitors. Specific behavioral (e.g., microhabitat selection) and morphological attributes (e.g., larger major claw) might benefit the thermoregulation of each crab species since no differences in body temperature were found between them *in situ*. Therefore, fiddler crabs that inhabit vegetated areas are more vulnerable to higher temperatures and may change its geographic range as a result of climate warming, while fiddler crabs that inhabit unvegetated areas are more tolerant to temperature rise and may have a competitive advantage under a temperature increase scenario.

1. Introduction

Intertidal environments present a wide thermal heterogeneity and organisms living in these habitats are exposed continuously to thermal stress [1–3] and desiccation risk [4–6]. Many intertidal organisms have a body temperature close to the lethal temperature and, thus, might be more limited to evolve new thermal limits when exposed to warmer temperatures [4,7]. Furthermore, thermal stress imposed by intertidal environments constrains activities and alters habitat use by ectotherms [1,8]. In this sense, intertidal ectotherms are expected to be severely affected by climate warming.

Global warming reached approximately 1 °C above pre-industrial

levels in 2017, and some regions have 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 of crustaceans. However, exposure to higher temperature could also reduce the metabolic rate, decreasing physiological energy costs and, providing a short-term tolerance above the critical temperature [11–13]. Thus, the metabolic adjustment to changing temperature suggests either the condition comprises the natural range of the species or a limitation of the species to adjust to that environmental condition [14].

Moreover, temperature increase can alter the foraging behavior by reducing feeding and handling time of preys, e.g., time breaking shells

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[15]. Behavioral response to warming is related to the physiological sensitivity of organisms to thermal stress [8]. Further, ectotherms can adjust body temperature behaviorally according to their tolerance [11, 8]. By selecting a microhabitat with a favorable condition or retreating into the burrow, especially in unvegetated areas, fiddler crabs can reduce body temperature [11]. However, resource availability (e.g., food) and mating opportunity may increase crabs' activities, such as waving display, leading to reduced refuge use (i.e., burrows) and increased exposure to thermal stress [3].

Claw-waving displays of male fiddler crabs have a primary function on female attraction to mate and territory defense [16]. During waving display, male fiddler crabs stay long periods outside their burrows, being exposed to heat stress [17]. However, the major claw of male fiddler crabs also seems to play a thermoregulatory role by dissipating heat to the air around the claw and, thus, influencing the body temperature of crabs [5,18].

Warming can affect physiology [19] and behavior of organisms, threatening the existence of species and resulting in significant ecological impacts [20]. Fiddler crabs have been used as a model in studies testing the effects of temperature on physiological response [6, 21–26]. For example, Vernberg and Vernberg [24] compared the response of several populations of fiddler crabs, including *Leptuca uruguayensis*, and observed that both upper and lower thermal limits are correlated with habitat temperature. Fiddler crabs from varying habitats can respond differently to temperature increase by presenting distinct desiccation rates and thermoregulatory abilities [6]. Since fiddler crabs have an important ecological function as an ecosystem engineer [27] and in structuring meiofaunal community [28], studies considering the physiological and behavioral responses of these organisms to warming are mandatory. *Leptuca uruguayensis* Nobili, 1901 (formerly known as *Uca uruguayensis*) and *Leptuca leptodactyla* Rathbun in Rankin, 1898 (formerly known as *Uca leptodactyla*) [29] have similar size and a close sympatric association [30], although *L. uruguayensis* inhabits mostly vegetated areas, while *L. leptodactyla* inhabits unvegetated areas [31,32]. When *L. uruguayensis* and *L. leptodactyla* inhabit the same area, spatial segregation is observed. *Leptuca uruguayensis* occurs at the lower level of the waterline, usually with moist soil, while *L. leptodactyla* occurs at the upper level of the water line [33]. In the microhabitat of *L. uruguayensis* (Table 1, Figure S1A, S2, and S3), vegetation presence reduces the luminous intensity, the soil and burrow temperature of fiddler crabs [30] and keep the soil moisture [34]. Thus, crabs would be less exposed to heat and desiccation stress under vegetation cover, and they would be expected to experience more significant physiological stress when in unvegetated areas [35]. The aim of the present study is to investigate the effects of temperature on the physiology and behavior of *L. uruguayensis* and *L. leptodactyla*. We hypothesized that species from vegetated and unvegetated areas would have different physiological and behavioral responses to warming. Thus, we expect *L. uruguayensis* to be more sensitive to warmer temperatures than *L. leptodactyla*, assuming that these species have different thermal tolerance according to their distribution.

