UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" – UNESP

INSTITUTO DE BIOCIÊNCIAS, CAMPUS DE BOTUCATU

PROGRAMA DE PÓS-GRADUAÇÃO EM CIENCIAS BIOLÓGICAS - ZOOLOGIA

FERNANDO RAFAEL DE GRANDE

As consequências do aumento de temperatura para a distribuição, tamanho e morfologia dos caranguejos-chama-maré.

Apoio**:**

Botucatu, 2021

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> Tese apresentada ao Programa de Pós Graduação em Ciências Biológicas (Zoologia), do Instituto de Biociências de Botucatu, Universidade Estadual Paulista, como parte dos requisitos para a obtenção do título de Doutor.

Orientadora: Tânia Marcia Costa

Botucatu – SP

2021

Dedico esta tese a todos os trabalhadores da ciência os quais, mesmo vivendo em um país que passa por um momento de obscurantismo e negligência científica, são um exemplo de dedicação e compromisso com a sociedade.

Agradecimentos

Agradeço em primeiro lugar a minha família, meus pais José D. De Grande e Sueli T. Comin De Grande e meu irmão José Samuel De Grande por todo o incentivo ao longo de minha carreira. Agradeço ao suporte financeiro provido pelos meus pais. Apesar de todas as dificuldades que enfrentaram, meus pais, pequenos agricultores que cursaram apenas até a quarta série do ensino fundamental rural, possibilitaram a formação de um cientista. Mas, sobretudo, agradeço a meus pais e também a meus avós já falecidos, Carmen Rocha Comin, Aparecido Comin e Eleonor Pereira De Grande, pelo amor e admiração que me ensinaram a ter pela natureza. Se um dia escolhi me dedicar ao estudo da natureza, foi graças aos ensinamentos deles. Não são todas as famílias em que as plantas são perpassadas como herança entre as gerações ou que as comemorações são feitas à sombra de árvores. Também não é comum ao invés de usar brinquedos, distrair as crianças com a beleza de uma flor ou engenhosidade do ninho de um pássaro. Eu me sinto sortudo por ter sido criado dessa maneira e espero que esses valores continuem sendo passado às próximas gerações, como vem sendo feito com meu querido sobrinho Lorenzo De Grande.

Agradeço também a minha esposa Priscila Granado por todo amor, companheirismo, suporte emocional e, ultimamente, também financeiro. Foi sempre um prazer discutir com ela ciência, didática e filosofia. Tenho muito prazer e felicidade em dividir e construir a vida com essa mulher incrível.

Agradeço a todos meus colegas de trabalho do Labecom. Na verdade, mais que colegas eu os considero como amigos com os quais tive a oportunidade de forjar minha carreira. Durante todos esses anos, grande parte de meu aprendizado se deve as discussões e parcerias que tive oportunidade de ter no Labecom. É uma honra ter trabalhado ao lado de profissionais tão competentes e éticos. Foi um prazer dividir tantas alegrias em nossas, saídas de campo, confraternizações, bares e churrascos. Trabalhar junto a esse time de profissionais nunca foi um fardo, e sim uma imensa diversão.

Agradeço aos meus primeiros alunos de Iniciação Ciêntífica Eidi Kikuchi, Jaqueline Santos Borges, Jonathann Arakaki e Carolina Guardino por toda oportunidade de aprendizado

que disfrutamos juntos. Com certeza, esses foram os melhores alunos que eu poderia desejar para o início de minha carreira.

Agradeço a todos meus amigos (seria difícil nomear todos eles) com os quais dividi tantas alegrias e dificuldades desde o ensino médio até meu doutorado. Poder contar com pessoas tão fraternas é uma dádiva. Agradeço especialmente meu amigo Leonardo Schultz da Silva com quem dividi república desde o primeiro dia de graduação até meu doutorado. Ao longo desse tempo todo, mais que um amigo, eu ganhei um norvo irmão.

Agradeço meus camaradas de militância com os quais divido a esperança e o desafio de construir um mundo melhor. A oportunidade que tenho junto à eles de aprender organizar nossas categorias de trabalho, bem como a oportunidade de conhecer diferentes perspectivas filosóficas, reforçam minhas convicções que a ciência é uma ferramenta poderosa. Espero um dia poder auxiliar para a utilização dessa ferramenta em prol dos trabalhadores.

Agradeço todos meus professores e demais servidores da Unesp de São Vicente e de Botucatu por todo o conhecimento transmitido e dedicação à nossa formação profissional. É uma honra ter estudado nessa Universidade.

Por fim, e especialmente, agradeço a minha querida orientadora a Profª. Drª. Tânia Marcia Costa, com quem tenho prazer de trabalhar a mais de uma década. Ao longo desses 12 anos que venho sendo formado pela professora Tânia, eu aprendi muito mais do que os conhecimentos científicos e técnicos que ela me transmitiu. Ela é para mim um exemplo de profissionalismo e competência. Sua capacidade de gerir pessoas é excepcional, o que proporcionou incontáveis oportunidades para minha carreira e para a carreira de meus colegas. Levarei como exemplo a ser seguido em minha vida profissional a habilidade didática dela, a criatividade em delineamentos experimentais, a capacidade de gerir um grupo de pesquisa e, sobretudo, a resiliência necessária para construir e se manter na carreira acadêmica. Tentarei ainda, levar para a minha vida a sensibilidade humanística dela, a empatia pelas dificuldades alheias, a ética profissional e a determinação. Se um dia eu me tornar professor universitário, e conseguir ser para meus orientados metade da orientadora que a professora Tânia é, então eu saberei que estarei formando ótimos cientistas.

Professora Tânia, de coração, meus sinceros agradecimentos!

Sumário

Apresentação

O aumento de temperatura em consequência das mudanças do clima vem causando alterações globais na distribuição territorial, fenologia e fisiologia dos organismos (Parmesan and Yohe, 2003; Parmesan, 2006; Pörtner and Farrel, 2008; Dydersky et al., 2018; He and Silliman, 2019; Price et al., 2019). Potencialmente, o aumento de temperatura pode causar alterações em todos os níveis de organização biológica: desde a expressão gênica, até alterações na diversidade de espécies de uma comunidade (Parmesan, 2006; Pörtner and Farrel, 2008; He and Silliman, 2019). Os organismos costeiros estão sendo ameaçados pelo aumento de temperatura da água e do ar devido ao derretimento do gelo polar, o aumento do nível médio do mar e o aumento de eventos extremos como, por exemplo, tempestades e ondas de calor (Stenseth et al., 2002; Perry et al., 2005; Parmesan, 2006; Vinagre et al., 2016; He and Silliman, 2019). Além das alterações do clima, os ambientes costeiros são ameaçados por outros estressores antrópicos como a poluição, a destruição de habitats e a redução da biodiversidade, uma vez que eles são um dos ambientes mais densamente povoados no mundo (He and Silliman, 2019). Ambos os fenômenos colocam os organismos costeiros em um estado crítico de conservação (He and Silliman, 2019) e, portanto, pesquisas que avaliem a vulnerabilidade, resistência e resiliência deles são fundamentais para estratégias de mitigação e preservação.

A temperatura das águas na costa leste da América do Sul é influenciada pela corrente do Brasil a qual apresentou um aquecimento de aproximadamente 2°C entre 1950 e 2008 (Wu et al., 2012). A temperatura do ar também vem aumentando nos últimos séculos na costa leste da América do Sul (Marengo et al., 2009). A costa do Brasil, por exemplo, apresentou um aumento de temperatura do ar entre 0,1 a 0,5°C por década entre 1975 e 2015 (Bernardino et al., 2015). O aumento de temperatura pode por em risco algumas espécies de importância econômica como, por exemplo, os peixes pampo-galhudo *Trachinotus goodei* (Anexo I: Kikuchi et al., 2019) e a merluza *Merluccius hubbsi* (Verba et al., 2020). A perda de espécies comerciais pode desestruturar as economias locais, sobretudo aquelas ligadas ao extrativismo e práticas tradicionais (Eide and Heen, 2002; Kalikoski et al., 2010; Verba et al., 2020). Outras espécies de importância ecológica, como as espécies estruturadoras de habitats, poderão

ampliar sua faixa de distribuição na America do Sul e alcançar maiores latitudes devido ao aumento de temperatura, como é o caso das árvores de mangue (Anexo II: Borges et al., 2019; Ximenes et al., 2016, 2018). A expansão das árvores de mangues poderá substituir os ecossistemas de marismas modificando toda a comunidade do entre-marés da região sul do Brasil (Anexo II: Borges et al., 2019). Por outro lado, a expansão da distribuição dos organismos devido ao aquecimento global ainda é um processo pouco compreendido. Algumas espécies podem se ajustar nas comunidades colonizadas e exercerem efeitos positivos ou nulos sobre as espécies nativas (Pessarrodona et al., 2018). Um exemplo disso é a recente expansão do caranguejo-chama-maré *Leptuca cumulanta* para o litoral sudeste do Brasil, o qual é capaz de viver em assembleias mistas sem prejudicar o congênere nativo *L. uruguayensis* (Anexo III: Arakaki et al., 2020).

Os caranguejos-chama-maré desempenham funções-chave de engenharia de ecossistemas nos ambientes estuarinos (Kristensen, 2008). Durante a atividade de alimentação e de escavação de tocas estes caranguejos promovem a bioturbação do sedimento, disponibilizando recursos essenciais para outros organismos (Kristensen, 2008). Eles são organismos gregários que podem ocorrer em alta densidade, com algumas espécies apresentando mais de 200 indivíduos por metro quadrado (Crane, 1975). A densidade e período sazonal de atividade dos caranguejos-chama-maré regulam a estrutura da comunidade bentônica, afetando tanto os produtores quanto os consumidores primários (Reinsel, 2004; Citadin et al., 2018). Além disso, os chama-marés podem ser considerados como conectores do fluxo de energia e nutrientes produzidos nas comunidades bentônicas da zona do entre-marés e os ambientes aquáticos e terrestres adjacentes, uma vez que eles são predados por peixes, outros caranguejos, aves e mamíferos (Kneib and Weeks, 1990; Rozas and LaSalle, 1990, Koga et al., 2001; Krumme et al., 2001; Rulison et al., 2012). Os chama-marés habitam diversos habitats como, por exemplo: manguezais, marismas, bancos de areia, baixios lodosos, praias estuarinas e costões rochosos (Shih et al., 2015; Checon and Costa, 2017; De Grande et al., 2018). Eles ocorrem na costa de todos os continentes (exceto os polos) habitando regiões tropicais, subtropicais e temperadas (Crane, 1975).

A ecologia térmica dos caranguejos-chama-maré é relativamente bem estudada. Até o momento sabe-se que os chama-marés dependem da temperatura, por exemplo, para seu

período de atividade sazonal na superfície do sedimento (Colpo and López-Greco, 2017), para o período de reprodução (Colpo and López-Greco, 2017), ocupação de micro-habitats (Principe et al., 2018) e distribuição latitudinal (Sanford, et al., 2006; Johnson, 2014; Rosenberg, 2018, Darnell and Darnell, 2018). Além disso, atualmente também é reconhecido que o quelípodo hipertrofiado dos machos de chama-maré funciona como um órgão termoregulador, auxiliando-os na dissipação de calor (Darnell and Munguia, 2011; Munguia et al., 2017). Desta forma, considerando a importância ecológica dos caranguejos-chama-marés nos ambientes costeiros, a ampla distribuição geográfica em que ocorrem ao redor do planeta e a responsividade deles à variação térmica, nós poderíamos elenca-los como possíveis organismos-modelo para entendermos os efeitos das mudanças do clima. No entanto, para que os chama-marés possam ser usados como organismos-modelo em estudos climáticos de regiões costeiras, algumas lacunas do conhecimento sobre esses crustáceos devem ser superadas, especialmente na América do Sul. Especificamente, ainda não é conhecido: se a distribuição latitudinal dos chama-marés na América do Sul é delimitada pela temperatura (a distribuição latitudinal dependente da temperatura foi estudada para os chama-marés apenas na América do Norte; veja: Sanford et al., 2006; Johnson, 2014; Rosenberg, 2018, Darnell and Darnell, 2018); se a variação de tamanho corporal entre as populações de uma mesma espécie é uma resposta fenotípica à temperatura; ou se a variação na capacidade de termoregulação entre os indivíduos de uma mesma espécie com diferentes morfotipos confere vantagens competitivas.

Em meu doutorado, defendo a tese que o aumento de temperatura provocado pelo aquecimento global afetará a ecologia e fisiologia dos caranguejos-chama-maré da América do Sul. No primeiro capítulo avaliei se os limites de distribuição latitudinal dos caranguejos-chama-maré na América do Sul são definidos pela diminuição gradativa da temperatura da água do mar. No segundo capítulo, avaliei se a variação latitudinal de tamanho do caranguejo-chama-maré *Leptuca uruguayensis* é causada pela variação de temperatura do ar. Por fim, no terceiro capítulo, eu avalio se os machos de *L. uruguayensis* com quelípodos regenerados (leptochelous) e não regenerados (brachychelous) apresentam diferenças na habilidade de termorregulação, e se tal habilidade pode explicar a distribuição diferencial desses morfotipos entre micro habitats vegetados e não vegetados.

Capítulo 1

**Cold water temperatures define southern boundaries of South American fiddler crabs'
distribution**

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Abstract

Temperature increase due to climate change has caused shifts in the range distribution of several organisms globally. In coastal intertidal environments most organisms have an amphibian life cycle and their latitudinal distribution boundaries may be delimited by their thermal tolerance during the larval pelagic stages. Fiddler crabs are key species in intertidal environments and their early larval stages occur in coastal marine waters. We evaluated the mean sea surface temperature (SST) gradient over the South American coast from 1982 to 2019 and compared it to the minimum and maximum critical thermal limits (CT_{min} and CT_{max}) of the larval stages of eight fiddler crab species to assess whether temperature delimits their territorial ranges. We found a clinal decrease in mean SST of 0.28 °C per latitudinal degree along the distribution of fiddler crabs in South America. Cold sensitivity differed among the larvae of fiddler crab species, which corresponds to the latitudinal temperature gradient observed in their southern boundaries. Thus, our results suggest that cold water temperature can define the southern boundaries of South American fiddler crabs' distribution. The results of CT_{max} cannot explain the northern latitudinal distribution of the fiddler crabs. Fiddler crabs showed a similar sensitivity to high temperatures (40.5 °C) regardless of species (except *Leptuca uruguayensis*) and these are higher than environmental temperatures observed in South America. We also observed an increase in mean SST of 0.9 °C in the last 37 years. This increase in water temperature may explain the recent distributional expansion of one South American fiddler crab species, *Leptuca cumulanta*. Therefore, we showed that differential thermal responses at the early larval stage have consequences on the geographic range of fiddler crab species. Our findings allow us to hypothesize that fiddler crabs' boundaries may expand to higher latitudes in the future due to global warming.

Keywords: global warming, thermal limits, larvae, latitudinal distribution, *Leptuca* spp., *Minuca* spp*.*

1.   **Introduction**

Global warming caused by increased burning of fossil fuels has shifted the range distribution of organisms around the word (Parmesan and Yohe, 2003; Parmesan, 2006;

Hickling et al., 2006; Chen et al., 2011; Dyderski et al., 2018; Price et al., 2019). Increased temperatures have allowed several species to expand their distribution to deeper waters and higher altitudes and latitudes (Walther et al., 2002; Parmesan and Yohe, 2003; Chen et al., 2011; He and Silliman, 2019). Globally, it is estimated that the distribution boundaries of species have moved towards the poles at a mean 16.9 km per decade (Chen et al., 2011). Range expansion to higher latitude environments due to climate change has already been observed, for example, in vascular plants (Smith, 1994; Cavanaugh et al., 2014; Dyderski et al., 2018), reptiles (Moreno-Rueda et al., 2012), fish (Perry et al., 2005), insects (Pöyry et al., 2009) and crustaceans (Rombouts et al., 2009; Johnson, 2014; Rosenberg, 2018), among other taxa. The arrival of an invasive expanding species in a new habitat can potentially negatively affect the structure of native community (Duckworth and Badyaev, 2007; Engelkes et al., 2008; Hejda et al., 2009; Scheffel et al., 2018). The expanding species can compete for essential resources, i.e. food or territories, causing a decrease in abundance, changes in richness and, in extreme cases, even the extinction of resident species (Thomas et al., 2006; Hejda et al., 2009; Yamano et al., 2011; Bogstad et al., 2015).

Range expansion can especially be observed in intertidal organisms, which have shifted their territorial boundaries toward higher latitudes faster than terrestrial species, and are thus proposed as early indicators of climate change (Helmuth et al., 2006; Whitfield et al., 2016). Most intertidal organisms have an amphibian life cycle in which early pelagic stages (i.e. the larvae or propagule stages) inhabit aquatic environments, while benthic late stages (i.e. the growth and reproductive stages) occur in intertidal environments (Little et al., 2009). The planktonic larval phase is advantageous for the geographical dispersion of these animals because the small size of the larvae allows them to be transported by sea currents over long distances (Thorson, 1950; Günther, 1992; Roman and Boicourt, 1999; Ellien et al., 2004; Peliz et al., 2007; Little et al., 2009) allowing genetic connectivity to be maintained between populations where adults are separated by thousands of kilometres (Laurenzano et al., 2013; Marochi et al., 2017). Such an adaptive strategy is especially important for sedentary benthic organisms, which have a limited capacity for displacement (Thorson, 1950; Günther, 1992; Little et al., 2009). However, during the larval stage, the body temperature of ectodermic organisms tends to be the same that of water environment, due to their tiny size, resulting in low thermal

inertia (Hoegh-Guldberg and Pearse, 1995). In consequence, water temperature can directly affect larval metabolism and survivorship, driving their distribution (Sandford et al., 2006; O'Connor et al., 2007; Madeira et al., 2016).

Expansion of the latitudinal range of two fiddler crab species has been reported in North America (see Johnson, 2014 and Rosenberg, 2018). On the West coast *Uca princeps* was originally found from Peru to Mexico; now, their distribution boundary has expanded to include California, United States (Rosenberg, 2018). On the East Coast of the United States, the latitudinal boundary of *Minuca pugnax* has expanded 80 Km northwards due to an increase of 1.3 °C in mean water temperature (Johnson, 2014). Experiments showed that *M. Pugnax* could live and grow for several winters during the benthic stage when they were placed in latitudes higher than their northern distributional boundary (Sanford et al., 2006). However, the survivorship of pelagic larvae decreased dramatically when they were reared in water temperatures below that observed at their distribution limit (Sanford et al., 2006). In South America latitudinal range expansion was reported for the fiddler crab *Leptuca cumulanta* Crane, 1943 (Arakaki et al., 2020). The original occurrence of this species was described by Crane (1975) as from Venezuela to Paraíba state, on the northeast coast of Brazil (6°45'S). Then Thurman et al. (2013) found *L. Cumulanta* on the coast of Rio de Janeiro State, on the southeast coast of Brazil (23°13'S), considering it to be an expansive species. Recently, Checon and Costa (2017) found this species further south on the southeast coast of Brazil, in São Paulo state (23°59'S).

Fiddler crabs are key species in estuarine systems due to their high abundance and ecosystem engineering role (Reinsel, 2004; Kristensen, 2008; Natálio, et al., 2017; Citadin et al., 2018). They are important for structuring benthic intertidal communities since their deposit-feeding activity and density can modulate the abundance and diversity of producer organisms (i.e. microphytobenthos; see Reinsel, 2004) and primary consumers (i.e. meiofauna, see Citadin et al., 2018). Fiddler crabs are connectors of the matter and energy flow between the intertidal habitat and adjacent environments, since they are preyed upon by fish (Rozas and LaSalle, 1990; Krumme et al., 2001) or other crustaceans from aquatic environments (Kneib and Weeks, 1990), and by birds (Koga et al., 2001; Ribeiro et al., 2019) or mammals (Rulison et al., 2012) from terrestrial environments. The bioturbation promoted in the sediment by their burrowing and

feeding activities can modify the environment by affecting the availability of resources to other organisms (Jones, et al., 1994; Reinsel, 2004; Kristensen, 2008; Smith, et al., 2009). Considering the ecological role of fiddler crabs, range expansion to higher latitudes could cause deep shifts in any new estuarine communities where they settle.

Ten species of fiddler crabs (Crane, 1975) occur on the east coast of South America: *Leptuca cumulanta, L. Leptodactyla* (Rathbun, 1898)*, L. Thayeri* (Rathbun, 1900)*, L. uruguayensis* (Nobili, 1901)*, Minuca burgersi* (Holthuis, 1967)*, M. mordax* (Smith, 1870)*, M. Rapax* (Smith, 1870)*, M. victoriana* (Hagen, 1987)*, M. vocator* (Herbst, 1804) and *Uca maracoani* (Latreille, 1802-1803)*.*The distributions of these species have different southern boundaries along a latitudinal gradient (Fig. 1). *Leptuca uruguayensis* is the only species with a northern distribution limit in South America, which is situated at 22°51'S in Rio de Janeiro, on the southeast coast of Brazil (Thurman et al., 2013). A plausible explanation for the differential distribution of the fiddler crabs' southern boundaries is that their thermal niches differ between species and, therefore, the latitudinal temperature gradient along the South American coast could act as a climate barrier. However, there are no comparative studies on the thermal limits of these species and thus this issue remains unclear.