2. Methods

2.1. Study area and animal collection

Intermolt male crabs of *L. uruguayensis* and *L. leptodactyla* were

collected during autumn station in a muddy sand flat in the margin of Itaguare River, Bertioga, Sao Paulo state, Brazil (23°77'S; 45°96'W) to test the effects of temperature increase on the physiology and behavior of fiddler crabs. The laboratory experiments were conducted during the same season, the autumn, considering the physiology of semiaquatic animals could change through the year. The area was partially covered by growing mangrove *Laguncularia racemosa* (with 2 m of maximum height), in the margin of a mangrove forest. Both species select different microhabitat according to the presence of vegetated and unvegetated areas. *Leptuca uruguayensis* inhabit microhabitat close to the vegetation (Figure S1A) while *L. leptodactyla* inhabit unvegetated microhabitat (Figure S1B; see Table 1 to further details about abiotic factors from the microhabitats of each species). Fiddler crabs can co-occur in the same area, and a microhabitat niche overlap may be observed [30,31], although spatial segregation can occur at a small scale [33], as observed in the distribution of crabs in the present study, regarding the presence of vegetation. Crabs were transported and acclimated in a plastic container with sediment collected at the study area. Sediment column had 10 cm depth (the maximum burrow depth of both species [36]), and soil moisture was kept with brackish water (salinity 15‰). Fiddler crabs are deposit feeders [27,37], extracting microalgae, vascular macrophytes, macroalgae, detritus, and fish carcasses from the substrate [38] and, thus, were able to feed during the experiment. Crabs were kept at 25 °C on a light regime of 12 h of light and 12 h of dark. After two days of acclimation to reduce stress from collection and 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* males were also conducted in a partially vegetated (Figure S1) muddy sand flat at Itaguare River.

2.2. Experimental design

We tested the effect of increasing temperature on physiology and behavior of two sympatric fiddler crabs. Based on our hypothesis, we expected that these species would have different responses to temperature increase, presenting distinct metabolic rates, energetic demands, and behaviors (e.g., surface activity and feeding). Two laboratory experiments were conducted with 3×2 factorial design (temperature: 27, 31, and 33 °C x species: *L. uruguayensis* and *L. leptodactyla*). The temperature range was defined considering the mean summer air temperature of Sao Paulo coast (27 °C) from SIMCOSTA database (Brazilian Coast Monitoring System) [39] and the predicted temperature increase of +4 °C (31 °C) and +6 °C (33 °C) at the end of 21st century and beyond, respectively [40]. The physiological response was evaluated by measuring oxygen consumption, ammonia excretion, hepatosomatic index, and temperature coefficient (Q_{10}) ($N = 12$). We also evaluated the behavioral response analyzing the feeding rate and burrow retreat ($N = 8$). 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 retreat into burrow). We selected only males to reduce the variability since males show one major claw and one small feeding claw while females have two small feeding claws. Also, males use the major claw for waving display; the behavior analyzed in the field experiment.

We conducted an additional experiment in the field, considering the importance of social interactions to claw-waving display behavior [16]

Table 1

Mean \pm 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)
<i>L. uruguayensis</i>	27.3 \pm 3.2 a	29.7 \pm 3.9 a	26,076.38 \pm 13,353.34 a
<i>L. leptodactyla</i>	27.9 \pm 3.4 a	32.4 \pm 4.8 b	65,058.28 \pm 30,129.87 b

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

and the thermoregulatory role of the major claw for male fiddler crabs [5]. Thus, we evaluated the effect of air temperature (ranging from 21 to 33 °C) on claw-waving display frequency of both *L. uruguayensis* and *L. leptodactyla* ($N = 30$ for each species). The effects of surface and body temperatures on waving were also analyzed. Waving display could increase the airflow around major claw and enhance heat dissipation. So, this behavioral mechanism could potentially improve the thermoregulatory benefit of major claw [5]. Therefore, we hypothesized that waving displays would be more frequent at higher temperatures.

2.3. Physiological responses

To evaluate the physiological response of temperature increase, male crabs were exposed to 3 temperatures: 27 °C (26.8 ± 0.7); 31 °C (30.8 ± 0.5); and 33 °C (32.5 ± 0.5). As male *L. uruguayensis* reaches sexual maturity at ~ 7.0 mm [41] and *L. leptodactyla* at 8.3 mm of carapace width (CW) [42], only adults were selected (*L. uruguayensis*: $CW = 9.7 \pm 0.4$ mm, major claw length (CL) = 17.4 ± 1.7 mm; *L. leptodactyla*: $CW = 10.2 \pm 0.5$ mm, $CL = 19.7 \pm 1.8$ mm). Sediment was collected from the same site of the animal collection, and organic fragments were manually removed. Microcosms ($15 \times 10 \times 12$ cm) were filled with a sediment column of 10 cm. Crabs were kept 16 days in a germination chamber (FANEM Mod. 347 CDG) under a 12/12 h light/dark cycle. Initial air temperature in the germination chamber was set 27 °C for 24 h of acclimation (after manipulation and transference to microcosms), then crabs were exposed for 15 days to 27 °C (control treatment), 31 °C or 33 °C, with a temperature increased in a rate of 1°C h^{-1} [43],[44]. Air temperature at 2 cm from the ground level was measured daily using a digital thermometer. To maintain the soil moisture, each microcosm received 100 mL of water with salinity 15‰, which represents the mean salinity in the area these species inhabit [31].

Although fiddler crabs are bimodal breathers (extracting oxygen from both air and water medium [22],[23]), in the present study, we measured the oxygen consumption of *L. uruguayensis* and *L. Leptodactyla* only in the water medium. Other studies with fiddler crabs also evaluated the oxygen consumption in only one of these media: in the air [21],[45],[46] or water [47]. Hence, crabs were transferred to individual respirometric chambers containing ~ 600 mL of water at the same salinity and temperature of the treatment. An acclimation period of 30–60 min reduces stress from manipulation, prior analyses of oxygen consumption [12],[21],[48–52]. In the present study, aeration was maintained for 30 min. Then aeration was removed, the oxygen concentration was measured using an oximeter (monitor and probe YSI, Models 53 and 5905, respectively) and chambers were sealed. After 4.5 h, the oxygen concentration was measured again. The oxygen consumption was calculated by the difference between initial and final oxygen concentration. Data were corrected against the control chamber (without animals) and standardized against the individual dry weight to be expressed as oxygen consumption by specific dry weight ($\mu\text{g mg}^{-1}$ dry weight h^{-1}) [50]. Crabs were oven-dried at 60 °C for 48 h to measure the individual dry weight. A comparison in percentage was made to elucidate the temperature effects in both species. Water samples (50 mL) were obtained from the respirometric chambers and frozen to posterior analyses of ammonia excretion by colorimetry method [53]. Ammonia excretion was also expressed as specific dry weight ($\mu\text{g mg}^{-1}$ dry weight h^{-1}). Crabs were killed by freezing, and the dorsal carapace was removed to separate the hepatopancreas, which was dried in air circulation oven at 60 °C for 48 h to obtain the dry weight. The hepatosomatic index was calculated as a proportion of hepatopancreas dry weight to the body dry weight [49]. Q_{10} corresponds to the differences in metabolic rate between two temperatures, usually with 10°C increment, but it can be extrapolated to observations at any two temperature [54] as observed in some studies with crustaceans that evaluated the rate of metabolic process with a temperature increase

(e.g., $+3^\circ\text{C}$ [55] or $+5^\circ\text{C}$ [48],[56]). This coefficient could indicate a thermal sensitivity of animals to the variability of climate conditions [57]. Q_{10} of oxygen consumption was calculated by the equation: $Q_{10} = (OC_2/OC_1)^{10/(t_2-t_1)}$, 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 [48],[58] of oxygen consumption between 27–31 °C, and 27–33 °C. The thermal coefficient will be equal 1 if the OC is independent of temperature and >1 if OC increases with temperature [58].

2.4. Behavioral responses

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: 27 °C (27.1 ± 0.6); 31 °C (30.5 ± 0.9); and 33 °C (33.0 ± 1.2). Social context, such as female presence and male density, influences the behavior of fiddler crabs, e.g., courtship signal [59] and refuge use [60]. To create a social context and stimulate the activity of crabs, nine individuals were placed in each microcosm: three males of *L. uruguayensis*, three males of *L. leptodactyla* and three females randomly selected from both species, i.e., two females from one species and one female from another. We randomly selected females from both species, considering that male fiddler crabs may court females of their species as well as of heterospecific species [60]. Thus, we used female from both species in each microcosm. Only adults were used ($CW > 6$ mm) [41]. Carapace width of both sexes and major claw length of males were measured using calipers (*L. uruguayensis*: male, $CW = 8.5 \pm 1.0$ mm, $CL = 14.6 \pm 2.1$ mm, female $CW = 8.7 \pm 0.9$ mm; *L. leptodactyla*: $CW = 9.5 \pm 0.7$ mm, $CL = 17.5 \pm 2.4$ mm, female $CW = 9.2 \pm 0.9$ mm). Sediment was collected from the same site of the 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.

The experimental temperature was set with daylight natural UVA heat lamps (100 W; Model Repti Zoo) placed at different distances from substratum to regulate different temperatures (27, 31 and 33 °C), set to 12 h light/dark cycle. Air and surface temperature were measured daily in four aleatory spots of each microcosm (the surface temperature at 27 °C: 27.9 ± 1.5 ; 31 °C: 32.1 ± 2.5 °C; 33 °C: 36.3 ± 2.9 °C). After two days of exposure, microcosms were video recorded for 40 min, but the initial 10 min were not used in the analyses. Two focal animals were randomly selected in each microcosm to observe behavioral responses. Feeding rate was measured by considering how many times the minor claw of one focal crab was lifted to the buccal cavity in five minutes, divided by 5 (feeding rate/minute) [61]. Additional 10 min of video were randomly selected to measure the period the second focal crab spent inside the burrow (burrow retreat), expressed graphically as a percentage of the time [62].