In this study, we compared the thermal limits of South American fiddler crabs' larvae to evaluate whether sea surface temperature (SST) delimits their territorial ranges. We hypothesized that temperature should act as a barrier if the larval thermal limits of different species of fiddler crabs correspond to the temperature of their latitudinal gradient of distribution. We tested if thermal limits differ between fiddler crab species and if these limits are associated with the latitudinal gradient of SST throughout South America. We also evaluated whether SST has been increasing, which could explain the distributional expansion of *L. Cumulanta* in recent years.

2. Materials and Methods

2.1 Sea surface temperature at the southern boundaries of fiddler crab territories

Daily optimum interpolation data of global mean SST from 1 January 1982 to 30 March 2019, with a spatial resolution of 0.25° x 0.25°, were obtained from the National Oceanic and Atmospheric Administration (NOAA) (www. ncdc.noaa.gov/oisst/; accessed June 25, 2020).

Data sets were geographically limited between the equatorial line (latitude 0°12'S) and the southernmost occurrence area of the fiddler crab species in South America (latitude 39°87'S; Fig. 1), and seasonally restricted to their reproductive period, during the warmer months (Table 1: from September to March). Based on the pre-selection of geographical limits and the reproductive period, it was estimated that the maximum distance that a larva could disperse (be carried by sea currents) during its development was an area of approximately 200 km offshore (Roman and Boicourt, 1999; Peliz et al., 2007).

Sea surface datasets were analysed in the R environment (R Development Core Team, 2020) using the packages "ncdf4" (Pierce, 2019), "fields" (Nychka, et al., 2017), "maptools" (Bivand and Lewin-Koh, 2019), "abind" (Plate and Heiberger, 2016), "sp" (Pebesba and Bivand, 2005) and "ggplot2" (Wichham, 2016).

2.2 Crab sampling methodology

Fiddler crabs were collected in the estuary of Santos/São Vicente, São Paulo State, on the south-eastern coast of Brazil (23°59'S – 46°24'W). This estuary experiences semidiurnal tidal systems and a subtropical climate without a dry season (Alvares et al., 2013). Water temperature varies between a minimum of 20 °C and a maximum of 28 °C (Porcaro et al., 2014).The mangrove forest in this estuary system is mainly composed of *Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia schaueriana* (Cordeiro and Costa, 2010). Other microhabitats are available for fiddler crabs throughout the mangrove forest, including small freshwater streams, unvegetated sandbanks and mudflats (Checon and Costa, 2017). Nine of the ten fiddler crab species found in Brazil inhabit this site: *L. cumulanta, L. leptodactyla, L. thayeri, L. uruguayensis, M. burgersi, M. mordax, M. rapax, M. vocator* and *U.maracoani*. We used all these species in our study except for *U. maracoani* (because we were unsuccessful in obtaining its larvae in laboratory conditions). For the species tested, we collected adult ovigerous females with embryos at the final stage of development (Table 2) (Costa and Negreiros-Fransozo, 1998; Oshiro, 1999). The final stage is preferred in order to avoid the interference of laboratory conditions on embryo development.

The thermal limits of the organisms may vary depending on the acclimatization temperature (Kikuchi et al., 2019). Since all the species used in this study lived in the same

area (i.e. at the same latitude), we assumed that the females and their egg clutches were acclimatized to the same latitudinal temperature. Thus, the thermal sensitivity of the species' larvae could be tested free of acclimatization effects. Considering that the breeding season of the fiddler crabs occurs mainly in the warmer months and larval release is associated with high tidal amplitude (Table 1; Christy, 1982; Castiglioni et al., 2007; Gusmão-Junior, et al., 2012), we collected all the species during low spring tides in the spring and summer of 2018-2019 (from November 2018 to January 2019). The ovigerous females were collected by digging out their burrows with a shovel and they were identified according to Bezerra et al. (2012).

2.3 Testing of thermal limits of Brazilian fiddler crab larval stages

In the laboratory the ovigerous females were individually placed in 600 ml beakers filled with 400 ml of water (salinity 27, according to the salinity range observed in the estuary of Santos/São Vicente: Moser et al., 2005). The females were kept under constant aeration and at room temperature of 25 °C (following Darnell and Darnell, 2018). Females were kept in these conditions until larval release, which occurred approximately 24 to 48 hours later, always during the night. The larvae of all the females of a species released on the same day (Table 2) were mixed in a single culture to avoid the inclusion of genetic variation as a factor in our model. Then, 24 swimming larvae (zoea I, 24 hours of age) per species were individualized and put in cell culture plates (24 wells of 1.0 ml, filled with water at salinity of 27 and at 25 °C). The plate bottom was line-marked with a white PVC plastic rectangle for easy observation of the larvae. The plate was placed in a water bath (Quimis®) where the thermal limits were tested. The temperature was increased or decreased from 25 °C at a constant rate of 0.3 °C min^{-1} (Darnell and Darnell, 2018) until the occurrence of equilibrium loss when larvae lose their ability to escape from potentially fatal conditions such as, for example, a predator (Lutterschmidt and Hutchison, 1997). The thermal tolerance of the larvae was determined by the Critical Thermal Minimum (CT$_{min}$) and Critical Thermal Maximum (CT$_{max}$) tests, defined as the arithmetic mean of the temperature at which individual reached the endpoint (Lowe and Vance, 1955; Mora and Ospina, 2001; Ravaux et al., 2016). The endpoint, measured by loss of equilibrium, was assumed when larvae could no longer remain in the water column because they stopped swimming (abdominal propulsion become disorganized and ceased) and then sank to the

bottom of the plate. The individual water temperature of each well was reported using a thermocouple coupled to a digital thermometer (Lutron[®], TM-947SD). After the critical thermal experiments the larvae were quickly returned to their acclimation temperature, and then larvae and their mothers were released at the sampled sites.

Since the larvae of the different species used here were subjected to the same thermal conditions before the experiment, interspecific differences observed between their thermal limits could reflect differences in thermal sensitivity between species, which could be used to explain the different distribution limits. The thermal tolerance of larvae (CT_{min} and CT_{max}: response variables) were compared between species (categorical variable: fixed factor with eight levels) using a one-way ANOVA analysis followed by the post-hoc Scheffe test (in case of significant difference between the treatments). A minimum of 95% was adopted as a level of significance. We used linear regressions to test whether the thermal limits of the larvae were associated with latitudinal SST, using the water temperatures from the southern boundary of each species.

3. Results

Along the distribution range of fiddler crabs in South America, the SST from September to March decreases 0.28 ± 0.30 °C (mean ± standard deviation) per latitudinal degree, from mean 28.35 °C near the line of the Equator in Brazil to mean 16.86 °C near the Bahia Blanca Estuary, Argentina. From 1982 to 2019, the average SST along the South America coast has been increasing at 0.24 °C per decade, showing a total increase of 0.9 °C in 37 years (Fig. 2).

The thermal tolerance of fiddler crab larvae to low temperatures differed between species (ANOVA results: $F_{7,184}$ = 157.76; MS = 89.53; P < 0.0001). The most cold-sensitive species was *L. cumulanta,* which reached loss of equilibrium at 16.5 °C (Fig. 3: A, Table 3). Subsequently, the species most sensitive to cold water were *M. vocator* and Florianopolis species, reaching CT_{min} at approximately 14.5 °C*. Minuca mordax* and *L. uruguayensis* were more tolerant of the cold, reaching their thermal limits at approximately 12.0 and 10.0 °C, respectively. Results from the linear regression showed that the CT_{min} of species during the larval stage are positively associated with the mean SST of the fiddler crabs' southern boundaries in South America (Fig. 4).

Larval tolerance of high temperatures also differed between species (ANOVA results: $F_{7,184} = 25.10$; MS = 15.10; $P < 0.0001$). The most sensitive species to warm temperatures was *L. uruguayensis*, which reached CT_{max} at approximately 38.0 °C, differing from the other species which reached CT_{max} at approximately 40.5 °C (Fig. 3: B; Table 3).

4. Discussion

South American fiddler crab species are territorially limited by cold water temperatures in a latitudinal gradient. The differences between species' territorial ranges can be explained by the fiddler crabs' sensitivity to cold water in the early pelagic stages. During the larval phase the different species of fiddler crab showed a cold sensitivity that corresponded to the gradient of their southern boundaries. For example, from the eight species we studied, the southern boundary of *L. cumulanta* is at 23°59'S (the northernmost southern limit of all the species' boundaries) and it was also the species that was least tolerant of cold water during the larval phase. In contrast, the southern boundary of *L. uruguayensis* is at 36°25'S (the southernmost limit) and this was the species most able to tolerate cold water during the larval phase.

We found that mean SST during the summer months has increased 0.9 °C in South America since 1982. The warming of coastal waters may drive the expansion of the thermal niches of the fiddler crabs towards the poles. Faced with global warming, we expect that fiddler crabs will expand their distribution range to higher latitudes.

At the southern distributional boundaries, fiddler crabs tend to breed during the warmer months (Table 1), and their larval development until the settlement stage typically occurs over a short period: *L. thayeri* ≈ 30 days (Anger, 1990); *L. uruguayensis* ≈ 24 days (Rieger, 1996) *M. rapax* ≈ 12 days (Simith et al., 2014); *M. vocator* ≈ 10 days (Simith et al., 2012). However, larval development and larval survivorship of fiddler crabs decrease at low temperatures (Sanford et al., 2006). For example, larval development time increased exponentially with decreasing temperature in laboratory tests using the North America fiddler crab *M. pugnax* (Sanford et al., 2006). The larval survivorship of this species is higher than 50% in temperatures above 18 °C, when it reaches the final megalopa stage in 33-51 days, but it decreases to below 10% at 16 °C, requiring 52-82 days to complete total development (Sanford et al., 2006). Mortality rates are extremely high during the pelagic stage, and thus larvae that develop quickly are more likely

to survive to settlement (Thorson, 1950; Ospina-Alvarez et al., 2018). Low water temperatures could affect the larval development of South American fiddler crabs in a similar fashion, consistent with our results. The literature lacks studies comparing the larval development of South American crabs at different temperatures. One source of evidence comes from comparing studies performed with *M. vocator* by Rieger (1999) and Simith et al. (2012). In the first study, larvae raised at 25 °C had lower survival and required four to six zoaeal stages to complete development, whereas in the second larvae raised at 29.5 °C achieved 100% survival and completed their development with five larval stages. Therefore, the decrease in survivorship and increase in time required for larval development in response to low temperatures (Sanford et al., 2006), added to the differential sensitivity to low temperature observed among fiddler crab species (in the present work), corroborate the hypothesis that the clinal water temperature gradient constitutes an effective climatic barrier forming the latitudinal boundaries of these intertidal crustaceans.

We found that coastal water temperature during the breeding season of fiddler crabs has been increasing in South America by a mean 0.24 °C per decade in the last 37 years. Our results corroborated studies which found that the annual mean water temperature of the Brazil current increased 1.93 °C from 1950 to 2008 (Wu et al., 2012) and that mean annual air temperature showed an increase that ranged from 0.1 to 0.5 °C per decade between 1975 and 2015 (Bernardino et al., 2015). The temperature increase on the South American coast may change the present thermal gradient and allow fiddler crabs to expand to higher latitudes, as observed for the North American fiddler crabs (Johnson, 2014; Rosenberg, 2018). Based on our data, the range expansion recently observed for *L. cumulanta* in the south-eastern estuaries of Brazil could be assumed to have occurred as a result of sea water warming over recent decades. Sightings of *L.cumulanta* began to be reported in south-eastern Brazil around the 2010s (Thurman et al., 2013; Checon and Costa, 2017). From 2010 to 2017, the density of *L. cumulanta* increased more than 20 times in one recently-colonized mangrove (Arakaki et al., 2020). Cabo Frio (23°00'S), in south-eastern Brazil, represents the boundary of the two major biogeographic marine ecoregions – the Tropical Atlantic and Temperate South America marine ecoregions (Spalding et al., 2007). Our results from 1982 to 2019 showed that SST between Cabo Frio and the current southern boundary of *L. cumulanta* (23°59'S) reached a mean of

24.45 °C after the year 2000. Before the year 2000, mean SST was less than 24 °C. The warmer water temperatures observed in the last two decades may have allowed larvae of *L. cumulanta* transported from lower latitudes to complete their development and settle in areas of the south-eastern coast of Brazil. To date, there are no studies comparing the larval development of *L. cumulanta*. Future studies comparing the larval development of *L. cumulanta* at 24 °C and other temperatures may confirm or refute this hypothesis.

The results comparing CT_{max} between the South America fiddler crabs, in contrast to CT_{min}, did not explain their latitudinal distribution. The maximum thermal limits of fiddler crab larvae are higher than the environmental temperatures observed in South America. The maximum SST temperature observed during the studied period (1982 to 2019) was 28.88 °C. All the studied species (except *L. uruguayensis*) showed similar thermal tolerance (40.5 °C). They occur in equatorial estuaries and, therefore, their territorial boundaries are not found at lower latitudes (Thurman et al., 2013). Their northern boundaries occur in Central or North America (see Thurman et al., 2013) and are probably defined by a cold water gradient from the northern hemisphere (as demonstrated for their southern boundaries in the present study) or variables such as, for example, habitat availability, competition with other species or geographic barriers caused by marine currents. *Leptuca uruguayensis* is the only species with a northern boundary at 22°51'S, in Rio de Janeiro, on the south-eastern coast of Brazil (Thurman et al., 2013). The CT_{max} of *L. uruguayensis* larvae was approximately 38 °C, the lowest CT_{max} of the species in the present study. However, maximum SST in Rio de Janeiro (24.83 °C) is approximately 14.0 °C, below the CT_{max} of *L. uruguayensis* larvae. The larvae of *L. uruguayensis* reared at 25 °C were able to complete their development until the megalopa phase (Rieger, 1996). The northern limit of this species is probably caused by adult sensitivity to high mean air temperatures (above 29 °C), which prevent these crabs from growing and reaching sexual maturity (De Grande et al., unpublished results).

Overall, we found that differences between the territorial boundaries of South American fiddler crabs can be explained by the sensitivity of their larvae to cold water in a latitudinal gradient. Since coastal water temperatures off South America have been warming due to climate change we can expect that fiddler crabs' territories may expand to higher latitudes in the future. Species' range expansions can shift the structure of newly colonised communities due to

interspecific competitive interactions (Yamano et al., 2011; Bogstad et al., 2015; Kortsch et al., 2015). Some range expanding species, such as *L. cumulanta*, may adjust to the new communities and be able to live in mixed assemblages, sharing habitats and resources without causing damage to native species (Arakaki et al., 2020). In other cases, the expanding species may negatively affect the native species, for example, the predator fiddler crab *M. rapax* may decrease the mean body size of the prey population, *L. uruguayensis* (De Grande et al., unpublished results). Future studies should continue to monitor fiddler crabs' territorial boundaries and evaluate the consequence of the arrival of a range expanding species on native organisms and their habitats.

5. Conclusions

Cold sensitivity of the larval stage of South American fiddler crabs causes the differential distribution of their southern boundaries. The increase in temperature of coastal waters due to global warming, such as the increase of 0.9 °C in 37 years observed in the present study, may cause fiddler crabs to expand their ranges to greater latitudes in South America. We suggest that the recent territorial expansion of at least one species of South American fiddler crab, *L. cumulanta*, has been caused by temperature increase. In summary, the latitudinal gradient of water temperature along the coast of South America acts as a climatic barrier between different species of fiddler crabs, which has been altered due to climate change.

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Tables

Table 1. Reproductive period of the fiddles crabs. Reproductive months of South America fiddler crabs species based in the presence of ovigerous females in the population.

Species	J	F	M	A	M	J	J	A	S	O	N	D	Sampled populations	Reference
Leptuca cumulanta	·		·	·			·	·	·	·	·	·	00°40'S, 46°40'W	Koach et al., 2005
Leptuca leptodactyla	·	·	·						·	·	·	·	25°52'S, 48°36'W	Masunari, 2012
Leptuca thayeri	·	·	·	·			·						3°43'S, 38°32'35W	Bezerra and Mathews-Cascon, 2007
Leptuca thayeri	·	·	·	·	·			·	·	·	·	·	23°29'S, 45°09'W	Costa and Negreiros-Fransozo, 2002
Leptuca thayeri	·	·	·						·	·	·	·	23°29'S, 45°09'W	Costa et al., 2006
Leptuca uruguayensis	·	·	·	·				·	·	·	·	·	23°29'S, 45°09'W	Costa et al., 2006
Leptuca uruguayensis	·	·	·							·	·		36°25'S, 65°57'W	Colpo and López-Greco, 2017
Leptuca uruguayensis	·										·		36°16'S, 57°06' W	César et al., 2007
Leptuca uruguayensis	·	·	·								·		37°46'S, 57°27'W	Spivak et al., 1991
Minuca burgesi	·								·				23°20'S, 45°43'W	Benetti et al., 2007
Minuca mordax	·	·	·					·	·	·	·	·	23°24'S, 45°00'W	Fransozo et al., 2009
Minuca rapax	·	·		·	·		·	·	·	·	·		00°40'S, 46°40'W	Koch et al., 2005
Minuca rapax	·	·		·	·	·	·	·	·	·	·		23°14'S, 44°42'W	Castiglione et al., 2007
Minuca vocator		·	·	·	·	·							00°40'S, 46°40'W	Koch et al., 2005
Minuca vocator	·	·	·	·	·	·	·		·	·	·	·	23°24'S, 45°01'W	Colpo and Negreiros-Fransozo, 2003
Minuca vocator	·	·	·										23°24'S, 45°03'W	Colpo and Negreiros-Fransozo, 2003
Minuca vocator	·	·	·	·					·	·	·	·	23°49'S, 46°09'W	Colpo and Negreiros-Fransozo, 2003

Table 2. Ovigerous females of fiddler crab species used in thermal limits experiments. Number of females (n) and mean size (carapace width) of specimens used to test the maximum and minimum thermal limits (CT_{max} and CT_{min}). Ovigerous females were used to obtain larvae used both in CT_{max} and CT_{min} experiments.