2.4.2. Waving display

Males in reproductive maturity were observed at low diurnal tides with different air temperatures during the reproductive season to evaluate the effect of temperature on major claw-waving displays. Major claw has the potential to benefit thermoregulation of fiddler crabs [5], and males with larger major claw usually have the body temperature lower than males with smaller one [1]. The waving display could promote dissipation of heat to the air around the claw [5], although this behavior has a primary function to mating attraction [16], in which male waves the major claw to attract females to mate [63]. In the present study, female's proximity was not considered, although it may affect the waving display engaged by male fiddler crabs. The observation was conducted in a mixed population (males and females inhabiting the same area), without manipulation of crabs to avoid stress

and the observer interference on their behavior and body temperature. Also, *L. uruguayensis* and *L. leptodactyla* were observed respectively at vegetated and unvegetated microhabitats, in the same area (partially covered by vegetation) in order to have similar air temperature and allow comparison between species (Figures S1A and S1B).

The luminous intensity is related to solar radiation incidence and heat condition. To characterize the microhabitat of each species, we measured the luminous intensity in five replicates per area before and at the end of the observation, in eleven different days (Figure S3). Then, intact adult crabs with all pereopods were observed for 10 min, and claw-waving display frequency was quantified. In the end of the observation, the temperature of internal major claw and body of the crab were measured, inserting the thermo-sensor (Lutron TM-946) of the digital thermometer into the major claw and body cavity, through the posterior margin of carapace [7]. We measured CW and CL using callipers (*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 sediment surface and at the air 2 cm above the ground level (Table 1 and Figure S2). The waving rate was calculated, dividing the waves of each crab by 10 (waving/minute).

2.5. Data analysis

Variables from the physiological (oxygen consumption, ammonia excretion, hepatosomatic index) and behavioral responses (feeding rate) were analyzed with a Generalized Linear Model (GLM), by Poisson distribution, considering crab species and temperature as fixed factors. Homoscedasticity was evaluated by Levene test. Data from the duration of burrow retreat were homoscedastic and were analyzed by a two-way Analysis of Variance (ANOVA). The number of waving display was analyzed using a Generalized Linear Mixed Model (GLMM), with the number of displays as dependent variable, crab species as fixed factor and temperatures of crab (body and major claw), sediment surface and air as random factors. Scatter-plot of predicted values against residuals had a shotgun pattern. For all univariate analyses, the post hoc Fisher's least significant difference (LSD) test was used to evaluate differences among levels of significant factors. Environment variables of the habitats of *L. uruguayensis* and *L. leptodactyla* were compared by Multivariate General Linear Model (MGLM). To interpret the multivariate test results, we analyzed its univariate components (luminous intensity, air, and surface temperature). Data of crabs from both species were compared by a MGLM, and its univariate components were analyzed (temperature of the body and major claw, CW, and CL).

3. Results

3.1. Physiological response

Temperature affected the physiology of both fiddler crab species in different ways. At 27 °C, *L. uruguayensis* showed higher oxygen consumption than *L. leptodactyla*. However, in comparison with control, temperature increase to 33 °C reduced 47% of the oxygen consumption by *L. uruguayensis*, while it increased 50% of the oxygen consumption by *L. leptodactyla* (GLM, species x temperature, Wald = 21.3871, $p \leq 0.001$) (Fig. 1A). Temperature increases to 31 and 33 °C reduced the ammonia excretion rate of *L. leptodactyla*. For *L. uruguayensis*, the ammonia excretion rate decreased from control to 31 °C and was intermediate at 33 °C (Fig. 1B) (GLM, species x temperature, Wald = 6.008, $p = 0.049$). Hepatosomatic index (Fig. 1C) was higher in *L. leptodactyla* (GLM, species, Wald = 6.05261, $p = 0.014$) than in *L. uruguayensis* regardless of the temperature treatment. However, with a temperature increase to 31 and 33 °C reduced this index in both species (GLM, temperature, Wald = 28.13991, $p \leq 0.001$). Q_{10} value between control temperature and 31 °C for *L. uruguayensis* was ~1, while it was ~3 for *L. leptodactyla*. However, under temperature increase to 33 °C, Q_{10} of *L. uruguayensis* was lower than 1, suggesting a lower metabolic