Species	N	Carapace Width (mm)
Leptuca cumulanta	3	7.07 ± 0.68
Minuca vocator	3	17.17 ± 0.42
Leptuca thayeri	5	19.66 ± 1.77
Minuca rapax	3	13.37 ± 1.24
Minuca burgersi	6	11.57 ± 1.19
Leptuca leptodactyla	3	8.60 ± 1.05
Minuca mordax	3	17.73 ± 0.78
Leptuca uruguayensis	3	7.10 ± 0.90

Table 3. Critical thermal minimum (CT_{min}) and maximum (CT_{max}) of South American fiddler crabs and environmental temperatures of their boundaries. The mean temperature at which each species reached loss of equilibrium in the CT_{min} and CT_{max} tests are shown for fiddler crab larvae (zoaea I, age 24h). The mean sea surface temperature (SST) of warmer months is shown for each estuary within the distribution boundaries of each species. We have assumed Florianópolis-SC as the same distribution boundary for *Leptuca leptodactyla, L. thayeri, Minuca burgersi* and *M. rapax.*

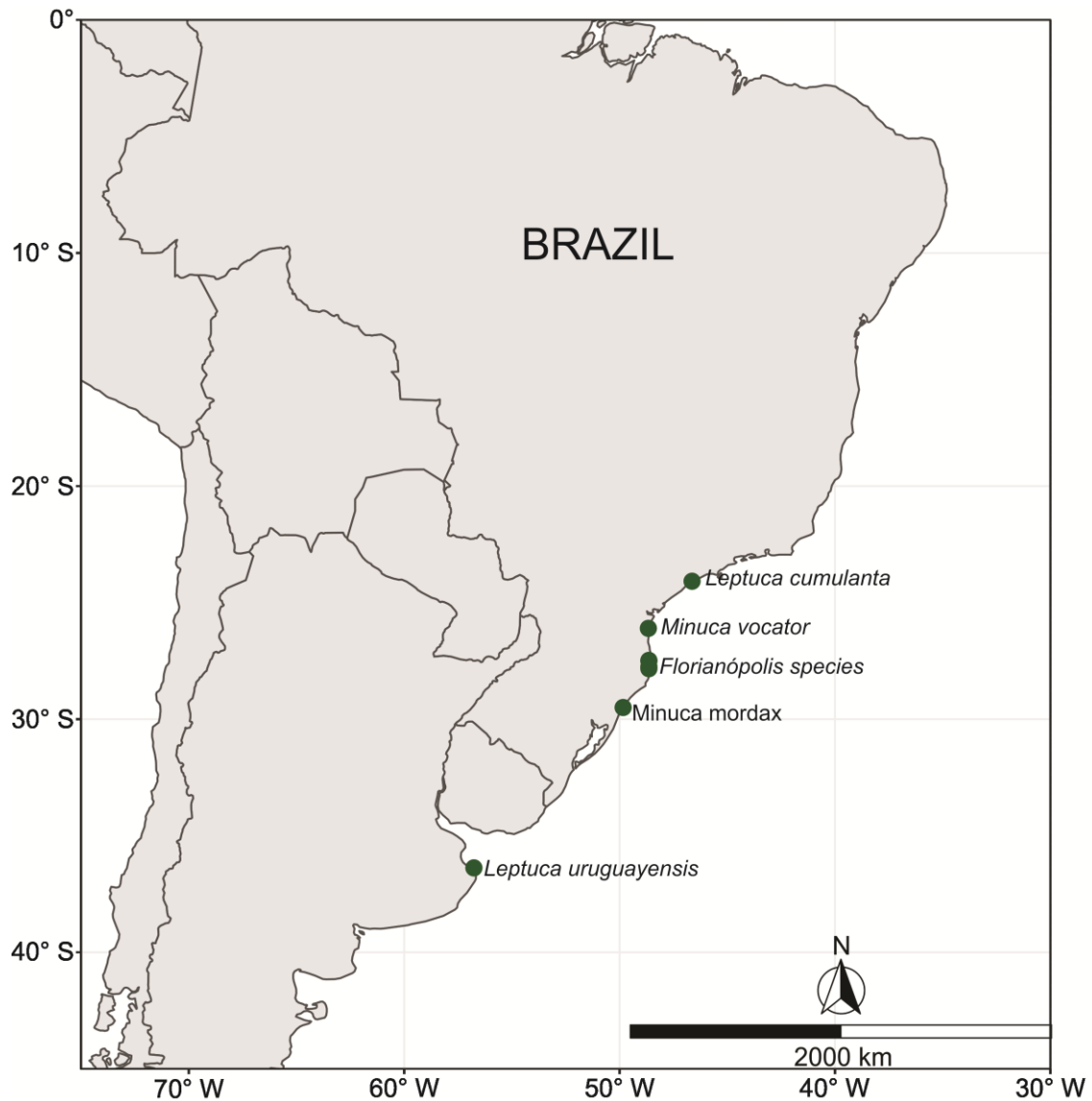| Species	Southern distribution boundary	Mean SST at southern boundary	CT_{min}	CT_{max}
Leptuca cumulanta	23°59'S (Checon and Costa, 2017)	24.22°C	16.59 ± 0.44 °C	40.12 ± 0.62 °C
Minuca vocator	26°02'S (Almeida, 2009)	23.96°C	13.97 ± 1.14 °C	40.29 ± 0.80 °C
Leptuca thayeri	27°26'S (Thurman et al., 2013)	23.70°C	14.59 ± 0.96 °C	40.93 ± 0.97 °C
Minuca rapax	27°35'S (Thurman et al., 2013)	23.70°C	15.14 ± 0.51 °C	40.47 ± 0.90 °C
Minuca burgersi	27°45'S (Thurman et al., 2013)	23.70°C	13.06 ± 0.68 °C	40.49 ± 0.68 °C
Leptuca leptodactyla	27°49'S (Thurman et al., 2013)	23.70°C	14.57 ± 0.65 °C	40.28 ± 1.02 °C
Minuca mordax	29°19'S (Thurman et al., 2013)	22.76°C	11.91 ± 0.54 °C	40.58 ± 0.43 °C
Leptuca uruguayensis	36°25'S (Colpo and Lópes-Greco, 2017)	19.01°c	10.35 ± 0.79 °C	38.31 ± 0.61 °C

Fig. 1. The southern boundaries of the distributions of eight fiddler crab species along a latitudinal gradient in South America according to Almeida (2009), Thurman et al. (2013), Checon and Costa (2017) and Colpo and Lópes-Greco (2017). Florianópolis species is the southern boundary of *Leptuca leptodactyla, L. thayeri, Minuca burgersi* and *M. rapax.*
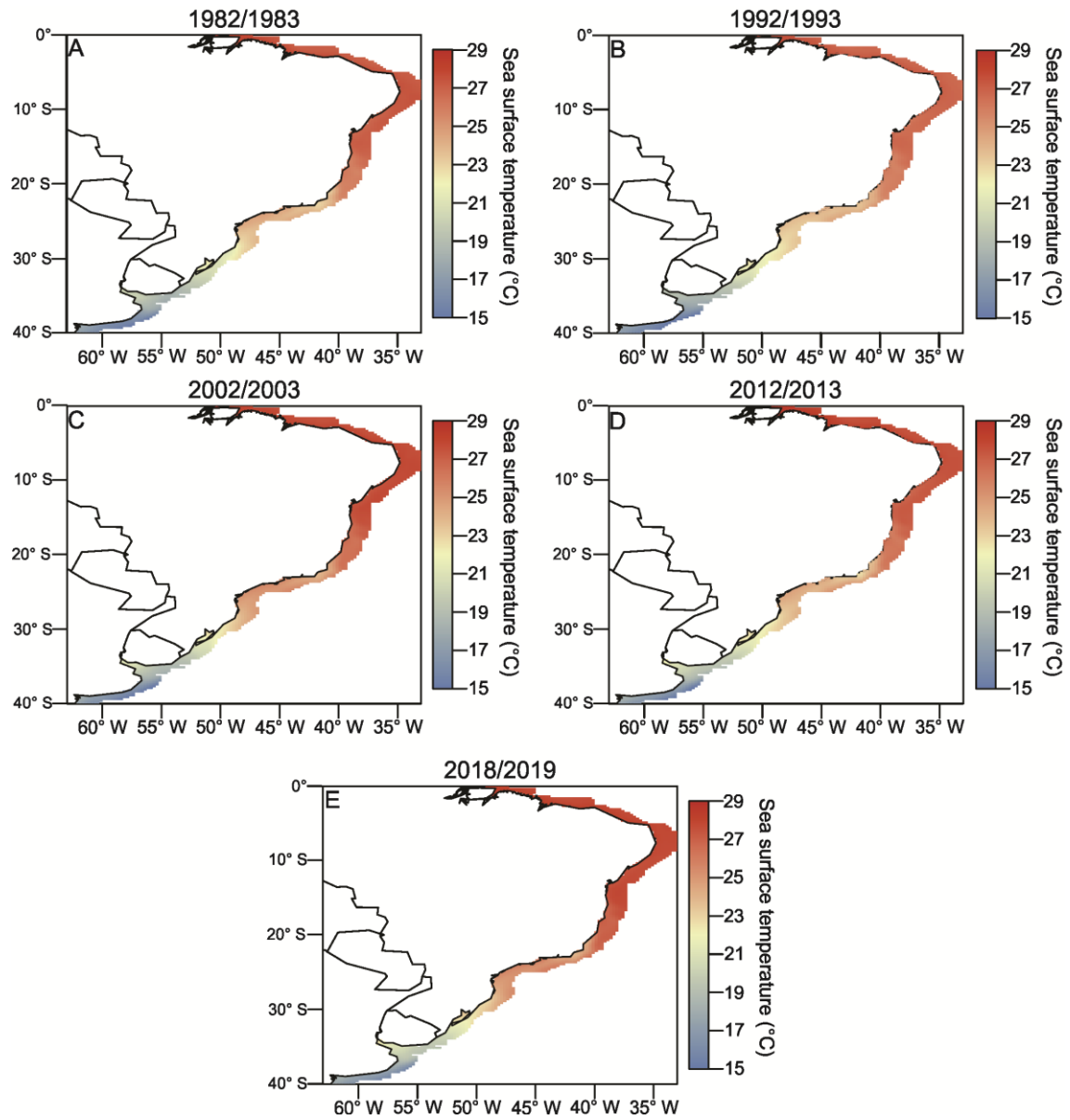
Fig. 2. Mean sea surface temperature along the fiddler crabs' distribution range on the South American coast, from 1982 to 2019 (A to E) and sea surface temperature averaged by year from 1982 to 2019 (F). Data sets were seasonally restricted to warmer months, the reproductive period of fiddler crabs, from September to March.
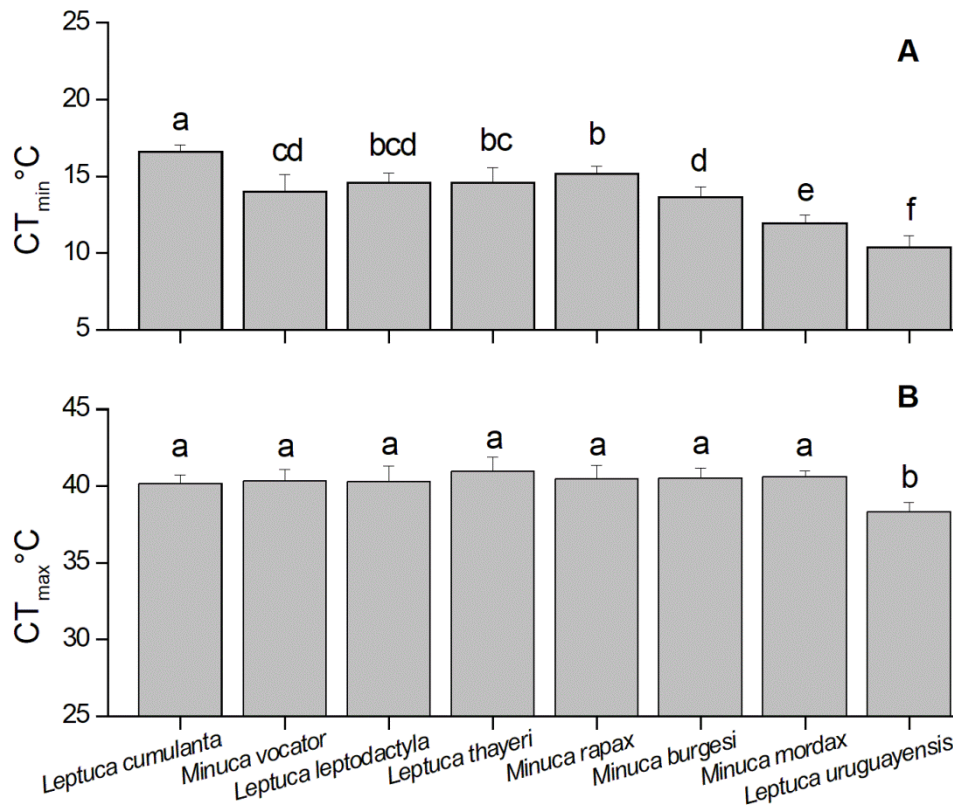
Fig. 3. Critical thermal limits of fiddler crab larvae (Zoaea I, 24 h age). A: mean temperature in which larvae species lost equilibrium in critical thermal minimum tests. B: mean temperature in which larvae species lost equilibrium in critical thermal maximum tests. Different lower case letters indicate statistical differences ($P < 0.05$) between species.
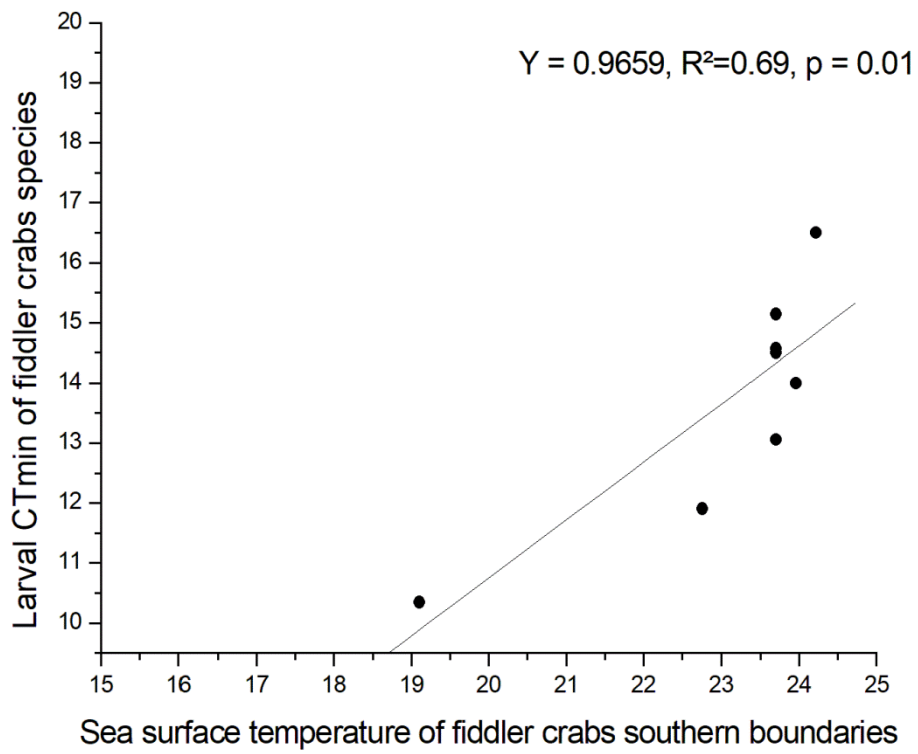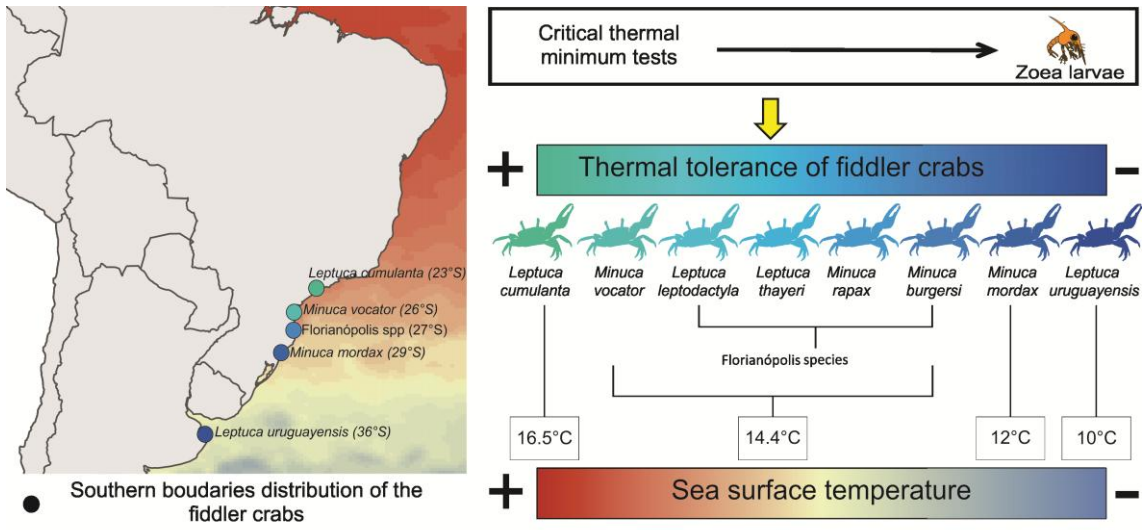
Fig. 4. Association between the thermal sensitivity of larvae of different species of fiddler crabs (Critical thermal minimum, CT_{min}) and their southern boundaries of distribution along the coast of South America.

Graphical Abstract

Highlights

- Cold water temperature defines southern boundaries of the fiddler crabs.

- Thermal sensibility of the fiddler crabs species delimit their distribution.

- Sea surface temperature increase 0.9°C in the last 37 years.

- Distributional range of *Leptuca cumulanta* expanded due temperature.

Capítulo 2

Size-at-age or Structure Shift: Which Hypothesis Explains Smaller Body Size of the Fiddler Crab *Leptuca uruguayensis* in Northern Populations?

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Size-at-age or Structure Shift: Which Hypothesis Explains Smaller Body Size of the Fiddler Crab *Leptuca uruguayensis* in Northern Populations?

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Abstract

The mean body size decrease is known as the third most important global consequence of climate change to wild life. Rising temperatures may lead to decreased mean body size of organisms and change their ecological role in the environment. Herein we investigated why the fiddler crab *Leptuca uruguayensis* is smaller at its northern distributional limit by using the 'size-at-age' and 'structure shift' as alternative hypotheses. For the first hypothesis, we evaluated whether the smaller mean body size of *L. uruguayensis* from a northern population is a phenotypic response to the thermal environment. For that, we tested whether the crabs grow less and reach the onset of sexual maturity earlier at high temperatures. We also evaluated their oxygen consumption at different temperatures to test whether higher metabolic rates due to warmer temperatures leads to smaller body sizes. For the second hypothesis, we evaluated whether smaller mean body size in a northern population is a result of differential survivorship between age-classes. We tested whether the temperature itself or a predator model with a

range distribution linked to temperature (*Minuca rapax*) could negatively select larger *L. uruguayensis* sizes. We showed that crabs grow less, reach sexual maturity earlier and have lower survive in response to high metabolic costs imposed by higher temperatures. The predator chose a large *L. uruguayensis* size, a finding that could mean selective pressure where prey populations overlap with this predator. Thus, global warming may decrease the mean body size of the fiddler crabs at lower latitudes, affecting their ontogenesis and by selective pressure against larger individuals.

1. Introduction

The mean body size of many organisms has declined in response to anthropogenic climate change (Gardner et al. 2011; Ohlberger 2013; Azra et al. 2020). Body size reduction has been suggested as one of the three most important consequences of global warming, together with shifts in phenology and species distribution (Gardner et al. 2011). A size decline has been observed at different levels of biological organisation from individuals to populations, and it is linked to an ecological consequence in the fitness or survival of the organisms (Kingsolver and Huey 2008; Peck et al. 2009; Ohlberger 2013; Azra et al. 2020).

In response to temperature, many organisms show a positive relationship between mean body size and latitude that has been explained by temperature–size rules (Atkinson 1994; Angilletta et al. 2002; Kingsolver and Huey 2008; Gardner et al. 2011; Ohlberger 2013). The temperature–size rule represents a form of phenotypic plasticity, in which the adult size (phenotypic) of a given genotype is affected by temperature (Angilletta et al. 2004; Kingsolver and Huey 2008). Although the temperature–size rule has been widely described (see Atkinson 1994), research within the field has remained largely descriptive without considering the interplay between physiological mechanisms and the broader ecological context (Ohlberger 2013). The understanding of how temperature determines a cline in body size along a latitudinal gradient can help researchers to predict the effects of climate change on organisms and, consequently, how it will change the natural systems.

Temperature can drive body size reduction in populations mainly due to the 'size-at-age shift' or 'structure shift' hypotheses (Ohlberger 2013). According to the size-at-age hypothesis, temperature affects the growth and development of an organism through direct effects on biochemical reactions, which regulate energetic processes such as metabolic cost and resource acquirement (Ohlberger 2013; Ern et al. 2015). According to the 'oxygen- and capacity-limited thermal tolerance theory', when the temperature rises beyond the thermal optimum, growth is impaired by insufficient energy or oxygen, a phenomenon that results in the decrease of body size at any developmental stage (Pörtner 2010). The size decline of individuals in a population is a phenotypic response to the thermal environment during the ontogeny of individuals (Ohlberger 2013).On the other hand, in the structure shift hypothesis, the decline in mean size occurs due to changes in the relative abundance of different age-classes or life-stages within the population (Ohlberger 2013). Size selection can be a direct result of differential survivorship between age-classes in response to temperature itself. For example, small juvenile individuals can be less susceptible to extreme temperature compared to larger adult ones (Peck et al. 2009). Size selection also can be indirectly associated with temperature. Increases in natural mortality of different age-classes at higher temperatures could be driven by changes in feeding rates and predation mortality (Audzijonyte et al. 2019).

The fiddler crab *Leptuca uruguayensis* shows differences in mean body size among populations from different latitudes (Hirose et al. 2013; Masunari et al. 2017). *Leptuca uruguayensis* is widely distributed along 2,400 km of the Atlantic coast of South America, from Rio de Janeiro State, Brazil (22°51'S42°02'W) to Buenos Aires, Argentina (37°45'S57°28'W) (Thurman et al. 2013). In northern populations, such as in São Paulo State, Brazil (23°24'S45°03'W), the mean carapace width (CW) of *L. uruguayensis* is around 8 mm, while in southern populations, such as Buenos Aires, Argentina (36°19'S56°47'W), it is 13 mm (Hirose et al. 2013). However, no restriction on the gene flow between the Brazilian and Argentine populations of *L. uruguayensis* has been observed (Laurenzano et al. 2012). Understanding the difference in fiddler crabs' body size is particularly important because they represent a key functional group in the structuring of the coastal benthic community (Kristensen et al. 2012; Johnson et al. 2019). Given that they are bioturbators, the variation in their size can potentially affect the quantities of resources available for other trophic levels when they turn over the

sediment with their burrowing and feeding activities (Johnson et al. 2019). The progress of global warming could result in a decline in body size of fiddler crabs at a latitudinal scale, which could change their engineering role on coastal communities.