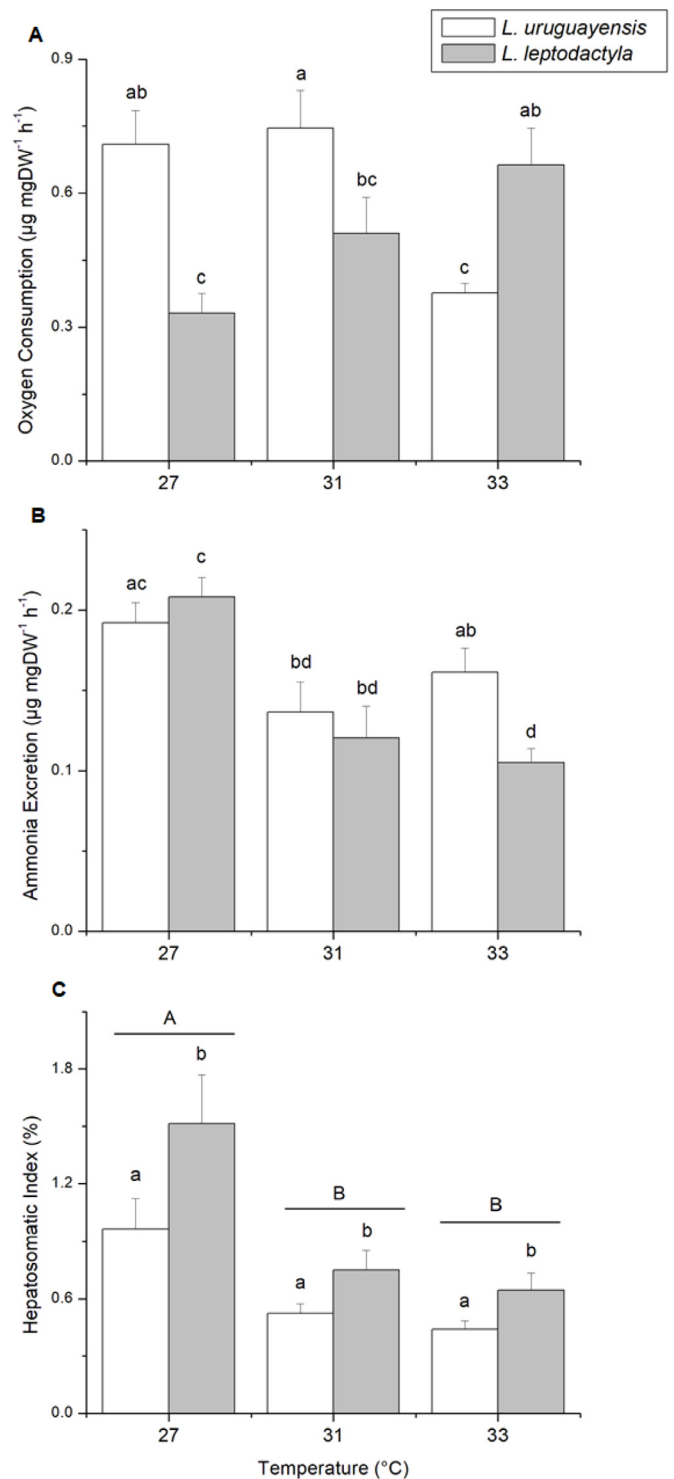


Fig. 1. Mean \pm SE oxygen consumption (A), ammonia excretion (B), and hepatosomatic index (C) of *Leptuca uruguayensis* and *L. leptodactyla* exposed to different temperatures. Different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments (species and temperatures). 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	<i>L. uruguayensis</i>	<i>L. leptodactyla</i>
27–31 °C	1.13	2.92
27–33 °C	0.03	3.73

adjustment, but Q_{10} of *L. leptodactyla* was ~ 4 (Table 2).

3.2. Behavioral response

3.2.1. Feeding rate and burrow retreat

Overall, temperature affected the behavioral responses of both fiddler crabs. Feeding rate of *L. uruguayensis* was reduced under warmer temperature (31 and 33 °C) (Fig. 2A). *Leptuca leptodactyla* showed a lower feeding rate than *L. uruguayensis* at control temperature (27 °C) and no differences in this behavior were observed between temperature treatments (GLM, species \times temperature, Wald = 12.21726, $p = 0.002$). Warming resulted in an increase of burrow retreat time for both crab species (two-way ANOVA, species \times temperature, $F_{2,42} = 21.1234$, $p \leq 0.001$), and no differences were found between fiddler crab species (Fig. 2B).

3.2.2. Waving display and temperature

Waving rate per minute of *L. uruguayensis* and *L. leptodactyla* under temperature ranging from 21 to 33 °C is shown in Fig. 3. We found that the frequency of claw-waving display was not affected by any factor (e.g., crab species, body and claw temperature of crabs, and temperature of the environment) (Table 3). Environmental variables differed between areas 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 greater in *L. leptodactyla* area than in *L. uruguayensis* area. No differences were found in air temperature, which was also similar to the summer mean from Sao Paulo coast (27 °C), the value used as a control on physiological and behavioral experiments at the laboratory (Table 1, Figure S2). The

temperature of body and claw were similar between species, but carapace width and major claw length were higher in *L. leptodactyla* than *L. uruguayensis* (MGLM, Wilks' Lambda = 0.154808, $F_{3,56} = 101.91$, $p < 0.001$).

4. Discussion

In the present study, we show that warmer temperatures induce different physiological responses in the two fiddler crab species. Under warmer temperatures, *L. uruguayensis* decreased the oxygen consumption, while *L. leptodactyla* increased it. Warmer temperature decreased the energy reserve from hepatopancreas and constrained the surface activity of both fiddler crab species. Furthermore, temperature increase reduced the feeding rate of *L. uruguayensis*, but it did not significantly affect the feeding by *L. leptodactyla*. In contrast, the waving display of both fiddler crabs was not affected by temperature.

Oxygen consumption increases with increasing temperature. However, further temperature increase (approaching the temperature limits) compromises the oxygen supply, inducing a progressive transition to hypoxia in the body fluids [13]. Although metabolic depression and anaerobic metabolism reduce the energy requirement and extend the thermal tolerance at short term, at long term, the temperature increase may compromise energy supply, growth, and survival of animals [11]. Thus, decreased metabolic rates with temperature increase ($Q_{10} < 1$) may reflect a thermal limitation of species [14],[26],[57], such as observed in *L. uruguayensis* at 33 °C. The crab *Pachygrapsus marmoratus* increases the oxygen consumption under temperature increase, then decreasing the oxygen consumption at temperatures above 28 °C [64]. Warming of +6 °C also decreased metabolic rates of the sea urchin *Heliocidaris erythrogramma*, which might suggest suppression of physiological processes to maintain fitness [65]. Furthermore, *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 imposed by warmer temperatures, since this species inhabits vegetated areas and, thus, being less exposed to heat. Also, on the coast of Sao Paulo, *L. uruguayensis* lives near to its thermal and

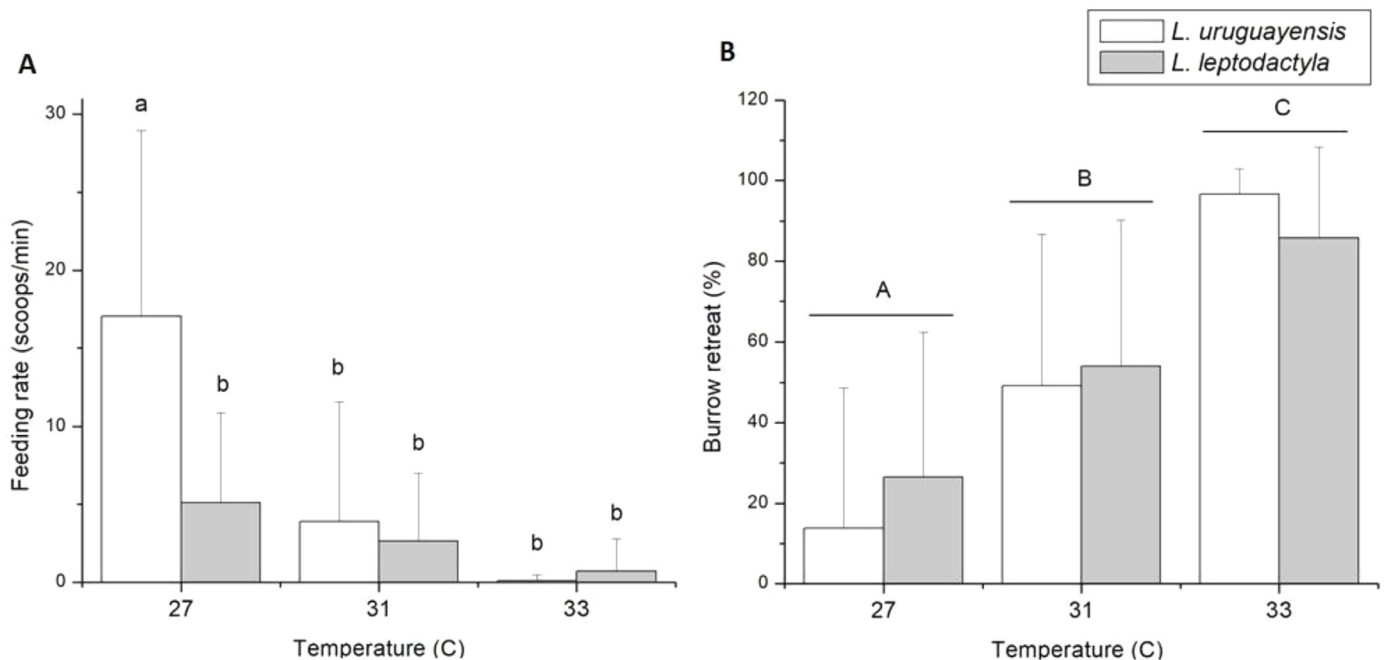


Fig. 2. Mean \pm SD feeding rate (A) and the proportion of time inside the burrow (B) of *Leptuca uruguayensis* and *L. leptodactyla* exposed to different temperatures. Different lower-case letters indicate significant differences ($p \leq 0.05$) among treatments (species and temperatures). Capital letters above horizontal bars indicate significant differences between temperature treatments.