In this study, we evaluated whether the smaller mean size of adult *L. uruguayensis* from a population close of the species' northern distributional limit is linked to temperature. Using laboratory experiments, we hypothesised the causes of size variability in response to temperature and explored the size-at-age and structure shift approaches. First, we tested whether *L. uruguayensis* grows less and reaches sexual maturity earlier at high compared to low temperatures. As complementary experiments, we evaluated the metabolic costs of living at different temperatures by measuring their oxygen consumption. For the second approach, we evaluated whether the temperature itself or a predator model with a range distribution linked to temperature could negatively select larger sizes of *L. uruguayensis*.

2. Material and Methods

2.1 Study species

For all the following experiments, we used fiddler crab specimens collected in the Portinho mangrove, Santos-São Vicente estuary, on the southeast coast of Brazil (23°59'16.74" S, 46°24'26.28" W). The area is characterised by well-established communities of fiddler crabs, including *L. uruguayensis* and *Minuca rapax* (Checon and Costa 2017). All crabs were collected manually during low tide, using a shovel to dig their burrow and then catching them.

In the Portinho mangrove forest, *L. uruguayensis* has a mean CW of approximately 6.5 mm and a mean density of approximately 15 ind/m² (De Grande et al. 2018). We collected two size classes of *L. uruguayensis* adults: large crabs, with a CW between 9 and 11 mm; and small crabs, with a CW between 6 and 8 mm. We also collected *L. uruguayensis* juveniles with a CW between 3 and 5 mm (Hirose et al. 2013; Martins and Masunari 2013) for an experiment about growth, sexual maturity and survivorship at different temperatures.

At Portinho, the predatory fiddler crab *M. rapax* reaches a density of approximately 2 ind/m² (Checon and Costa 2017). Two size classes of male *M. rapax* were collected: large predator crabs, with a CW between 19 and 22 mm; and small predator crabs, with a CW

between 13 and 16 mm. *Minuca rapax* was used as a predator model in the last experiment presented in methods section.

2.2 Temperatures used in the experiments

The temperatures used in the following experiments were determined considering the thermal amplitude experienced by *L. uruguayensis* from Argentina to Southeast Brazil. For the first experiment (size-at-age shift experiment), we used 20°C and 25°C, considering the mean air temperatures between the latitudes of 37°S – Mar Chiquita (Colpo and López-Greco 2017) and 22°S – Rio de Janeiro (Bernardino et al. 2015; Ximenes et al. 2016). We also used one treatment of a temperature increase of 29°C (25°C +4°C), based on values predicted for the years 2081–2100 by general circulation models (GCMs) under the Intergovernmental Panel on Climate Change (Stocker et al. 2013) RCP 8.5 scenario, for a mean temperature at the northern limit of *L. uruguayensis.* Although the air temperature in winter drops below the minimum 10°C in Argentinean populations (Colpo and López-Greco 2017), preliminary tests showed that *L. uruguayensis* in the laboratory does not survive for 24 h at 15°C under the experimental conditions used in the section 2.3.1. Thus, we established the temperatures of 20°C, 25°C and 29°C used in the subsequent experiment about oxygen consumption (section 2.3.2).

2.3 Testing the size-at-age shift hypothesis

2.3.1 Growth, sexual maturity and survivorship of *L. uruguayensis* at different temperatures

We tested the hypothesis that *L. uruguayensis* grows less and reaches sexual maturity more quickly at high temperatures. We expected lower survival at higher temperatures. The experiment was conducted using male and female *L. uruguayensis* juveniles with a mean CW ± standard deviation (SD) of 4.64 ± 0.55 mm and 4.53 ± 0.66 mm, respectively. Crabs were raised for almost two years (645 days) in three temperature treatments: 20°C, 25°C and 29°C. For each temperature, 24 males and 24 females were used.

The fiddler crabs were raised in individual containers (10 x 10 x 4 cm) filled with 20 ml of saltwater (salinity 27, according to the salinity observed in the estuary of Santos - São Vicente: Moser et al. 2005). A section of pipe (length: 2 cm; diameter: 0.5 mm) was placed as a shelter

for the crabs in each container. The temperature management required for each treatment was done using germination chambers (Fanem® 347 CDC). Crabs were placed in germination chambers at 25°C (approximately the same temperature in which they were collected in the field).Then, in the 20°C and 29°C treatments, the temperature was gradually decreased and increased, respectively, at a rate of approximately 0.3°C h^{-1}. Every two days, the crabs were fed with crushed feed for marine fish Poytara® (Allen et al. 2012; Darnell et al. 2015), their containers were cleaned and the water was changed.

Although we used juvenile crabs with approximately the same CW size, their age could be slightly different; therefore, the number of moult to reach the onset of sexual maturity could be variable. Thus, we estimated their growth by calculating the average percentage CW increase per individual reached after the moults (i.e. the average growth percentage observed between all moults for one individual), as well as the average intermoult time per individual. The CW when crabs reach sexual maturity and the time required to reach sexual maturity (days) were evaluated among treatments. The survivorship over time of each treatment was also observed. Given that lethal tolerance levels are estimated at 50% mortality (Peer et al. 2016; Thurman et al. 2017), a survival rate of ≤50% was considered critical for the successful survival of *L. uruguayensis.*

The morphological maturity of males and females was determined according to the ratio between their CW and propodus length (PL) and abdomen width (AW), respectively. These dimensions are commonly used in allometric growth studies because they indicate secondary sexual characters closely associated with their reproductive activities and constitute elements of sexual dimorphism in adulthood (Castiglioni and Negreiros-Fransozo 2004; Martins and Masunari 2013; Masunari et al. 2017). We assumed as the criterion of sexual maturity the ratio PL/CW ≥ 1.0 for males and AW/CW ≥ 0.5 for females. These values indicate that the hypertrophied male cheliped may be used in mating activities, such as a fight for females, courtship and handling the female during mating, while the increase in female AW provides a chamber where the eggs may be attached to pleopods and incubated (Castiglioni and Negreiros-Fransozo 2004). Once the crabs were kept in laboratory conditions for a long time they were not able to return to wild, thus after the experiment they were sacrificed by freezing.

2.3.2 Oxygen consumption

We tested the hypothesis that the temperature increase imposes high metabolic costs to *L. uruguayensis* and, consequently, causes an increase in oxygen uptake. The oxygen consumption of male *L. uruguayensis* juveniles, with a mean CW ± SD of 5.35 ± 0.47 mm, was determined at temperatures of 20°C, 25°C and 29°C. We used six crabs per treatment (N = 6). The fiddler crabs were housed in plastic containers (10 x 10 x 4 cm) filled with 20 ml of saltwater (salinity 27) and placed in germination chambers at 25°C. Then, the temperature was gradually decreased or increased at approximately 0.3°C h^{-1}, according to the appropriate treatment. After reaching the correct temperatures, the crabs remained 48 h in the germination chamber under starvation to acclimate them to the experimental temperatures.

Following Vianna et al. (2020), we used glass chambers (Erlenmeyer flask) filled with 50 ml of saltwater (salinity 27) and placed them in a water bath so that water temperature was equal to the experimental temperatures (20°C, 25°C and 29°C). The crabs were individually inserted into the glass chambers and allowed to acclimatise for 30 min with aeration. Then aeration was removed and we measured the initial oxygen concentration with an oximeter (Model 53 and probe YSI Model 5905 monitor). The chambers were closed and, after 2h, the final oxygen concentration was measured. Control chambers without animals were kept under the same experimental conditions. The oxygen consumption was calculated by the variation between initial and final oxygen concentration (μg O_2mg^{-1} dry weight h^{-1}). Data were corrected against the control chamber and standardised against the individual dry weight.

2.4 Testing the structure shift hypothesis

2.4.1 Size selection by temperature

In this experiment, we evaluated whether larger individuals of *L. uruguayensis* are more sensitive to temperature than smaller individuals and, thus, explain their smaller mean size in northern populations. We tested the critical thermal maximum (CTmax) of *L. uruguayensis* adults of two CW classes: small size, mean ± SD of 7.68 ± 0.30 mm (n = 10); and large size, mean ± SD of 10.14 ± 0.16 mm (n = 10). Crabs were acclimated for 48h in the germination chamber at 25°C in individual containers (10 x 10 x 4 cm) filled with 20 ml of saltwater (salinity 27). Then, crabs were individually transferred to circular glass containers (40 mm diameter),

filled with 5 ml of saltwater, and placed in the water bath. These water volumes were sufficient to cover the fiddler crabs' legs and the ventral portion, ensuring that body temperatures was approximately the same as the water temperature (Darnell et al. 2015; Darnell and Darnell 2018).The temperature was raised from 25°C to 50°C at a rate of 0.3°C min^{-1} until the fiddler crabs lost their motor coordination. This rate results in steady heating of the water in the individual containers and fiddler crab body temperatures (Darnell et al. 2015; Darnell and Darnell 2018). At temperatures above 40°C, the container with the crab was quickly removed from the water bath every minute, and the crab was placed on its back inside its container. Individuals able to recover their position were immediately returned to the water bath to continue heating, whereas those that could not recover within 15 s were removed from the water bath and they returned to a normal condition (Allen et al. 2012). We calculated CTmax as the mean temperature at which individuals were unable to recover themselves (Allen et al. 2012). No crab died during the CTmax test, and the fiddler crabs were released in their natural environment after the experiment.

2.4.2 Size selection by the predator *Minuca rapax*

We tested whether the smaller size of *L. uruguayensis* in northern populations could be explained by the choice behaviour of the predator *M. rapax*. We used this species because the southern distribution of *M. rapax* overlaps with the northern distribution of *L. uruguayensis.* Given that *L. uruguayensis* has a smaller body size in northern populations, we hypothesised that *M. rapax* chooses to prey on larger individuals, a phenomenon that could promote *L. uruguayensis* populations with a smaller mean body size. The distribution of *M. rapax* is potentially associated with temperature since previous studies had shown that the distribution of other fiddler crab species, including those of the *Minuca* genus, had expanded to high latitudes due to the global temperature increases (Johnson 2014; Rosenberg 2018). Given that an enlarged fiddler crab claw can affect predator ability and/or preference (see Bildstein et al. 1989), we only used male crabs, both as prey and predator, to avoid sexual dimorphism as a source of variation in our results.

As in the previous experiments, we used two CW classes for adult *L. uruguayensis*: small size, mean ± SD of 7.07 ± 0.68 mm (n = 720); and large size, mean ± SD of 9.84 ± 0.69

mm (n = 720). A preliminary field study showed that *M. rapax* preys on other fiddler crabs with a CW from 40 to 68% of their own CW (Carvalho and Costa, unpublished data). Based on these preliminary observations, we used *M. rapax* of two CW classes: mean ± SD of 14.76 ± 1.03 mm (n = 120), for which the CW of small-sized prey corresponds to 47.56% of a predator, and mean ± SD of 21.14 ± 1.21 mm (n =120), for which the CW of large-size prey corresponds to 46.73% of the predator. If *M. rapax* always chooses prey close to its half size, the mean size of northern *L. uruguayensis* populations could not be explained by the predation effect because the predator would exert the same selective pressure on different prey size classes. The hypothesis that predators select large *L. uruguayensis* would be accepted only when *M. rapax* of both size classes choose large prey or when at least one class size chooses large prey. The experiment was conducted in individual glass containers (40 x 23 x 25 cm) containing 400 ml of saltwater (salinity = 27) that were kept under room temperature (approximately 25°C). The predators were acclimated in these containers for 48 h before the prey were introduced, and were not fed to prevent previously consumed food from interfering with the results.

The experimental design to test the chosen behaviour was based on Olabarria et al. (2002), De Grande et al. (2018) and Arakaki et al. 2020. In this approach, the choice proportion of a particular item, when it is with *n* others in a multiple-choice situation, is compared against the choice proportion of the same item among *n* possibilities, when it is in a no choice-situation. Thus, it is possible to compare one choice among diverse prey, which may have different nutritional properties, be eaten in different amounts and require different levels of a predator's energy to handle the prey (see Underwood et al. 2004).

We provided *M. rapax* individuals of each size class the following prey treatments (Fig. 1): (T1) a multiple-choice treatment, including the large and small size *L. uruguayensis* groups (i.e. one group of three large and another group of three small individuals); (T2) no-choice treatment, with two groups of three large *L. uruguayensis* only (i.e. six large individuals); (T3) another no-choice treatment, but with two groups of three small *L. uruguayensis* only (i.e. six small individuals); and (T4) another multiple-choice treatment, with small and large *L. uruguayensis* (i.e. three small and three large individuals). We offered six preys per predator during 12 h based on a pilot study we performed in which we observed that 12 h was sufficient for *M. rapax* to eat more than one *L. uruguayensis* (allowing choice possibility). Given that some

treatments demanded prey of the same size (T2 and T3), we separated them into two groups and marked the crab's abdomen with non-toxic paint (for example: for T2, the abdomen of three crabs of the reference group were painted red and the abdomen of the other three crabs of the opposite group were painted blue). To avoid any effect that the paint might cause on the palatability of the prey, all the crabs were painted with different colours in all treatments. For both *M. rapax* classes, each treatment was replicated 30 times (n = 30).

The hypothesis that *M. rapax* will choose larger *L. uruguayensis* was accepted when:

$$H: \frac{n_{L_t 1}}{N_1} > \frac{n_{L_t 2}}{N_2}, \frac{n_{S_t 3}}{N_3} > \frac{n_{S_t 4}}{N_4},$$

where in $n_{L_t i}$ or $n_{S_t i}$ is the number of prey eaten in the reference group (identified by a subscript t; Fig. 1), referring to prey sizes (*large crabs: L* or *small crabs: S*); N is the total number of consumed prey from both groups; and t = 1, 2, 3 and 4 indicates the treatment. Choice treatments were 1 and 4, and the ones without choice were 2 and 3. If *M. rapax* chose large *L. uruguayensis*, the proportion of prey eaten for the reference group L_t in T1 must have been greater than that observed for L_t^2 and S_t^3, where there was no choice and the same proportion was expected. In T4, crabs would tend to feed off large prey, a phenomenon that would decrease the proportion of small prey eaten for S_t^4. After the experiment, predators and surviving prey were released into their natural habitats.

2.5 Statistical analysis

The percentage of CW increase, the intermoult time, the time for crabs to reach the sexual maturity and the mean CW after crabs reach their sexual maturity were compared using a subset of generalised linear mixed models (GLMMs) in R, using the packages *lme4* and *glmmADMB*. The percentage CW increase was used as a response variable in gamma models with log link function.

Models of the intermoult time and time for crabs to reach sexual maturity were first performed with a Poisson distribution and link log, but counting data were over dispersed. Then, models were refitted with a negative binomial error distribution with a link log because it is appropriate to deal with overdispersion data. The size at which crabs reached the sexual maturity was compared by gamma models with log link function. In most models, temperature treatments (three levels: 20°C, 25°C and 29°C) and sex (two levels: male and female) were

used as fixed factors. We used the number of moults done by each individual nested with individual identification because the number of moults was variable across individuals, and we set them as random effects in GLMMs models. However, few crabs reached sexual maturity (N = 18), and none from the 29ºC treatment. Hence, the models of time and size at which crabs reach sexual maturity were compared using just two temperature treatments (20°C and 25ºC) and sex (two levels: male and female) as fixed factors. In these models, individual identification was fitted as a random effect.

We used a Cox proportional hazards regression to estimate the effect of temperature on crabs' survival. The models were specified in hazard rate terms, considering the daily tendency of death occurrence (i.e. according to the hazard baseline) of the individuals until they reach a survival rate of ≤50% that was deemed to be critical for success (CS). In this analysis, we included the event observations as a response variable (individuals that died until ≤50% CS during the experiments) and censored observations (individuals that died after the CS percentage >50% or survived at the end of the experiment, i.e. reach sexual maturity). We used the temperature treatments (three levels: 20°C, 25°C and 29ºC) and sex (two levels: male and female) as factors in this analysis. Model assumptions were examined using the R function 'cox.zph' (*survival* package), and the variance inflation factors (i.e. collinearity) were assessed using the 'vif' function (*car* package) and were < 2, indicating no collinearity. We also used likelihood ratio tests (LRTs) to assess the effect of each variable by backward elimination of factors. Cox regressions were applied using the *survival* package.

Males' oxygen consumption was compared among temperature treatments using GLMMs with gamma distribution with a log link function. We used the male oxygen consumption (variation between the initial minus the final O_2 concentration corrected against the control) of each male as the response variable. Temperature treatments (three levels: 20, 25 and 29ºC) were fitted as a fixed factor. Males' identification was fitted as a random effect.

A Student's T-test was used to compare the CTmax between large and small male *L. uruguayensis* fiddler crabs, after confirming normality and data homogeneity of variances with Shapiro-Wilk and Levene's tests, respectively.

For the predation experiment, the proportional number of *L. uruguayensis* males consumed by large or small *M. rapax* was evaluated using GLMM binomial models with a logit

link function. We used as response variable the proportion data of one focal crabs' group (*L. uruguayensis* from $n_{L_t i}$ or $n_{S_t i}$) eaten in relation to the total number of consumed crabs by a given predator (*N*) according to the two-choice treatments. Models were evaluated separately for each class of predators – large and small *M. rapax*. The models were performed with treatments (four levels: T1, T2, T3, and T4) as fixed effects and container glass identification as a random effect.

In all GLMM analyses, we performed model comparisons for each response variable, assessing fixed factor significance by comparing simpler models (i.e. with only intercept and random effects) with more complex models (i.e. one or two fixed factors and random effects), using LRTs. When necessary, significant differences among levels within fixed factors were performed via the *multcomp* package with Tukey adjustment for multiple comparisons. We considered 0.05 as the significance level in all analyses.

3. Results

3.1 Size-at-age shift

3.1.1 Growth, sexual maturity and survivorship of *L. uruguayensis* at different temperatures

Temperature treatments had a significant effect on the fiddler crabs' CW growth (GLMM, X_2^2 = 12.827, *P* = 0.001). At 20°C, the mean fiddler crab growth was 9.62% by moult, which differed from growth at 25°C (*Z* = 2.501, *P* = 0.01, adjusted *P* value, Tukey method) and 29°C (*Z* = 3.644, *P* = 0.0008), where the mean growth was 7.52% and 5.93%, respectively. However, there was no difference between 25 and 29°C (*Z* = 1.417, *P* = 0.15, Fig. 2A). Sex (GLMM, X_1^2 = 3.639, *P* = 0.05) and the interaction between sex and temperature (GLMM, X_2^2 = 4.918, *P* = 0.08) had no effect on CW growth. The average intermoult time also differed among the temperature treatments (GLMM, X_2^2 = 37.600, *P* = 0.0001), regardless of sex (GLMM, X_1^2 = 0.434, *P* = 0.51) and the sex and temperature treatment interaction (GLMM, X_2^2 = 4.754, *P* = 0.09). For the 20°C treatment, *L. uruguayensis* spent on average more time in the intermoult period (approximately 132 days) compared to 25°C (*Z* = 3.122, *P* = 0.004) and 29°C (*Z* = 6.593, *P* = 0.001), in which crabs spent a mean of 83 and 66 days, respectively. There was no difference between 25 and 29ºC (*Z* = 2.184, *P* = 0.06, Fig. 2B).

The size and time at which *L. uruguayensis* reached the onset of sexual maturity differed among temperatures, regardless of sex (Table 1). On average, the crabs made three moults until they reach the onset of sexual maturity. At 20°C, the crabs reached a larger CW (mean ± SD = 6.63 ± 0.28 mm) and required 1.58 more time (mean ± SD = 448.83 ± 108.84 days) to reach morphological sexual maturity compared to 25°C (CW = 6.18 ± 0.43 mm; time = 283.91 ± 117.09 days). At 20°C, 2 males and 4 females reached the onset of sexual maturity, while at 25°C, 5 males and 7 females reached the onset of sexual maturity. No crab reached sexual maturity at 29°C. The Cox proportional hazard model showed that was a significant treatment—but not sex—effect on *L. uruguayensis* survivorship (Table 2). The increase in temperature had a strong negative effect on critical success (≤50%) in individuals conditioned at 29°C (Fig. 3). As temperature decreased, the risk of death was reduced by 0.30 at 25°C and 0.29 at 20°C with respect to the critical limit when compared to 29°C (Table 2). At 29°C, *L. uruguayensis* reached critical success in approximately 110 days, while in 25°C and 20°C, critical success was reached in approximately 275 and 365 days, respectively.

3.1.2 Oxygen consumption

The oxygen consumption of *L. uruguayensis* males differed among treatments (GLMM, $X_2^2 = 5.999$, $P = 0.04$). At 29°C, oxygen uptake of juvenile males was 1.00 µg O_2 mg^{-1} dry weight h^{-1}, significantly higher compared at 20°C, in which oxygen consumption was 0.33 µg O_2 mg^{-1} dry weight h^{-1} ($Z = 2.542$, $P = 0.03$, adjusted P value, Tukey method, Fig. 4). At 25°C, oxygen consumption was 0.48 µg O_2 mg^{-1} dry weight h^{-1}. We did not observe significant differences in oxygen consumption between 20°C and 25°C ($Z = -0.846$, $P = 0.39$), or between 25°C and 29°C ($Z = 1.697$, $P = 0.13$).