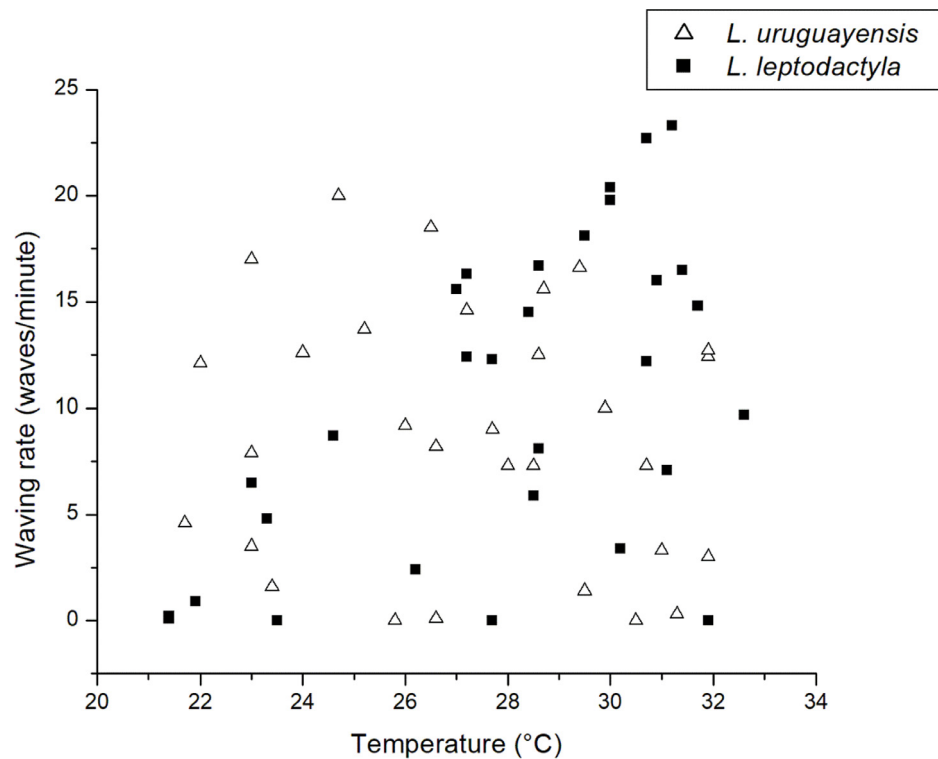


Fig. 3. Waving rate (waves/minute) of *Leptuca uruguayensis* and *L. leptodactyla* (triangle and square symbols, respectively) under different temperatures in the field.

Table 3

GLMM of waving displays of *Leptuca uruguayensis* and *L. leptodactyla* under different temperatures.

	df	Wald	p
Intercept	1	1.287887	0.256438
Surface temperature	1	2.863006	0.090638
Air temperature	1	0.601441	0.438029
Body temperature	1	1.210300	0.271273
Claw temperature	1	0.151276	0.697319
Species	1	0.666084	0.414420

geographic range limits [66] (22°52'S; 42°01' W), and warming environment can alter its geographic range, preventing its occurrence at lower latitudes, such as the study area (23°77'S; 45°96'W).

Leptuca leptodactyla was able to adjust its metabolic rate to temperature rise ($Q_{10} > 3$), increasing the metabolic rate under a higher energy demand condition. Similar increase on oxygen consumption was observed in the prawn *Macrobrachium tenellum* with an increase of 3 °C (from 26 to 29 °C) [55] and in the sand fiddler crab *Leptuca pugilator* exposed to an increase of 6 °C (from 20 to 26 °C) [21]. *Leptuca leptodactyla* is most abundant in unvegetated areas, and this crab can tolerate higher temperatures, occurring in areas with sediment temperature reaching up to 38 °C [32], as also observed in the present study (see Table 1 and Figure S2). Therefore, *L. leptodactyla* might have a higher thermal limit than *L. uruguayensis*, which could confer a competitive advantage under higher temperatures. Vernberg [25] compared the metabolic response to the temperature of populations of fiddler crabs (*Minuca rapax* and *Minuca pugnax*) from different latitudes and observed that crabs might exhibit the metabolic response correlated to their geographical distribution. In the present study, we observed a similar pattern, which the metabolic rates of the temperate species (*L. uruguayensis* [24]) were higher at intermediate temperatures, while the rates of the tropical species (*L. leptodactyla* [67]) were higher at elevated temperatures, indicating metabolic adaptation to temperature elevation. Thus, thermal tolerance can be correlated to the thermal

history of the population, as also demonstrated by Vernberg and Vernberg [24].

We evaluated oxygen consumption in submerged crabs, similar to a high tide condition. Unfortunately, very little is known about the oxygen consumption of bimodal breathing crabs in both medium, air and water simultaneously. Taylor and Butler [68] showed that oxygen consumption and heart rate of crabs *Carcinus maenas* acclimated to 15 °C and exposed to water or air for three hours were not significantly different. Henry [69] suggested that the rates of oxygen uptake in water and air are similar for facultative air-breathing crustaceans when exposed for a short term to air. Additionally, during periods of air exposure, the oxygen stores of the hemolymph are consumed. The intertidal crabs *Pachygrapsus marmoratus* and *Tubuca urvillei* consume oxygen at almost equal rates in air and water while *Carcinus mediterraneus* consumes up to 44% more in air medium [[23],[70]]. Fusi et al. [64] showed that when in water, oxygen consumption of the crab *P. marmoratus* increases during warming but in the air remains lower than in the water. The authors suggested that air-breathing ectotherms show higher thermal tolerances in the air due to the higher availability of oxygen in this environment. Therefore, it is possible that in fiddler crabs, warmer temperatures induce different physiological responses in the air and water medium, as well as in low and high tide, and these effects deserve further investigations in future studies. The temperature increase to 31 °C reduced ammonia excretion of both *L. uruguayensis* and *L. leptodactyla*. This result might be related to the reduction of feeding or change on nitrogenous end-product excretion, e.g., urea [51], that was not identified by the ammonia excretion analyses. Moreover, energetic substrate selection would produce different end-products. For example, if amino acids are the primary substrate, it would produce a more considerable amount of nitrogenous waste [71] compared to catabolism of mainly non-nitrogen compound [72]. Thus, crabs may have increased the catabolism of non-nitrogen compounds, like lipids, under higher temperature. However, at 33 °C, the ammonia excretion rate of *L. uruguayensis* was similar to those at control and 31 °C treatments, which might indicate a compensatory metabolic response to cope with elevated temperatures that crabs are unable to endure,

followed by a decrease on oxygen consumption. In contrast, *L. leptodactyla* 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 of the energy source accessed when energy demand increases [73] or feeding reduces [74]. Warmer temperature reduced the hepatosomatic index of both species, what is probably related to changes in feeding rate. *Leptuca uruguayensis* reduced the feeding rate, while *L. leptodactyla* did not reduce feeding rate but increased the oxygen consumption. These results indicated increased use of energy reserve from hepatopancreas in both species. Temperature increase might also compromise the function of this organ, likely reducing the production of digestive enzymes.

Temperature rise constrains surface activities of intertidal invertebrates. At the hottest hours of the day, the body temperature of fiddler crabs also increases, and surface activity is reduced, especially in unvegetated areas [1]. Thermal refuge, such as burrows, reduces body temperature and alleviates thermal stress [1], [8], [30]. Temperature rise increased the time inside the burrow in both fiddler crab species. Also, warmer temperature reduced the feeding rate of *L. uruguayensis*. However, *L. leptodactyla* showed lower feeding rate in all temperature treatments, what could be a response specific of *L. leptodactyla*. This species usually occurs in unvegetated areas [31], [32], so its activities must be dependent on sun heat exposure, which could explain the lower feeding rate of *L. leptodactyla* at laboratory experiments. Although fiddler crabs are bimodal breathers [22], [23], the availability of oxygen is higher in the air, which may extend the heat tolerance and sustain greater metabolic and activities rates, despite desiccation stress may become more limiting in air exposure [75], [76]. In this sense, *L. uruguayensis* and *L. leptodactyla* showed similar behavioral response with temperature increase.

Waving display has a primary function of mating attraction and territory defense [16]. 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 transferred between major claw and body of fiddler crabs [5]. Males with larger major claw experience lower body temperature [1]. However, the thermoregulatory function of major claw may be related solely to heat dissipation promoted by the presence of the enlarged structure and, thus, the temperature would not affect the waving display frequency.

Physiological stressors are the main factor driving the spatial distribution of fiddler crabs, regardless of sympatric interactions [35]. Accordingly, Checon and Costa [33] observed spatial segregation at a small scale of the sympatric species *L. uruguayensis* and *L. leptodactyla*, which was related to the tide influence. *Leptuca uruguayensis* occupied areas closer to the waterline, that is supposed to keep soil moisture and lower thermal variability. By contrast, *L. leptodactyla* was more frequent at the upper-level area, submerged only at spring tides [33]. We observed that *L. uruguayensis* selected a cooler microhabitat with lower sediment surface temperature and luminous intensity. Microhabitat selection can contribute to reducing body temperature when environmental temperature approach lethal temperature [1], [2]. Although *L. leptodactyla* was found on a warmer substrate, body temperature was similar between fiddler crab species, suggesting crabs were able to thermoregulate despite the differences in microhabitat temperature. Males of *Astruca mjoebergi* with larger major claw show lower body temperature than males with smaller major claw [1]. In the study area, *L. leptodactyla* had a larger major claw, attribute that benefit thermoregulatory capability. Also, fiddler crabs with wider carapace show a slower desiccation rate [6], which might alleviate dehydration in *L. leptodactyla* at unvegetated habitat. Furthermore, this species usually displays whitening carapace, chelipeds, and pereopods [30], that benefits cooling in crabs [17], [77]. Therefore, each species has attributes

or behaviors that may contribute to regulate their body temperature and make it possible for both species co-occur in the same area.

5. Conclusions

In general, climate warming may potentially compromise physiological functions by increasing maintenance energy cost and constrain surface activities and social interaction, such as mating. We observed that two fiddler crab species were affected differently by warmer temperature, and we can predict that climate warming would impact negatively the performance of fiddler crabs that inhabits vegetated areas, restricting it to higher latitudes. In contrast, fiddler crabs of unvegetated areas may have a competitive advantage in lower latitudes, that could contribute to the occurrence of this species in a warming environment. In the present study, we evaluated the oxygen consumption in the water medium. Future studies should be performed comparing the oxygen consumption of fiddler crabs also in the air medium.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Supplementary materials

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