3.2 Structure shift

3.2.1 Size selection by temperature

The temperature at which *L. uruguayensis* adults lost their motor coordination did not differ between the size-class treatments (Paired *t* test, $t20 = -1.4063$, $P = 0.17$). The CTmax (mean ± SD) for large and small males was 42.76 ± 0.39°C and 43.04 ± 0.49°C, respectively.

3.2.2 Size selection by the predator *M. rapax*

The larger class of *M. rapax* chose to prey on larger *L. uruguayensis* (GLMM, X_3^2 = 12. 228, *P* = 0.006). The *post hoc* comparison showed that the proportion of large *L. uruguayensis* preyed upon in the two-choice treatment T1 (large-small crabs) was higher compared to the T4 treatment (small-larger crabs, *Z* = 3.398, *P* = 0.0034, Fig. 5A). There was no difference in choice among the other treatments (Table 3). The smaller class of *M. rapax* preyed equally on both size classes of *L. uruguayensis* (GLMM, X_3^2 = 1.541, *P* = 0.67).The proportion of consumed *L. uruguayensis* of each size class did not differ among the treatments of these last experiments (Fig. 5B)

4. Discussion

The temperature–size rule is widely described throughout wild populations from different taxa; it refers to a positive relationship between body size and latitude (Atkinson 1994; Angilletta et al. 2002; Kingsolver and Huey 2008; Gardner et al. 2011; Ohlberger 2013). In fiddler crabs, the phenomenon has been reported for *Minuca pugnax* (Johnson et al. 2019), *Leptuca pugilator* (Darnell and Danell 2018) and *L. uruguayensis* (Masunari et al. 2017; Hirose et al. 2013). Based on the current study, the smaller size of *L. uruguayensis* from the northern population of its distributional range can be explained as a phenotypic response to the air temperature and a result of differential survivorship between age-classes in response to predator choice. Therefore, the latitudinal cline in *L. uruguayensis* body size can be framed in both size-at-age and structure shift hypothesis. Following the first hypothesis, *L. uruguayensis* grow less and reach sexual maturity earlier at higher temperatures due to the increase in their metabolic costs. Yet, according to the structure shift hypothesis, the predator *M. rapax* can negatively select the larger sizes of *L. uruguayensis*. This eventuality could mean a decrease in average body size in prey populations where both species share territory. Thus, rising temperatures due to climate change are expected to cause a decrease in the average size in southern populations of *L. uruguayensis* by impairing their growth and/or allowing their predator to expand southward.

In our study, we showed that *L. uruguayensis* grows less and faster in warmer air temperatures. *Leptuca uruguayensis* shows a gradual response in ontogenetic development due to temperature. First, crabs have their growth impaired at warm intermediate temperatures

(25°C); then, at extremely high temperatures (29°C), besides the impaired growth, there is also the loss of reproductive capacity. Similar gradual responses are known for other crustaceans, including amphipods (Pöckl 1992), lobsters (Anger 1984) and other brachyuran crabs (Azra et al. 2020). Further, at 29°C, *L. uruguayensis* males and females showed the worst survival probability, falling below the critical level of 50% two times faster than the survival performance at 25°C and three times faster than at 20°C. Corroborating these results, *L. uruguayensis* oxygen consumption at 29°C increased two times compared to 25°C, and three times compared to 20°C. Thus, the growth, time to sexual maturity onset and survival performance of *L. uruguayensis* might be impaired by metabolic costs imposed by high temperatures and a consequent decrease in mean body size.

Our findings indicate that temperatures of 20°C and 25°C were still within the limits in which *L. uruguayensis* could keep their energy needs above a critical level and be able to reach sexual maturity. However, the metabolic increase at 25°C over a long period of time may impose an energetic cost for *L. uruguayensis*, a phenomenon that results in fast growth and small body size. An average temperature of 29°C may be considered as infeasible for the long-term establishment of *L. uruguayensis* populations. Similar to our study, large adult *L. uruguayensis* males (CW ≈ 9.7 mm) of a population also from São Paulo State had higher oxygen consumption at 27°C and 31°C, when compared to the sympatric and phylogenetically close species *L. leptodactyla* (Vianna et al. 2020).The authors suggested that higher oxygen consumption of *L. uruguayensis* may reflect the physiological challenge imposed by warmer temperatures in their northern distributional limits. The rise in oxygen consumption may be interpreted as a compensatory response to the increased energy demands for cellular respiration at high temperatures (Pörtner 2010). When *L. uruguayensis* crabs are exposed to high temperatures, they compensate for their metabolic demand by increasing oxygen uptake, allowing them to live and grow for a limited period without reaching sexual maturity. In this sense, mean temperatures above 29°C may be a critical upper threshold for *L. uruguayensis* survival and might act as the factor limiting their northern latitudinal distribution.

Extreme temperatures did not act directly on the selection of the fiddler crab size class. *Leptuca uruguayensis* males of different sizes showed the same thermal limit, data that refute our initial hypothesis in which high temperatures could select against individuals with a large

body size at the species' northernmost distribution limits. In our study, *L. uruguayensis* showed a CTmax within the range described in previous studies for thermal limits of other fiddler crab species, which range from 40°C to 43°C (see Allen et al. 2012; Munguia et al. 2017; Darnell and Darnell 2018).

The northern distribution limit of *L. uruguayensis* (Rio de Janeiro) coincides with the boundary of two major biogeographic ecoregions, the Tropical Atlantic and Temperate South America marine ecoregions (Spalding et al. 2007). Due to the change in temperature and rain regime, this climatic transitional zone has been reported as a boundary for several species, such as the nesting of loggerhead *Caretta caretta* (Montero et al. 2019) and the mangrove tree *Conocarpus erectus* (Ximenes et al. 2016). At the northern latitudinal limit of *L. uruguayensis*, in Rio de Janeiro (22°S), the annual mean temperature is approximately 23°C (Ximenes et al. 2016), but during summer the temperature commonly increases above 35°C (Marques Filho et al. 2016). Corroborating our results, the mean temperatures experienced by *L. uruguayensis* in Rio de Janeiro (< 25°C) may allow them to reach sexual maturity, and the maximum temperatures are below their thermal limit (< 43.0°C). Air temperatures are higher beyond the northern distribution limit of *L. uruguayensis* (Dereczynski et al. 2019). Studying the biology of the crab *Ucides cordatus* in mangroves from Vitória Bay, Brazil (20°S), Goes et al. (2010) observed air temperatures above 29°C over four consecutive months, conditions that could represent sufficient time for *L. uruguayensis* survival to reach a critical level (based on the current study). Therefore, high average temperatures in latitudes lower than 22°S may limit the northern distribution of *L. uruguayensis*.

In addition to temperature effects, the presence of the predator *M. rapax* could contribute to the small body size of *L. uruguayensis* in northern populations. Predator preferences for a particular size class can affect the average body size throughout prey populations (Boulding et al. 2017; Selden et al. 2017). In our choice experiments, large *M. rapax* chose to prey on large *L. uruguayensis*, while small *M. rapax* did not select prey size to eat. Large *L. uruguayensis* in north populations, where their distributional range overlaps with that of *M. rapax*, are under negative predator pressure, a phenomenon that could explain the smaller average body size. Further studies should evaluate whether the predation rate of *M. rapax* is strong enough to affect the size class distribution of *L. uruguayensis* in wild populations.

From 1970 to 2010, temperatures along the southeastern and southern coast of Brazil increased approximately 0.24°C and 0.15°C, respectively, per decade (Bernardino et al. 2015). Considering our findings, we expect that future warming due to climate change will decrease body size in southern *L. uruguayensis* populations, as well as shift their distribution range by a decline of northern populations where average air temperatures exceed 29°C. We could also expect the same effect for other fiddler crab species if their metabolic costs increase with temperature. Decreasing the mean body size of mature female crabs may affect the species fitness because body size is among of the factors limiting fertility (Azra et al. 2020). Once organism's size influences its impact on ecosystem, and fiddler crabs are ecosystem engineers that affects intertidal environments functioning, the smaller mean sizes of the fiddler crabs in low latitude could decrease their bioturbation per-capita impacts (Johnson et al. 2019). Future studies could help clarify these issues. Given that global warming has caused a poleward range expansion in fiddler crabs (Johnson 2014; Rosenberg 2018), it is expected that *M. rapax* will reach the southern populations of *L. uruguayensis*, an eventuality that might contribute to the decrease in the mean body size of the latter. Both species studied here should be continuously monitored in future studies to predict changes in their body size and distribution, as well as the results of these changes in natural environments.

5. Acknowledgments

6. References

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Table 1 Generalised linear mixed model (GLMM) comparisons of time (upper portion) and size (lower portion) in which the fiddler crabs *Leptuca uruguayensis* reached morphological sexual maturity, using log-likelihood ratio tests

Comparison	GLMMs Models	Log-likelihood	df	Models compared	X^2	P
Time until reach the onset sexual maturity						
Temperature and sex effects	M1: with temperature as fixed effect and ID as random effect	-114.50	1	M1 *x* M3	3.848	**0.04**
	M2: with sex as fixed effect and ID as random effect	-115.93	1	M2 *x* M3	0.990	0.31
	M3: with intercept only and ID as random effect	-116.43	1			
Temperature *x* sex interactions	M4: with temperature and sex interaction (temperature *x* sex) and ID as random effect	-112.84	1	M4 *x* M5	1.820	0.17
	M5: with temperature + sex effects and ID as random effect	-113.75	1			
Size at the onset sexual maturity						
Temperature and sex effects	M6: with temperature as fixed effect and ID as random effect	-3.17	1	M6 *x* M8	10.556	**0.001**
	M7: with sex as fixed effect and ID as random effect	-7.55	1	M7 *x* M8	1.793	0.18
	M8: with intercept only and ID as random effect	-8.44	1			
Temperature *x* sex interactions	M9: with temperature and sex interaction (temperature *x* sex) and ID as random	-1.46	1	M9 *x* M10	2.365	0.12

effect			
M10: with temperature + sex effects and ID as random effect	-2.64	1	

Table 2 Output of the Cox proportional hazards model to investigate the temperature and sex effects on *Leptuca uruguayensis* survivorship until critical success

Factors	Hazard ratio	95% CI	z Wald statistic	P>(|z|)	LRT (X^2)	P
Temperature at 29ºC[a]	NS	–	–	–		
Temperature at 25ºC	0.30	0.16 – 0.56	-3.737	**0.001**	16.902	**0.001**
Temperature at 20ºC	0.29	0.15 – 0.55	-3.800	**0.001**		
Sex – male[b]	0.97	0.62 – 1.50	-0.140	0.88	0.019	0.88

[a]Reference level is temperature at 29ºC

[b]Reference level is female

Table 3 Pairwise comparison between the sequential order groups offered to larger *Minuca rapax* predators. Larger males of the prey *Leptuca uruguayensis* are represented by a capital 'L' and small males by capital 'S'

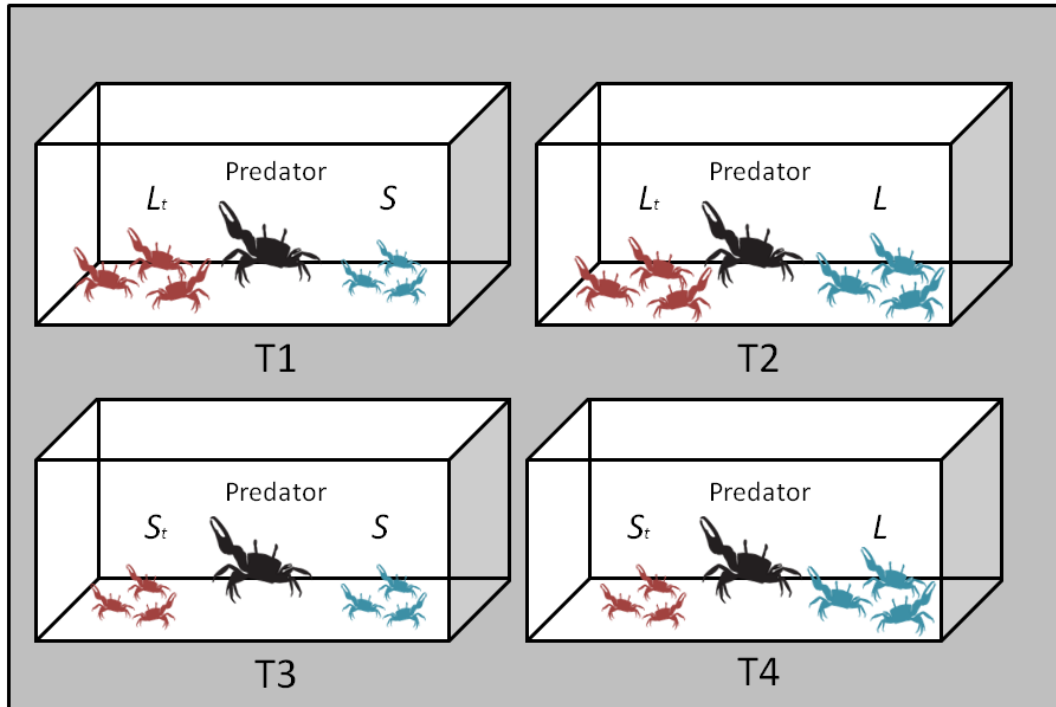| Groups	Estimate	Std. Error	Z value	P
Large predator Tukey post-hoc comparison				
LS - LL	0.3310	0.2860	1.158	0.65
SL - LL	-0.6558	0.2984	-2.198	0.12
SS - LL	-0.1508	0.2872	-0.525	0.95
SL - LS	-0.9869	0.2904	-3.398	**0.003**
SS - LS	-0.4818	0.2788	-1.728	0.30
SS - SL	0.5051	0.2916	1.732	0.30

Figures

Fig. 1. Experimental design. Four treatments (T1-T4) used to evaluate the choice proportion of the predator fiddler crab *Minuca rapax* (black crabs) for the prey *Leptuca uruguayensis* of two class sizes: large prey (L) and small prey (S). Choice (proportion > 0.5) for a reference group "t" (red crabs) was expected in T1 when the reference group is composed of large *L. uruguayensis* and the opposite group (blue crabs) is composed of small *L. uruguayensis*. No choice treatments (T2 and T3) were composed of *L. uruguayensis* of the same class sizes, so was expected same choice proportion (0.5) between the reference group (red crabs) and the opposite group (blue crabs). Avoidance (proportion < 0.5) is expected in T4 when the reference group (red crabs) was composed of small *L. uruguayensis* and the opposite group (blue crabs) was composed of large *L. uruguayensis*.
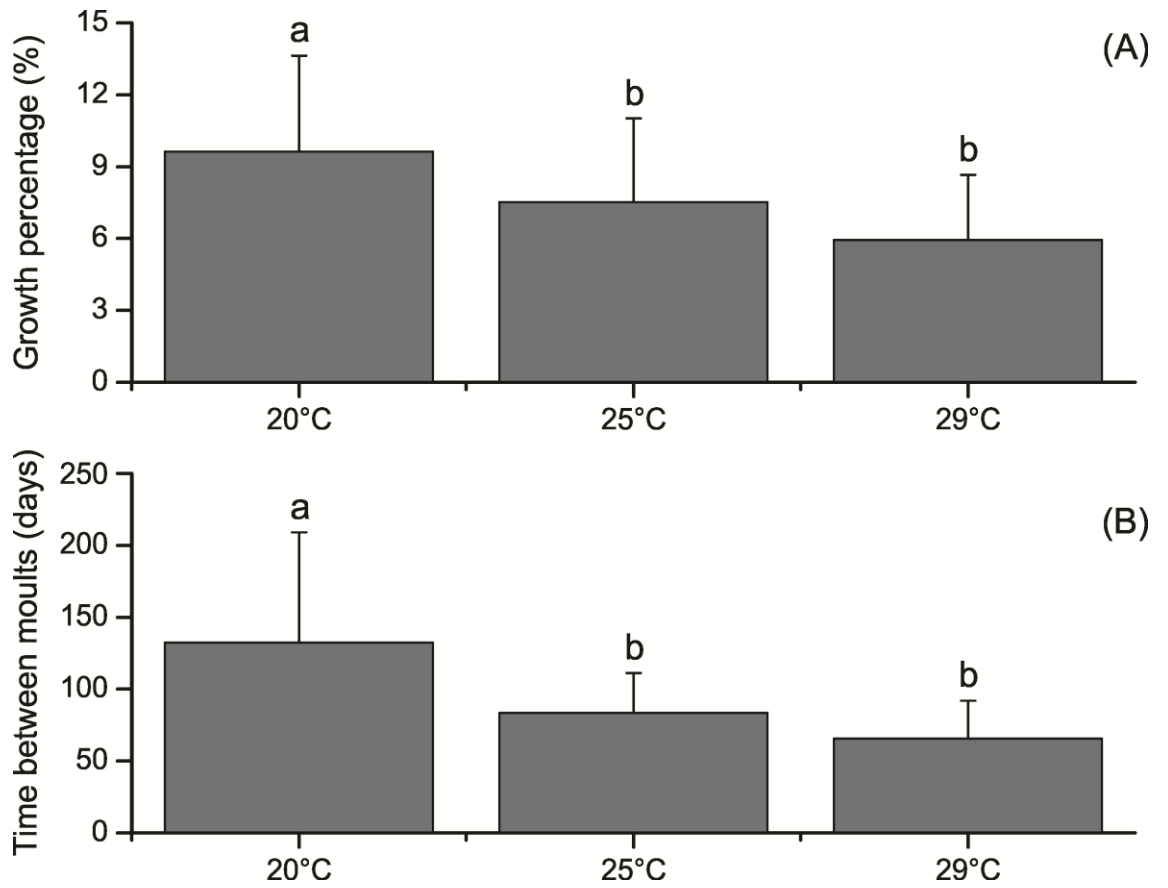
Fig. 2. Growth percentage and intermoult time of the fiddler crab *Leptuca uruguayensis* at different temperatures. (A) Average percentage and standard error increase per moult in carapace width of male and female *L. uruguayensis* at 20, 25 and 29°C; (B) average time and standard error between the moults of male and female *L. uruguayensis at* 20, 25 and 29°C. Different lower case letters indicate significant statistical differences between treatments.
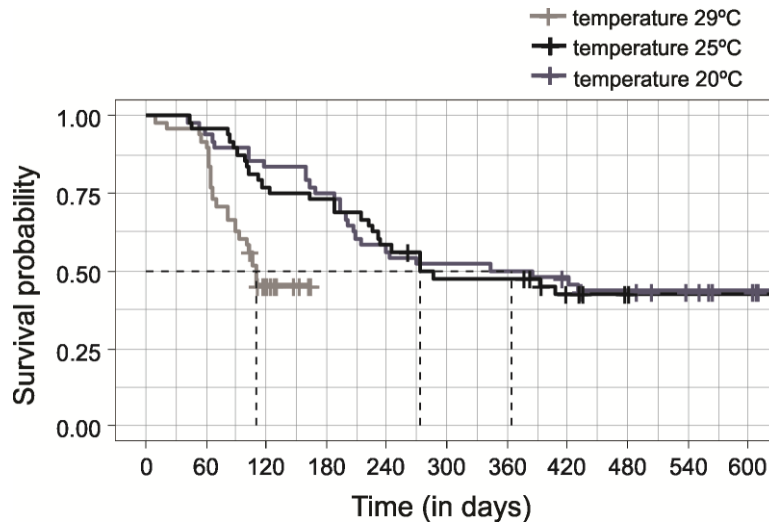
Fig. 3. Survivor probability of the fiddler crab *Leptuca uruguayensis* over time at the experimental temperatures. The survival probability of the crabs was estimated until the critical population level of 50% (dotted line) at 20, 25 and 29°C.
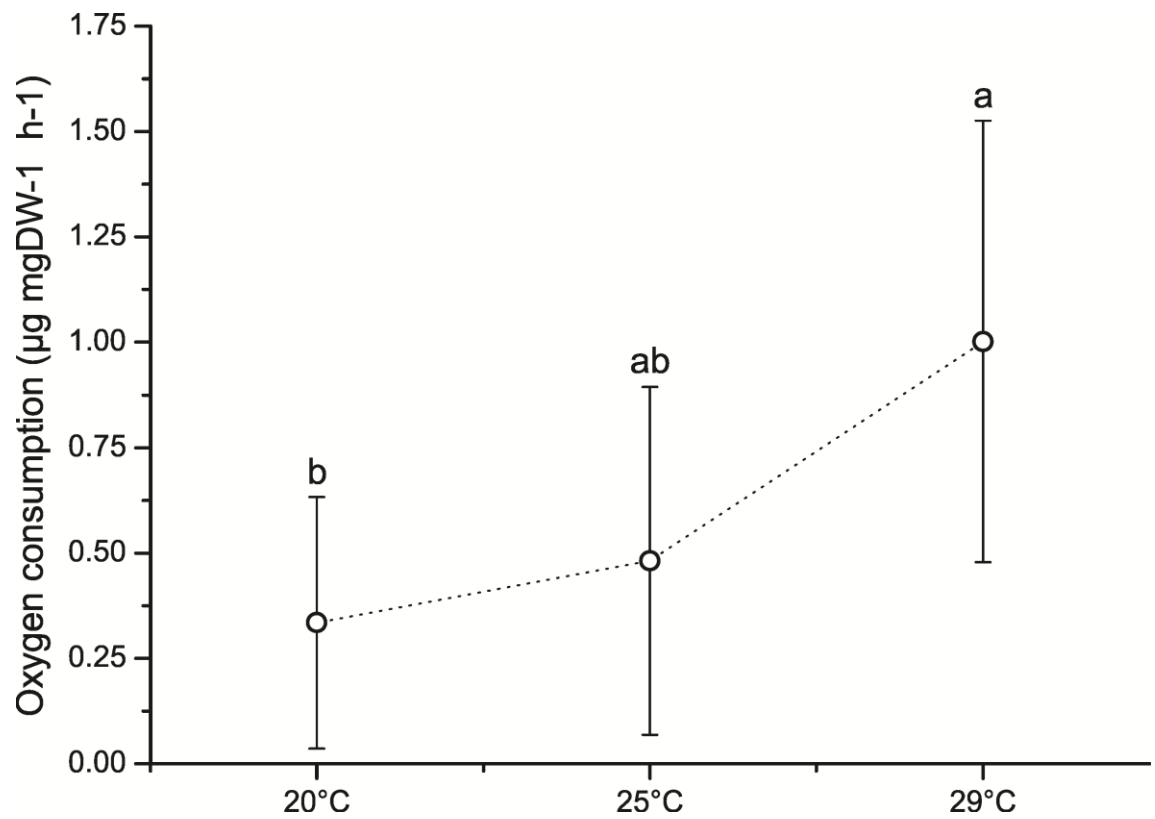
Fig. 4. Oxygen consumption of male juvenile fiddler crabs (*Leptuca uruguayensis*) at different temperatures. The average µg O_2 mg^{-1} dry weight h^{-1} was evaluated among the experimental temperatures of 20, 25 and 29°C. Different lower case letters indicate significant statistical differences between treatments.
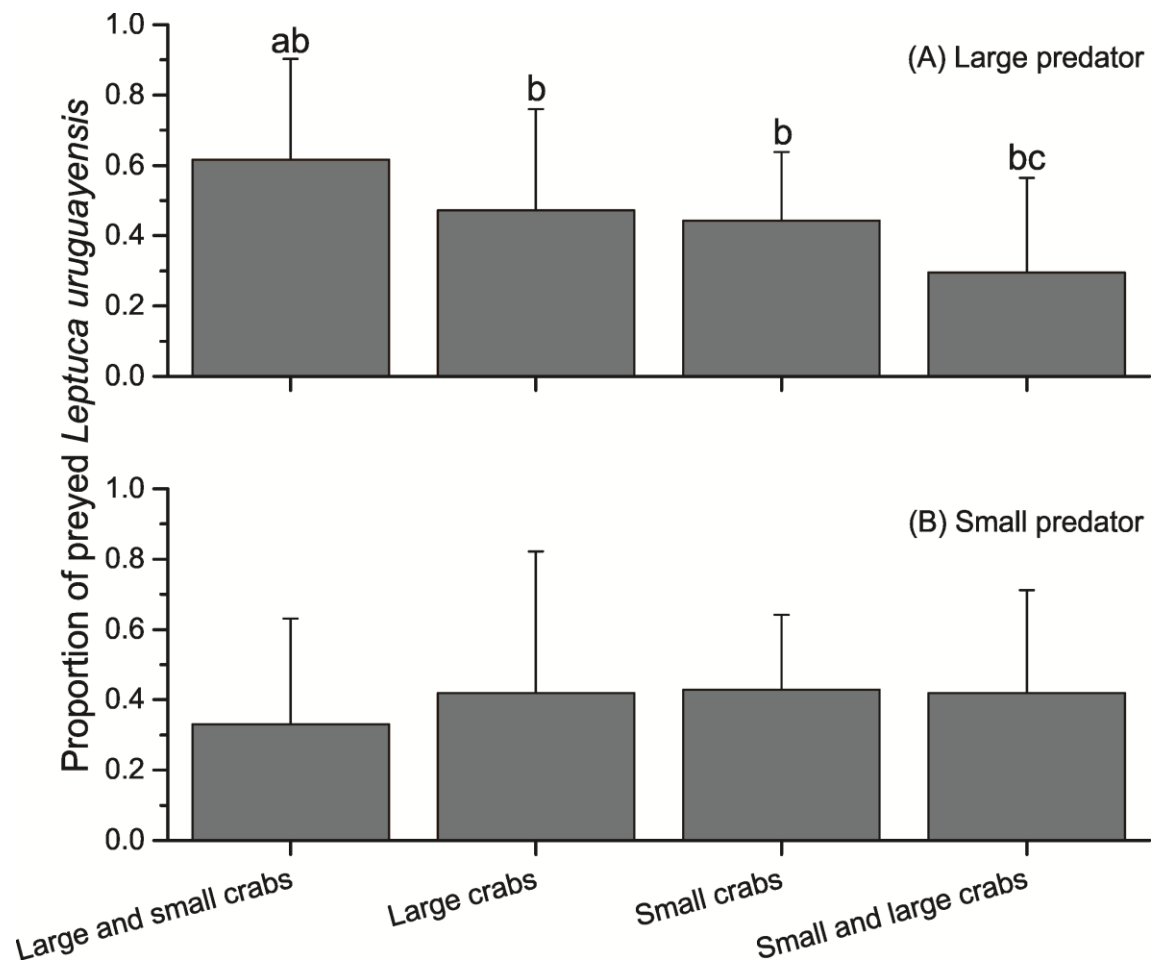
Fig. 5. The choice proportion of the predator fiddler crab *Minuca rapax* for the prey *Leptuca uruguayensis* of two class sizes of carapace width (small prey: 7.07 ± 0.68 mm; large prey: 9.84 ± 0.69 mm). (A) The proportion of *L. uruguayensis* preyed upon by large *M. rapax* (carapace width: 21.14 mm); (B) the proportion of *L. uruguayensis* preyed upon by small *M. rapax* (carapace width: 14.76 mm). Different lower case letters indicate significant statistical differences between treatments.

Graphical Abstract

Highlights

- *Leptuca uruguayensis* grow less and in warmer temperatures.

- *Leptuca uruguayensis* reach sexual maturity earlier in warmer temperatures.

- High temperatures reduce the survival of *L. uruguayensis*.

- The predator *Minuca rapax* chose a large *L. uruguayensis* size.

Capítulo 3

Losers can win: Thermoregulatory advantages of regenerated claws of fiddler crab males for establishment in warmer microhabitats

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Abstract

Fiddler crab males present a hypertrophied claw, which is used for sexual and aggressive displays, fights with competitors, and has been proposed as an important thermoregulatory organ for heat control. Two claw morphologies can be observed within fiddler crab populations: brachychelous claws (unregenerate) and leptochelous claws (regenerated). The leptochelous morphotype presents less muscle mass and longer fingers, resulting in a less advantageous weapon in fights. Considering their slender shape, we hypothesized that the leptochelous morphotype would present lower thermal inertia and be more efficient at body heat dissipation. We evaluated the role of the fiddler crabs' claw shape as a heat sink and how this influences their distribution between unshaded and shaded microhabitats. We tested in the field whether the proportion of *Leptuca uruguayensis* males with leptochelous claws was higher in unshaded microhabitats than shaded ones. In the laboratory, we tested if heat transfer between the body and claw is higher in leptochelous males than in brachychelous. The wave behaviour between both morphotypes was compared in the field during the hottest period of the day. *L. uruguayensis* with regenerated claws comprised more than 60% of the male's population of unshaded microhabitats compared to 18% in shaded microhabitats. Leptochelous males showed a mean heat transfer between body to claw 35% higher than that observed in brachychelous males. During the hottest period of the day, brachychelous males waved approximately 30% more than leptochelous males. Therefore, regenerated claws may be advantageous for the establishment of *L. uruguayensis* males in warmer and unshaded microhabitats because they are more efficient for heat loss and allow crabs to cool down, spending less time waving. Our study shows the relevant context of winners and losers in the face of climate change and highlights the importance of morphological variations in thermoregulatory structures for the occupation of thermal niches.

Keywords: claw shape; thermoregulatory organs; thermal niches; differential distribution; climate change.

1. **Introduction**

Climate change, especially global warming, has been considered one of the main contemporary forces acting on adaptive changes in wildlife (Walther et al., 2002; Parmesan and Yohe, 2003; Richardson, 2008; Daufresne et al., 2009; Dyderski et al., 2018; Price et al., 2019). Organisms must provide mechanisms that allow them not to exceed their thermal limits in extreme edge environments (Marshall et al., 2010; Fusi et al., 2015; Munguia et al., 2017). In response to temperature increase, organisms can shift their geographical distribution boundaries to new places or restrict their occupation to suitable microhabitats available in their original habitats (Richardson, 2008; Pöyry et al., 2009; Turlure et al., 2010; Chen et al., 2011; Osland et al., 2019). When organisms persist in their original habitats, behavioural, physiological, and morphological responses are important to maintain their thermal optimum and ensure maximum performance (Angilletta et al., 2002; Fusi et al., 2015; Munguia et al., 2017). Despite this, little attention has been given to the morphological variation of organisms and the consequence for thermal regulation (Gardner et al., 2011). Morphological variations in thermoregulatory structures, like horns, bills, or claws, may be important for predictions about a populations' resilience to global warming (Gardner et al., 2011).

Intertidal organisms have been proposed as early indicators of global warming because they have changed their distributional boundaries and advanced toward higher latitudes faster than most recorded territorial shifts in terrestrial species (Helmuth et al., 2006; Whitfield et al., 2016). Intertidal ectotherms are particularly sensitive to climate change because many of them live close to their physiological limits (Somero, 2010, 2002; Lima et al., 2016). The population changes observed in intertidal organisms (i.e. variation in distribution range, habitat occupation, size, or shape) could be an early indicator of climate change impacts. For example, Ortega et al. (2016) showed a decrease in abundance and individual size and an increase in body abnormalities of the yellow clam *Mesodesma mactroides* in Uruguay related to sea warming.

Fiddler crabs are intertidal organisms present in all tropical and subtropical coastal regions of the world, which makes them a good model to assess the impact of rising temperatures on natural populations. For example, the latitudinal distribution of *Minuca pugnax* has been displaced 80 km to the north due to the temperature increase of 1.3°C from 2012 to 2013 (Johnson, 2014). Another range-expanding fiddler crab is *Uca princeps*, which was originally found from Peru to Mexico and now can be found in the state of California, United

States (Rosenberg, 2018). In tropical regions, fiddler crabs experience temperatures higher than their thermal limit and behaviours, such as the use of burrows or the selection of microhabitats, have been demonstrated as adaptive strategies to manage with the temperature rise (Munguia et al., 2017; Chou et al., 2019; Darnell et al., 2019). Within the intraspecific level, fiddler crabs present a clinal latitudinal-gradient of carapace size, in which low latitude populations present smaller individuals due to a rising temperature gradient (Hirose et al., 2013; Masunari et al., 2017; Azevedo et al., 2018; Darnell and Darnell, 2018).

Fiddler crabs have pronounced sexual dimorphism, in which females possess two small and symmetrical claws used for feeding and males have a small feeding claw and other large hypertrophied claw (Rosenberg, 2002). Males use the hypertrophied claw in reproductive and agonistic displays, as well as in fights for territory defense (Jennions and Backwell, 1998; Backwell et al., 2000; Booksmythe et al., 2010). The hypertrophied claw is also a thermoregulatory organ that functions as a heat sink, transferring heat from the body to the claw and dissipating it to the air (Windsor et al., 2005; Darnell and Munguia, 2011). Claw size can also explain more than 10% of body temperature variations in males (Munguia et al., 2017). At this point, claw size has been proposed as an important factor in intraspecific distribution due to its effect on thermal microhabitat selection or the period of activity of males (Munguia et al., 2017; Chou et al., 2019; Darnell et al., 2019).

In some species of fiddler crabs, claws may also vary in shape on an intraspecific level (Rosenberg, 2002; Masunari et al., 2017). Male fiddler crabs, like other crustaceans, can suffer autotomy of their claws and legs during agonistic interactions with other males or when escaping from predators (Hopkins, 1993; Backwell et al., 2000). When males lose their hypertrophied claws, the new claw, known as leptochelous claw, regenerates with a different shape compared to non-regenerated brachychelous claws (Backwell et al., 2000; Figure 1). Leptochelous shape can occur at high frequencies within the population, accounting for 44% of males of *Austruca annulipes* (Backwell et al., 2000). A leptochelous shape is less robust than brachychelous due to less muscle mass, thinner dactilus and pollex, longer length, and lack of teeth (Backwell et al., 2000; Rosenberg, 2002). Leptochelous claws may be a less advantageous weapon in fights against males with brachychelous claws (Backwell et al., 2000, Lailvaux et al., 2009). However, the brachychelous shape has a high energy cost to males and

can consume 43% more oxygen than regenerated claws in *Gelasimus vomeris* (Bywater et al., 2014).

Since the leptochelous shape has less mass and less robustness, we hypothesized that it could present lower thermal inertia and consequently more efficiency in body heat loss than non-regenerated claws (brachychelous). Leptochelous claws could be advantageous for crabs that endure the hottest day periods or occupying warmer and exposed habitats (i.e. unshaded areas), increasing their activity time above the surface and enhancing their chance to find a mate. We have observed that fiddler crabs' claws are larger in unshaded microhabitats than in shaded ones (personal observation). Thus, it would be reasonable to suppose that the phenotypic plasticity of claws could be associated with the temperature difference between microhabitats and therefore, warmer areas could favor the establishment of fiddler crabs with regenerated claws.

This study aimed to evaluate the thermal responses between two claw-shapes of the fiddler crab *Leptuca uruguayensis* and the consequence of this morphological heterogeneity between populations from different mangrove microhabitats. We tested the following hypothesis: (1) The proportion of males with leptochelous claws is higher in unshaded than shaded mangrove microhabitats; (2) Heat transfer from the body to the claw is higher in fiddler crabs with leptochelous claws; and (3) Wave activity is higher in males with brachychelous claws than leptochelous during the hottest day period.

2. Material and Methods

2.1 Variation in claw shape between mangrove microhabitats

Sampling was conducted in Santos/São Vicente Estuary, city of Praia Grande, São Paulo State, southeastern coast of Brazil. The mangrove forest in this estuary system is mainly composed of *Rhizophora mangle* Linnaeus (1753), *Laguncularia racemosa* (L.) Gaertn (1807) and *Avicennia schaueriana* Stapf & Leechm (Cordeiro and Costa, 2010). Throughout the mangrove ecosystem forest, clearance can be found featuring a mosaic of non-vegetated and vegetated microhabitats in which fiddler crabs live. *Leptuca uruguayensis* inhabits non-vegetated and vegetated sandbanks, sharing territory with *L. leptodactyla, L. cumulanta, L.*

thayeri, and *Minuca rapax* (Machado et al., 2013; Checon and Costa, 2017; De Grande et al., 2018).

We tested whether the proportion of leptochelous claws in males of *L. uruguayensis* varied between unshaded (non-vegetated) and shaded (vegetated) mangrove microhabitats. Four sites along one *L. uruguayenis* population were sampled: two in unshaded microhabitats (site 1: 23°59'12.05'' S - 46°24'11.49'' W and site 2: 23°59'16.54'' S - 46°24'29.38'' W) and two in shaded microhabitats (site 3: 23°59'12.70'' S - 46°24'14.09'' W and site 4: 23°59'16.43'' S - 46°24'28.49'' W). Each site measured approximately 10 m² and the distance between sites varied as follows: sites 1 and 3 were 20 meters apart from each other and sites 2 and 4 were 50 meters away from each other. Sites 1 and 3 were approximately 500 meters away from sites 2 and 4. The temperature (°C) and luminous incidence (Lux) was recorded on 75 random points of sediment surface to characterize each site. Each site was sampled during low tide, from 10 am to 12 am, 15 times per sampled day, on 5 random days from October to November of 2019. Temperatures and luminous incidence were recorded using a thermocouple coupled to a digital thermometer (Lutron[TM], TM-947SD) and a digital light meter (Lutron[TM], LM-800A).

Each site was sampled using five random squares (50 x 50 cm), from the inside of which we captured all the fiddler crabs by digging their burrows with a garden shovel. Fiddler crabs were identified at the species level and the number of *L. uruguayensis* males and females used to calculate the species density in the respective areas. The males of *L. uruguayensis* per square were caught and taken to the laboratory for further analysis while other crabs were released into the wild. In the laboratory, the number of males with leptochelous and brachychelous morphotype per square was used to calculate the proportion of males with regenerated claws by subpopulation. The carapace width and propodus length of *L. uruguayensis* males were measured to estimate crab size (carapace size) and relative claw size (ratio between claw length and carapace width). To estimate the proportion of claw mass in relation to the total body mass of crabs (relative claw weight), we sacrificed the males by freezing. Then, their claws were carefully removed, separating the propodus from the carpus. The claws and bodies were dried for 48 hours at 60°C and then their dry weight was measured.

2.2 Heat transfer between body to claw in leptochelous and brachychelous males

We tested whether heat loss in adult males of *L. uruguayensis* with leptochelous claws was higher than in males with brachychelous claws. Crabs with a carapace width between 10 and 12 mm were collected in unshaded microhabitat during spring low tides, in Santos/São Vicente Estuary, site 2 (see section 2.1) and taken to the laboratory. They were individualized and housed in containers (4.0 cm diameter) with 10 ml of saltwater (salt 30), where they were kept at room temperature (25°C) for 48 hours. Crabs were fed with crushed feed for marine fish Poytara® (this diet is commonly used for fiddler crabs under laboratory conditions; see Allen et al. 2012; Darnell et al. 2015). Following Windsor et al. (2005), we set an experiment to evaluate heat transfer from body to claw, immersing the males' claws in an ice bath and monitoring their body temperature. The experiment was composed of: 1) a treatment group of fiddler crabs males with leptochelous claws (n = 5); 2) a treatment group of males with brachychelous claws (n = 5); 3) a control group of males with leptochelous claws (n = 5); 4) a control group of males with brachychelous claws (n = 5). We restrained individual crabs on a wood rod using elastic-plastic tie-downs (see Windsor et al., 2005). After 5 minutes for acclimation, we monitored the temperatures over a 30 minute trial by recording the males' temperature using a thermocouple coupled to a digital thermometer (Lutron^{TM}, TM-947SD), which was inserted inside the carapace in the cardiac region (Windsor et al., 2005; Hui et al., 2019). Another thermocouple was installed approximately 3 cm away from the body and used to register the environmental air temperature. During the initial 15 minutes, males were exposed to room temperature of 24.87 ± 0.41°C (mean ± standard deviation) and we recorded their body and air temperature in the last 5 minutes, every 2 seconds, using a digital data logger coupled to the digital thermometer. Then, the males' claws of treatment groups were immersed in the ice bath at a temperature of 1.16 ± 0.24°C for another 15 minutes and their body and air temperature were measured again every 2 seconds in the last 5 minutes. For the control groups, crabs were exposed to room temperature for another 15 minutes and their temperature was recorded for the last 5 minutes. The relative claw size was estimated using carapace width and propodus length, as previously measured. We used the same procedure explained in section 2.1 to estimate the relative claw weight.

2.3 Wave activity between males with brachychelous and leptochelous claws

In the field, we tested whether males of *L. uruguayensis* with leptochelous claws (n = 15) expend less time in wave activity during the hottest period of the day than males with brachychelous claws (n = 24). The time expended outside of the burrow was also evaluated between the crab's morphotypes. The males were observed during spring low tides, from 10 am to 12 am, in Santos/São Vicente Estuary, in an unshaded microhabitat (site 2 - see section 2.1). Wave behaviour can be associated with reproductive displays, so we only quantified the stereotyped waves of males who did not receive visits from females looking for mates during the observation period. Normally, fiddler crab males' wave faster when females approach (How et al., 2007; Callander et al., 2012), so it was possible to distinguish this behavior of stereotyped waves, the target of this study, as they do near the burrow entrance while feeding. Males with different claw morphotypes were previously visually selected. A thermocouple coupled to a digital thermometer (Lutron[TM], TM-947SD) was placed on the sediment surface approximately 1.0 cm from burrow entrance of the males. An observer was positioned approximately one meter from the focal male burrow and waited 5 minutes for the animal to retake its activities on the sediment surface. Then the focal male was observed for 10 minutes and the number of waves, the time of the male remained outside of the burrow, and the temperature of sediment were reported every 2 minutes. At the end of observations, the burrows were dug and males captured and taken to the laboratory. The carapace width and propodus length of *L. uruguayensis* males were measured to estimate relative claw size. To estimate the relative claw weight, we used the same procedure explained in section 2.1.

2.4 Statistical analyses

In section 2.1, we used Generalized Linear Mixed Models (GLMMs) to compare the luminosity index and incident solar radiation across shaded and unshaded microhabitats. The models incorporated the sediment surface temperature or luminosity index as response variables and the type of microhabitat (two levels: shaded and unshaded) as a fixed effect. The sampled areas (Area ID) was nested with measuring day (Day ID) and used in the models as random effects. Both models were fitted with Gamma distribution and log link.

We analyzed whether the proportion of leptochelous males differed between shaded and unshaded microhabitats using GLMMs. The number of leptochelous males was divided by

the total number of males (leptochelous and brachychelous) excavated in each sampled square and the proportion was set as the response variable. The type of microhabitat (two levels: shaded and unshaded) was set as a fixed effect. The sampled areas (Area ID) were used as a random effect. Models were fitted with a binomial family and logit link and the overdispersion parameter was checked.

We used General Linear Models (GLMs) to compare whether the males sampled in the field differed in relative claw size or relative claw weight between the types of microhabitats or claw morphology. The ratio between claw length/carapace width, as well as the proportion between the claw mass/body total mass, were used as response variables. The type of microhabitat (two levels: shaded and unshaded) and morphology type (orthogonal to a type of microhabitat, two levels: leptochelous and brachychelous) and their interaction were fitted as a fixed effect. We also compared whether fiddler crab males differed in size among the four sampled sites. We used the carapace width (CW) as a response variable and site [four levels: shaded-leptochelous (SL), unshaded-leptochelous (UL), shaded-brachychelous (SB), and unshaded-brachychelous (UB)] as a fixed factor. These models were fitted to a Gaussian distribution with an identity link function.

In section 2.2, we used repeated measures GLMs to test whether heat loss differed between leptochelous and brachychelous males immersed in ice bath and control groups. We used the difference between air temperature and crabs body temperature as a response variable. The morphology type (two levels: leptochelous and brachychelous), treatment condition (orthogonal to a morphology type, two levels: control and ice immersed groups), time (before and after immerse claw in ice), and their interaction were set as fixed factors in the model. Also, we conducted a subset of GLMs to test whether relative claw size and claw weight differed between leptochelous and brachychelous males or treatment groups used in the lab experiment. Claw size and claw weight were used as a response variable and the morphology type (two levels: leptochelous and brachychelous) and treatment condition (orthogonal to morphology type, two levels: control and ice immersed groups) were fitted as fixed factors. Models were fitted to a Gaussian distribution with an identity link function.

In section 2.3, we used Welch's F-tests to compare differences between relative claw size and relative claw weight between leptochelous and brachychelous males. Further, GLMs

were used to compare male activity time above the surface and waving behavior between the different morphotypes. Activity time above the surface and waving count displays were set as response variables. Models included the morphology type (two levels: leptochelous and brachychelous) as a fixed effect and the mean of the surface temperature variation as a covariate. The activity time above surface was log-transformed, and models were fitted using a Gaussian error distribution and identity link function. Waving behavior count models were fitted using a Poisson distribution and log link and the overdispersion parameter was checked in the final models.

Analyses were performed in R (R Core Team, 2020), using the package lme4 (Bates et al., 2015). All data subsets were checked to meet the assumptions of variance homogeneity and when necessary, it was log-transformed or suitable family distributions were applied to deal with non-normal data. In all models, the significance of fixed effects was investigated using likelihood ratio tests (LRTs) and compared with nested simple models and intercept only models. When necessary, post-hoc tests at different levels between factors were performed using the *multcomp* (Hothorn et al., 2008) and *emmeans* (Lenth et al., 2020) packages, with Tukey´s contrasts for multiple comparisons. All statistical analyses considered 95% as the significance level.

3. Results

3.1 *Variation in temperature, light, and crab claw morphotype between mangrove microhabitats*

The temperature of the sediment surface in unshaded microhabitats of *L. uruguayensis* was higher than in shaded sites (GLMM, microhabitat type: $X^2_1 = 14.9600$; $p < 0.0001$). In unshaded sites 1 and 2, the mean temperature reported on the sediment surface was 34.08°C (minimum: 27.2°C; maximum: 40.4°C). In shaded sites 3 and 4, the mean temperature of the sediment surface was approximately 7°C less than in unshaded microhabitats, showing a mean temperature of 26.70°C (minimum: 25.0°C; maximum: 30.2°C). Similarly, the luminous incidence in unshaded microhabitats was higher than in shaded ones (GLMM, microhabitat type: $X^2_1 = 38.7800$; $p < 0.0001$). In sites 1 and 2, the luminous incidence had a mean ± standard deviation (SD) of 89055.39 ± 28852.98 lx, while in sites 3 and 4, it was 2606.31 ±

1849.44 lx. The mean density of *L. uruguayensis* (including males and females) in sites 1 and 2 was 37.6 ± SD 23.03 and 37.6 ± SD 14.58 individuals per m², respectively; in site 3 and 4, it was mean 28.0 ± SD 4.0 and 14.4 ± SD 4.5 individuals per m², respectively.

The CW also differed between fiddler crab males from the sampled sites (GLM, site: $F_{(3,91)}$ = 3.0452; p = 0.03). Post-hoc tests showed that shaded-leptochelous (SL) males had larger carapace sizes (mean ± standard deviation: 9.58 ± 0.92) than shaded-brachychelous (SB) males (8.33 ± 1.15; SB x SL: z = -2.643, p = 0.03). There was no difference between CW sizes among other males according to the sampled sites (UL: 8.79 ± SD 1.32 and UB: 8.33 ± SD 0.86, Table 1).

The proportion of males with leptochelous claws in unshaded microhabitats was also higher than in shaded microhabitats (GLMM, microhabitat type: X^2_1 = 6.0898; p = 0.01). In unshaded sites 1 and 2, the proportion of males with leptochelous claws was 0.62 ± 0.30 individuals per square (mean ± standard error), while in shaded sites 3 and 4 the mean proportion of leptochelous claw was 0.18 ± 0.22 individuals per square (Figure 2, A).

Relative claw size did not differ between leptochelous and brachychelous morphotypes in both unshaded and shaded microhabitats (GLM, morphology type: $F_{(1,92)}$= 0.0452; p = 0.83). However, in unshaded microhabitats 1 and 2, the mean claw length of males was 1.62 ± SD 0.18 times larger than their carapace width, differing from male crabs of shaded microhabitats 3 and 4, with claw lengths 1.45 ± SD 0.22 times larger than their carapace width (GLM, microhabitat type: $F_{(1,92)}$= 12.888; p < 0.001, Figure 2, B). There was no effect of their interaction (GLM, morphology type x microhabitat type: $F_{(1,91)}$= 0.0448; p = 0.83).

The relative claw weight also did not differ between leptochelous and brachychelous morphotypes in both unshaded and shaded microhabitats (GLM, morphology type: $F_{(1,92)}$ = 2.7022; p = 0.10; Figure 3, C). In unshaded sites 1 and 2, the claw corresponded to 32 ± SD 4.0% of the weight of the males' body, differing from the crabs of shaded microhabitats 3 and 4, whose claw corresponded to 29 ± SD 6.0% of their body weight (GLM, microhabitat type: $F_{(1,92)}$ = 11.7160; p < 0.001). There was no statistical difference in the interaction between morphotype and microhabitat type (GLM, morphology type x microhabitat type: $F_{(1,91)}$= 0.2410; p = 0.62).

3.2 *Heat transfer from body to claw in leptochelous and brachychelous males*

There was a significant effect of the interaction between morphotype, treatment, and time in *L. uruguayensis* body heat transfer (repeated measures GLM; morphology type x treatment x time: $F_{(1,16)} = 6.6910$; p = 0.01, η2 = 0.295). In general, post-hoc tests showed that there was no difference between leptochelous and brachychelous morphotypes in body heat loss in control conditions during the first 15 minutes and final 15 minutes of the experiment (Figure 3, Table 2). In leptochelous males from control treatments, the mean body temperature was 23.63 ± SD 0.39ºC (mean ± standard deviation) during the first 15 minutes and 23.71 ± SD 0.36ºC in the final 15 minutes. In brachychelous males from control treatments, the mean body temperature was 23.79 ± 0.22ºC during the first 15 minutes and 23.76 ± SD 0.58ºC in the final 15 minutes. However, in treatment groups, the leptochelous and brachychelous males differed in body heat transfer before (in air condition, during the first 15 minutes) and after that their claw was immersed in ice during the final 15 minutes (Figure 3, Table 2). Leptochelous males had a mean body temperature 23.65 ± SD 0.31ºC before and 21.29 ± SD 0.29ºC after their claw was exposed to the ice bath, while brachychelous males had a body temperature 23.83 ± SD 0.64ºC before and 22.30 ± SD 0.42ºC after claws' ice exposition (Table 2).

There was no difference in relative claw size between morphotypes (GLM, morphology type: $F_{(1,16)} = 0.0003$; p = 0.99), treatments (GLM, treatment: $F_{(1,16)} = 0.0000$; p = 0.98), and their interaction (GLM, morphology type x treatment: $F_{(1,16)} = 0.0166$; p = 0.89). Similar results were found for relative claw weight, where no differences were found between morphotypes (GLM, morphology type: $F_{(1,16)} = 0.0112$; p = 0.91), treatment (GLM, treatment: $F_{(1,16)} = 0.2348$; p = 0.63), and their interaction (GLM, morphology type x treatment: $F_{(1,16)} = 0.1693$; p = 0.68).

3.3 *Wave activity between males with brachychelous and leptochelous claws*

Herein, we found that wave activity differed between males of *L. uruguayensis* with leptochelous and brachychelous claws, depending on the temperature of the sediment surface (GLM, morphology type x surface temperature: $X^2_1 = 25.3830$; p < 0.000001). Males with brachychelous claws waved, in mean, 3.4 ± SD 4.77 times per minute and their wave activity increased with temperature (Person's correlation: t = 3.0402; r = 0.42; p = 0.004). Males with leptochelous claw waved, in mean, 2.6 ± SD 4.1 times per minute and their wave activity was not associated with a temperature increase (t = 0.5254; r = 0.14; p = 0.60).

The time of surface activity did not differ between leptochelous and brachychelous morphotypes during the observations (GLM, morphology type: $F_{(1,56)} = 0.8903$; $p = 0.34$). The surface temperature variation was not related with male surface activity (GLM, surface temperature: $F_{(1,56)} = 2.0575$; $p = 0.15$). There was no significant effect of the interaction between morphotype and surface temperature (GLM, morphology type x surface temperature: $F_{(1,55)} = 0.0031$; $p = 0.95$). Both crab morphotypes expended, in mean, 89.91 ± SD 15.82% of their time out of burrows.

The claw length/carapace width ratio also did not differ between leptochelous and brachychelous morphotypes of males used in this experiment, which showed claws, in mean, 1.72 ± 0.16 times larger than their carapace width (Welch test: $F = 1.7907$, df = 31.4623, $p = 0.19$). Furthermore, the proportion of claw weight/total body weight did not differ between males with leptochelous and brachychelous claws (Welch test: $F = 1.4929$, df = 30.3418, $p = 0.23$). In both morphotypes, the claws of males corresponded to 35 ± SD 0.4% of their total body weight.

4. Discussion

Hypertrophied organs may play an important role in the thermoregulation of some animal species. Among the most well-known examples, we can mention toucan bills (Tattersall et al., 2009) and the ears of elephants (Weissenböck et al., 2010) and fennec foxes (Maloiy, 1982). In fiddler crabs, the hypertrophied claw of males can work as a heat sink during waving behaviour, helping them to cool the body during the hottest periods of the day (Windsor et al., 2005; Darnell and Munguia, 2011). In the present study, we showed that the thermoregulation function varies due to claw morphology, which may cause the predominance of *L. uruguayensis* males with regenerated claws in warmer unshaded microhabitats. Heat transfer from the body to claw was higher in *L. uruguayensis* males with regenerated leptochelous claws. They spent less time waving in the hottest periods of the day than males with non-regenerated brachychelous claws. Therefore, leptochelous claws, a trait *a priori* unfavorable in fights against males with non-regenerated claws (Backwell et al., 2000, Lailvaux et al., 2009), may be advantageous in a thermoregulatory context. Leptochelous claws allow the establishment of fiddler crabs in warmer microhabitats because their slender claws can be more efficient in dissipating heat into the air, which allows them to cool down by spending less time waving. Our

findings highlight the importance of morphological variations in thermoregulatory organs for the occupation of thermal niches, a relevant issue considering the context of winners and losers in the face of climate change.

Mangrove ecosystems form a mosaic of thermal niches along sedimentary substrates under the shade of vegetation or exposed directly to the sun (Koch et al., 2005; Munguia et al., 2017; Chou et al., 2019). In Australian mangroves, for example, the temperature of the sediment surface of shaded microhabitats may be approximately 8°C lower than in unshaded microhabitats during the hottest period of the day (Munguia et al., 2017). In the present study, we found a difference of approximately 7°C between shaded and unshaded sediment surfaces from microhabitats during the hottest day period. Mangrove organisms must have physiological, behavioural, or morphological adaptations that allow them to deal with such thermal variability (Koch et al., 2005; Medina et al., 2016; Munguia et al 2017; Ng et al., 2017; Principe et al., 2018; Levinton, 2020; Vianna et al., 2020). Some species have become thermal specialists, able to live only in one of these microhabitats (Nobbs, 2003; Koch et al., 2005; Principe et al., 2018). For example, the mangrove cicada *Arunta interclusa* (Sanborn et al., 2004), barred mudskipper *Periophthalmus argentilineatus* (Bennett et al., 2018), and fiddler crab *L. thayeri* (Principe et al., 2018) are found only in cooler shaded areas of mangroves. While other species, as for example, the California horn snail *Cerithidea californica* (Lorda and Lafferty, 2012) and fiddler crab *Uca maracoani* (Koch et al., 2005), are adapted to live in warmer areas exposed to the sun. Some generalist species are able to transit between both shared- and unshared habitats, temporarily occupying a microhabitat that meets their thermal requirements (Munguia et al., 2017; Chou et al., 2019; Principe et al., 2018; Hendy et al., 2020). In these species, thermal niches are resources disputed actively among conspecifics, and winning competitors may improve their fitness (Munguia et al., 2017; Chou et al., 2019). The fiddler crab *Austruca mjoebergi*, for example, can live in both shaded- and unshaded microhabitats, but they tend to choose to live in shade ones, where they can avoid extreme temperatures and thus, stay longer on the sediment surface (i.e. outside their burrows) and increase their opportunity to mate (Munguia et al., 2017; Chou et al., 2019; Darnell et al., 2019). In our study, we found that *L. uruguayensis* also can be found in both microhabitats, but the proportion of males with regenerated claws was higher in warmer unshaded microhabitats. Furthermore, males from

89

unshaded microhabitats had larger and heavier claws than males from shaded microhabitats, regardless of claw morphotype. The differential distribution observed herein for *L. uruguayensis* males with different claw morphology may be explained by the confirmation of the hypothesis that claw shape affects thermal inertia of the crabs' body.

The increase of time spent on the sediment surface is advantageous for the fiddler crab, increasing the feeding time and chance to mate (Munguia et al., 2017; Chou et al., 2019). The relative claw size was negatively correlated with body temperature in *Austruca mjoebergi* (Munguia et al., 2017), which may contribute to their increased time on the sediment surface. The larger relative claw size of *L. uruguayensis* from unshaded microhabitats observed in our study could be associated with the maintenance of body temperature suited to their thermal limits. In our laboratory experiment, *L. uruguayensis* males from the leptochelous morphotype showed a mean heat transfer between body and claw 35.31% higher than that observed in brachychelous males. Compared to brachychelous claws, leptochelous claws have less muscle mass volume, thinner dactilus and pollex, longer length, and lack of teeth (Backwell et al., 2000; Rosenberg, 2002). Since the crabs used in this experiment were standardized to the same relative claw size and the same proportion of claw weight, the higher heat dissipation in leptochelous claws may be explained by their slender shape. The slender shape favors a higher contact surface, accelerating heat transfer from the body to the chelae and chelae to the cold water. In honeyeater birds, for example, slender-shaped beaks were also suggested as a morphological adaptation that allows them to live in warmer habitats due to the facilitation of heat loss (Friedman et al., 2019). Both morphological traits observed in the present study, i.e. the larger relative claw size and leptochelous claw morphotype, may favor the establishment of *L. uruguayensis* males in warmer unshaded microhabitats.

However, losing water slower than the body may contribute to the claws' accumulation of heat, making it harder to exchange heat with the surrounding air (Levinton, 2020). A recent study found that water loss from the body of the fiddler crab *L. pugilator* was greater (10.0% ± 0.55) than claws (5.85% ± 0.45), probably due to the imperviousness of the claw (Levinton, 2020). These results are not consistent with the thermoregulatory organ hypothesis (Munguia et al. 2017). Despite this, if the leptochelous claw shape may retain less heat than brachychelous

claws, it could favor the greater proportion of males with regenerated claws in unshaded areas, as suggested by our results. Future studies should evaluate how different shaped fiddler crab claws could heat up and allow the survival of these organisms in microhabitats of different stress conditions.

Our results from the behavioral experiment in the field sustained the idea that the leptochelous morphotype was advantageous for male *L. uruguayensis* to live in unshaded microhabitats. Brachychelous males waved approximately 30% more than leptochelous males with the same relative claw size and same relative claw weight. However, the time of surface activity did not differ between leptochelous and brachychelous morphotypes during the observations. Since brachychelous claws are less efficient for heat dissipation than leptochelous claws, males with non-regenerated claws could endure the hottest day period by increasing their wave behavior without reducing their surface time. The increase in wave behavior could mean higher energetic costs to the fiddler crabs (Jennions and Backwell, 1998; Matsumasa and Murai, 2005; Sanches et al., 2018). Once brachychelous males have stronger claws (i.e. closing and pull resisting forces) and are relatively superior competitors' in fights against leptochelous males of the same size (Backwell et al., 2000, Lailvaux et al., 2009), they can monopolize preferable shaded microhabitats. This could explain the difference in the distribution observed between both morphotypes. Leptochelous males established in shaded microhabitats showed larger carapace width than brachychelous males. Larger sizes confer competitive advantages in fiddler crab fights (Jaroensutasinee and Tantichodok, 2003; Morrell et al., 2005), so larger leptochelous males would be able to defend their burrows against small males on shaded microhabitats. Future studies investigating the selection of habitats and potential competition between the two morphotypes and size advantages may contribute to support this hypothesis.

In view of climate changes, our findings highlight the importance of morphological variability as a phenotypic response that may help organisms to deal with rising temperatures. Studies have associated morphological variations with temperature (Shirley et al., 1987; Picard et al., 1996; Daufresne et al., 2009; Symonds and Tattersall, 2010; Gardner et al., 2011; Gizzi et al., 2016; Ortega et al., 2016; Friedman et al., 2019). However, morphological traits

associated with temperature were observed at an interspecific level and on a latitudinal scale as, for example, in the beaks of birds (Symonds and Tattersall, 2010; Friedman et al., 2019) and horns of bovids (Picard et al., 1996). At the intraspecific level, morphological variation associated with temperature is also known on large spatial scales, i.e. morphological differentiation between population from different latitudes, or on a long time scale, i.e. between generations over the years, as for example in clam shells (Gizzi et al., 2016; Ortega et al., 2016). In our study, the morphological variation in claws derived by behavioural and life-time processes had important consequences on distribution, thermal, and behavioural responses at the intraspecific level and in the same population. The morphological variation of *L. uruguayensis* during a lifetime can result in a differential spatial distribution between thermal microhabitats and favor leptochelous males that deal better with high temperatures. Future studies with other organisms at the intraspecific level, including other species of fiddler crabs, can help us to understand which phenotypes are likely to win or lose the climate change scenario and which microhabitats may function as thermal refuges.

5. Conclusion

Although regenerated claws can be disadvantageous weapons for fiddler crabs, we showed that regenerated claws of *L. uruguayensis* males can favor their occupation in warm unshaded microhabitats. In the field, we found that *L. uruguayensis* populations were heterogeneously distributed along shaded and unshaded microhabitats, with the predominance of males with larger leptochelous claws in warmer unshaded microhabitats. When exposed in the same conditions on unshaded areas, leptochelous males spent less time waving in the hottest day periods than males with non-regenerated brachychelous claws. Under laboratory conditions, leptochelous males were more efficient at body cooling than brachychelous males. Leptochelous claws can be advantageous for fiddler crabs' establishment in warmer microhabitats due to greater efficiency of heat loss, allowing cooling by spending less time waving. Our findings highlight the importance of morphological variation in thermoregulatory structures for the occupation of thermal niches, a relevant issue considering the context of winners and losers in the face of climate change.

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Tables

Table 1 Tukey post-hoc pairwise comparison between carapace width male sizes of *Leptuca uruguayensis* according to sites sampled: shaded-leptochelous (SL), unshaded-leptochelous (UL), shaded-brachychelous (SB), and unshaded-brachychelous (UB). (*) shows statistically differences between the group comparisons.

Site comparisons	Estimate	Std. Error	z statistic	P value
SL - UL	0.7953	0.4769	1.668	0.3309
UB - UL	-0.4554	0.3136	-1.452	0.4561
SB - UL	-0.4550	0.2830	-1.608	0.3640
UB - SL	-1.2509	0.4920	-2.542	0.0510
SB - SL	-1.2504	0.4730	-2.643	0.0389 *
SB - UB	0.0005	0.3077	0.002	1.00

Table 2 Tukey post-hoc pairwise comparison between the *Leptuca uruguayensis* morphotype, according to the time (before and after) and treatment exposure (control and ice). Males with brachychelous claw morphotypes are represented by the capital letter "B" and leptochelous represented by the capital letter "L". (*) shows statistical differences between group comparisons.

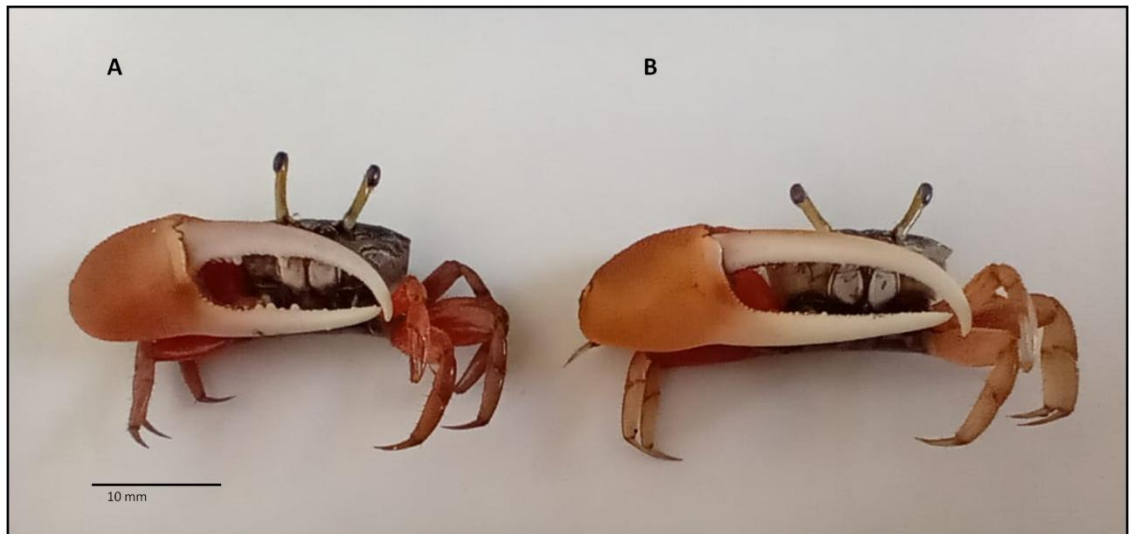| Morphotype	Time	Estimate	Std. Error	t statistic	P value
Control treatment					
B - L	before - before	0.0325	0.231	0.141	0.99
B - B	before - after	0.0271	0.211	0.129	0.99
B - L	before - after	0.0793	0.231	0.343	0.98
L - B	before - after	-0.0054	0.231	-0.023	1.00
L - L	before - after	0.0467	0.211	0.222	0.99
B - L	after - after	0.0521	0.231	0.226	0.99
Morphotype	Time	Estimate	Std. Error	t statistic	P value
Ice treatment					
B - L	before - before	0.1321	0.231	0.572	0.93
B - B	before - after	-1.3164	0.211	-6.239	0.0001*
B - L	before - after	-2.2561	0.231	-9.756	0.0001*
L - B	before - after	-1.4486	0.231	-6.264	0.0001*
L - L	before - after	-2.3883	0.211	-11.320	0.0001*
B - L	after - after	-0.9397	0.231	-4.064	0.0036*

Figures

Figure 1: Different males' claw morphotypes of fiddler crabs *Leptuca uruguayensis.* A)

Non-regenerated cheliped: brachychelous claw. B) Regenerated cheliped: leptochelous claw.
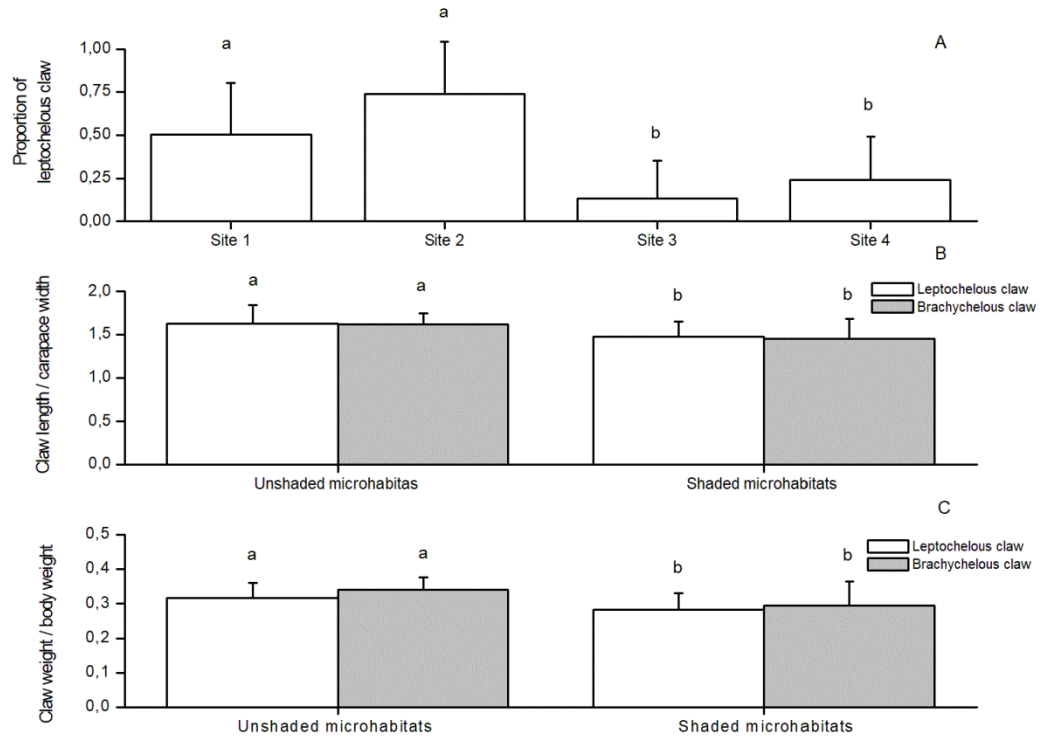
Figure 2: A) Proportion of *Leptuca uruguayensis* males with leptochelous claws in unshaded (site 1 and 2) and shaded microhabitats (site 3 and 4). B) Relative claw size between leptochelous and brachychelous males from unshaded and shaded microhabitats. C) Relative claw weight between leptochelous and brachychelous males from unshaded and shaded microhabitats. Different lowercase letters indicate statistical differences between treatments ($p < 0.05$).
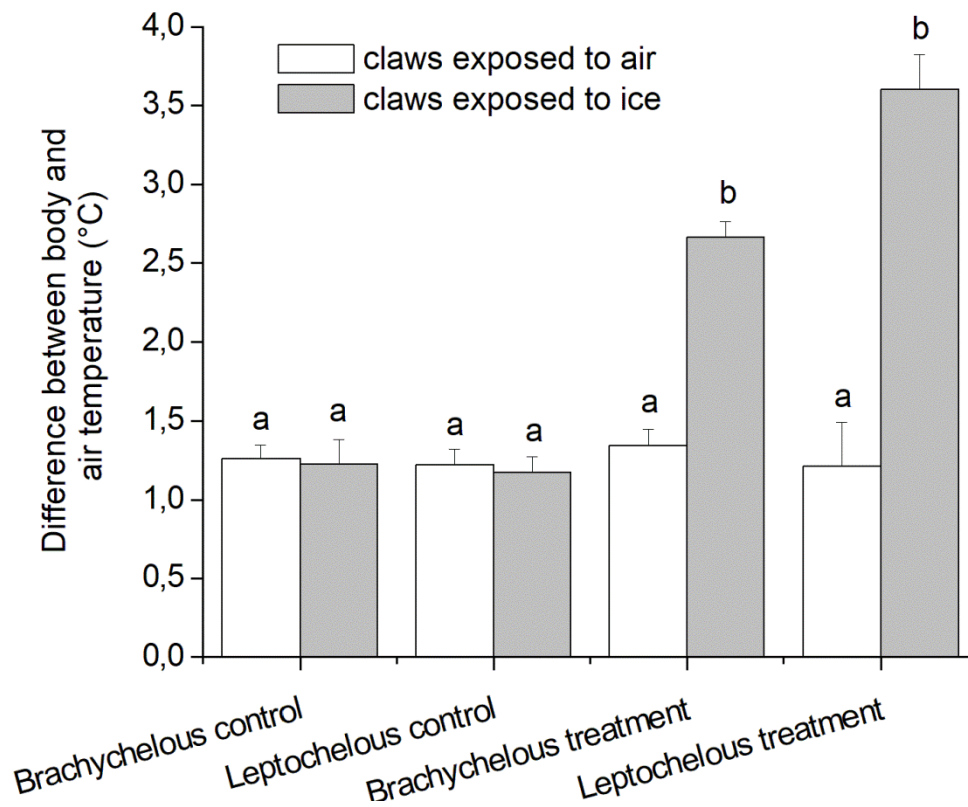
Figure 3: Heat transfer between body and claw in leptochelous and brachychelous fiddler crab males. Difference between body and air temperature of *Leptuca uruguayensis* males, which had their claws exposed to air only (control) and to air and then immersed in an ice bath (treatment). The white bars indicate the temperature difference during the first 15 minutes of the experiment when all crabs had their claws exposed to the air, regardless of the treatment. The grey bars indicate the temperature difference during the final 15 minutes of the experiment when the crabs from the treatment groups had their claws immersed in the ice bath. Different lowercase letters indicate statistical differences between treatments ($p < 0.05$).

Highlights

- *Leptuca urguayensis* of different claw morphotypes has differential habitat distribution

- Regenerated claws of fiddler crab males are more efficient for heat loss

- Brachychelous males waved more with rising temperatures

- Leptochelous males are more abundant in warmer non-vegetated habitats

Vitae

Ms. Fernando Rafael De Grande is a zoology PhD student at São Paulo State University – UNESP, Bioscience Institute, Botucatu Campus, Brazil. His main areas of scientific interest are the effect of climate change on key coastal ecosystems species and the behaviour and ecology of coastal organisms. His current projects is: "Effects of global warming on the distribution, body size, and morphology of South American fiddler crabs"

MSc. Bruno Rafael Fogo is a Marine Biologist that graduated from São Paulo State University –UNESP, Bioscience Institute, São Vicente, Brazil and has a Master's degree in Zoology from São Paulo State University – Biosciences Institute, Botucatu, Brazil. He has experience in animal behavior of estuarine organisms. His recent research investigated how body color patterns might affect thermoregulation and behavioural responses in fiddler crabs.

Dr. Tânia Marcia Costa is an associate professor at São Paulo State University – UNESP, Bioscience Institute, Coastal Campus, São Vicente, Brazil. Her main areas of scientific interest are the effect of climate change on key consumers, the function of coastal ecosystems, and behaviour and ecology of aquatic and coastal organisms (including species of interest to aquaculture). She is a researcher at UNESP'S Aquaculture Center (CAUNESP) and assistant editor at Nauplius. Her current projects is: "Impact of climate change and biodiversity loss on trophic interactions in coastal ecosystems"

Considerações finais

Neste trabalho nós demonstramos que os limites sul de distribuição das diferentes espécies de caranguejos-chama-maré da América do Sul são definidos pelas baixas temperaturas da água durante a fase larval. Nós também demonstramos que a variação latitudinal de tamanho corporal do caranguejo-chama-maré *Leptuca uruguayenis* é uma resposta metabólica desse crustáceo à variação de temperatura e a pressão predatória exercida por um predador cuja distribuição está associada à temperatura (i.e. *Minuca rapax*). Por fim, nós demonstramos que a variação morfológica dos quelípodos de machos de *L. uruguayensis* pode influenciar a termorregulação e, consequentemente, determinar a ocupação entre microhabitats, de forma que os quelípodos regenerados são mais vantajosos para a ocupação de habitats mais quentes, como os habitats não vegetados. Desta forma, frente ao aumento de temperatura das águas costeiras da América do Sul, que vêm sendo observado em consequência das mudanças do clima, nós podemos sugerir que os limites de distribuição dos caranguejos-chama-maré poderão ser ampliados para maiores latitudes nas próximas décadas. Como discutido no capítulo 1 essa expansão pode, inclusive, já estar ocorrendo para *L. cumulanta* e nós temos monitorado os efeitos da expansão dessa espécie sobre as espécies residentes (Anexo III: Arakaki et al., 2019). Ainda, baseado em nossas descobertas, nós podemos sugerir que as populações de chama-maré poderão sofrer uma redução do tamanho corporal médio, principalmente nas regiões mais quentes de baixas latitudes. Considerando que o tamanho da toca e quantidade de sedimento removido para a construção desta são dependente do tamanho corporal dos caranguejos chama-maré (Gusmão-Junior et al., 2012), e que construção de tocas é uma das principais atividade de engenharia de ecossistema promovida por estes caranguejos (Kristensen, 2008; Natálio et al., 2017), nós sugerimos que o potencial bioturbador associado ao tamanho corporal dos chama-marés sobre as comunidades bentônicas seja monitorado em estudos futuros. Por fim, baseado nas descobertas apresentadas no último capítulo, nós sugerimos que o aumento de temperatura poderá favorecer caranguejos machos com quelípodo regenerados o que, hipoteticamente, poderia favorecer os indivíduos com quelípodos leptoquélicos nas populações de *L. uruguayensis.* Ainda que o morfotipo leptoquélico não seja causado pelo aumento de temperatura, sua predominância nas populações pode indicar uma resposta dos caranguejos ao aumento de

temperatura. Futuros estudos que avaliem a proporção de quelípodos regenerados entre populações de *L. uruguayensis* em uma escala latitudinal poderão contribuir para a sustentação dessa hipótese. Por fim, e considerando as conclusões obtidas nos três os capítulos, nós sustentamos a tese de que o aquecimento global acarretará em consequências ecológicas e fisiológicas sobre os caranguejos-chama-maré da América do Sul. Considerando o conjunto de nossas descobertas, sugerimos que a alta capacidade dos caranguejos-chama-maré de responderem a variação de temperatura, faz destes animais um bom organismo-modelo para o monitoramento e avaliações futuras sobre os efeitos das mudanças do clima sobre a biota de ambientes costeiros.

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Anexos

Apresento nessa sessão três artigos associados ao tema de minha tese (i.e. os efeitos do aumento de temperatura sobre organismos costeiros) que foram publicados em periódicos internacionais. Tais artigos foram construídos durante o desenvolvimento de meu doutorado e são o resultado de coorientações e parcerias que realizei ao longo de minha trajetória profissional.

Anexo I

Título do trabalho: Thermal response of demersal and pelagic juvenile fishes from the surf zone during a heat-wave simulation.

Aluno coorientado: Eidi Kikuchi.

Parceiros: Rafael Mendonça Duarte e Teodoro Vaske-Júnior.

Periódico: Journal of Applied Ichthyology.

Abstract: Experimental measurements were collected in the laboratory to evaluate the maximum thermal limit and thermal plasticity of Neotropical juvenile fish with different life habitats (demersal and pelagic) from surf zone in response to a "heat-wave experiment". Trials were conducted using two temperature acclimations (T_a), including the current average temperature of Southeastern Brazil (T_a: 14 days at 25 ˚C) and the "heat-wave experiment" (T_a: 14 days at 30˚C), simulating a heat-wave event that occurs when the daily maximum temperature of more than five consecutive days exceeds the average maximum temperature by 5˚C. Typical species of the surf zone were used: the demersal White sea catfish (*Genidens barbus*) and Gulf kingcroaker (*Menticirrhus littoralis*), and the pelagic fishes Great pompano (*Trachinotus goodei*) and Long-fin mullet (*Mugil brevirostris*). The thermal range and plasticity values for the both life-habitats species were verified through current and heat-wave acclimation. The thermal tolerance at high temperatures (CT_{max}) of these species differed between T_a, habitat and species. Fish showed a species-specific response to temperature increase, regardless of their habitat even under similar abiotic conditions. However, at the heat-wave simulation, the demersal fish presented a greater thermal plasticity in relation to the pelagic fish. Despite the higher thermal tolerance when exposed to heat-wave simulation, all fish species displayed a lower thermal edge safety that is markedly close to their maximum thermal limits.

Anexo II

Título do trabalho: Do lower air or water temperatures limit the southern distribution of the white mangrove *Laguncularia racemosa* in South America?

Aluno coorientado: Jaqueline Santos Borges.

Parceiros: Tânia Marcia Costa.

Periódico: Estuarine, Coastal and Shelf Science.

Abstract: Rising temperatures due to climate change are expected to drive shifts in species composition, phenological patterns and the productivity of mangrove trees. During early life history stages, such as dispersal and settlement, temperature may affect the survival of propagules and, consequently, drive the distribution of mangrove species. The aim of this work was to evaluate whether low water and air temperatures experienced by propagules during the dispersal and settlement stages, respectively, limit the latitudinal distribution of the white mangrove *Laguncularia racemosa*. Based on the distribution range of *L. racemosa*, we investigated four water and air temperatures: 10, 15, 20 and 25 °C. First, we evaluated the effect of seawater temperature on the buoyancy time of propagules. Then we tested the effect of seawater temperature and buoyancy time (24, 48, 72 and 96 h) on the germination rate of *L. racemosa*. Finally, we evaluated the effect of air temperature (10, 15, 20 and 25 °C) on the germination of propagules during the stranded stage. The propagules in higher water temperatures (20 and 25 °C) submerged faster than in lower temperatures (10 and 15 °C). The percentage germination of propagules in water temperatures of 20 and 15 °C was higher than those in 25 and 10 °C. However, the percentage germination was greater than 70%, regardless of the water temperature or buoyancy time tested. Furthermore, the percentage germination of propagules in air temperatures of 25 and 20 °C was higher (above 70%) than in air temperatures of 15 and 10 °C (below 50%). Lower water temperature increased the dispersal time of propagules, but it was not crucial to the germination rate of *L. racemosa*, regardless of the buoyancy time of propagules. In contrast, a lower air temperature reduced the germination of propagules during the stranded stage. Therefore, water temperature is not a limiting factor for the success of *L. racemosa* during the dispersal stage, however, we found that the mangrove tree is highly sensitive to air temperature during its stranded stage. We hypothesized that the increased buoyancy time of propagules during lower temperatures is an adaptive advantage, which allows *L. racemosa* to be transported to warmer areas or to tolerate cold temperatures for longer periods before settlement.

Anexo III

Título do trabalho: Battle of the borders: Is a range-extending fiddler crab affecting the spatial niche of a congener species?

Aluno coorientado: Jonathann Yukio Arakaki.

Parceiros: Alexandre L. Arvigoa, Juan C.F. Pardoa, Bruno R. Fogo, Fábio H.C. Sanches, Caio Akira Miyaia, Murilo Z. Marochi e Tânia M. Costa.

Periódico: Journal of Experimental Marine Biology and Ecology.

Abstract: Climate change is drastically altering environmental conditions and resource availability. Many organisms are shifting their distribution boundaries. Fiddler crabs, for instance, are important ecosystem engineers in coastal environments that have been extending their distribution range poleward. In this study, we evaluated the influence of a range-extending species, *Leptuca cumulanta*, which has recently overlapped the distribution of the resident species *Leptuca uruguayensis*. Through a set of field and laboratory experiments, we characterised the degree of territorial overlap between *L. cumulanta* and *L. uruguayensis* from the lower to upper intertidal zone in a mangrove area. We also analysed whether the presence of *L. cumulanta* prevents habitat choosiness or influences agonistic behaviours in L. uruguayensis in territorial fights. We found that both species overlap territories at the same level in the intertidal zone. However, we observed that both habitat choice and agonistic behaviours of L. uruguayensis were unresponsive to the presence of *L. cumulanta*. The low interference between recent heterospecific neighbours sharing the same space supports coexistence of fiddler crabs *L. uruguayensis* and *L. cumulanta* in the early stage of overlapping.