UNIVERSIDADE ESTADUAL PAULISTA – UNESP

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS – ZOOLOGIA

Juan Carlos Farias Pardo

Espécies-chave estuarinas em um mundo em mudança: como as fases mais vulneráveis dos caranguejos violinistas respondem ao aquecimento e acidificação global?

Apoio:

FAPESP CAPES CNPq

São Vicente,

2018

UNIVERSIDADE ESTADUAL PAULISTA – UNESP

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS – ZOOLOGIA

Juan Carlos Farias Pardo

Espécies-chave estuarinas em um mundo em mudança: como as fases mais vulneráveis dos caranguejos violinistas respondem ao aquecimento e acidificação global?

Dissertação 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 Mestre.

Orientadora: Profa. Dra. Tânia Márcia Costa

Coorientador: Prof. Dr. Stefano Cannicci

São Vicente,

2018

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP

BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Pardo, Juan Carlos Farias.
Espécies-chave estuarinas em um mundo em mudança : como as fases mais vulneráveis dos caranguejos violinistas respondem ao aquecimento e acidificação global? / Juan Carlos Farias Pardo. - Botucatu, 2018

Dissertação (mestrado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu
Orientador: Tânia Marcia Costa
Coorientador: Stefano Cannicci
Capes: 20403003

1. Caranguejo - Pesquisa. 2. Mudanças climáticas. 3. Fatores abióticos. 4. Aquecimento global. 5. Stress (Fisiologia).

Palavras-chave: Ectotérmicos; Estressores múltiplos; Mudança Climática.

Dedico esse trabalho aos meus pais, Juan Luis e

Maria das Graças, e à minha vó, Maria Esther.

Agradecimentos

Agradeço aos meus mentores e queridos pais, Juan Luis e Maria das Graças, por me ensinarem como lidar com a vida e pelo imenso apoio durante toda minha trajetória;

À minha avó, Maria Esther, por todo amor oferecido e por ainda me inspirar até os dias de hoje;

À minha família, em especial para minha irmã, Ana Carla, por me ensinar, mesmo não sabendo que o faça, a ser uma pessoa melhor;

À minha orientadora Profa. Dra. Tânia M. Costa por todo suporte desde o momento que entrei na universidade. Um exemplo de gestora, pesquisadora, professora e pessoa. Sou imensamente grato por todo o período de orientação e amizade;

Ao meu coorientador Prof. Dr. Stefano Cannicci pelo auxílio científico durante o período de pesquisa no exterior;

À Universidade Estadual Paulista "Júlio de Mesquita Filho" pelo suporte e estrutura para a elaboração desse estudo;

Ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia) de Botucatu e seus funcionários, em especial ao Davi pela prestatividade e Prof. Antônio Castilho pela coordenação do Programa;

Aos amigos do Laboratório de Ecologia e Comportamento Animal (LABECOM) por todo auxílio em saídas de campo e laboratório, conversas científicas (ou não) e, principalmente, pela amizade;

À Universidade de Hong Kong pelo apoio durante o período de estágio no exterior, em especial a Sylvia, Cecily e Dr. Gray Willians pelo apoio técnico e estrutural;

À todos amigos das diversas fases da minha vida pelo imenso apoio;

À Profa. Dra. Alessandra Augusto pelas críticas construtivas feitas no trabalho e por compartilhar a infraestrutura do seu laboratório;

Aos membros do Laboratório de Sustentabilidade (LABSUSSA) em especial a Andressa e a Julia pelas dicas nas análises e amizade;

Aos membros da banca, Glauco Machado e Rafael Duarte, por aceitarem o convite de avaliar o nosso trabalho;

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (processo nº 2017/00802-0, 2018/00577-0) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (processo nº 131970/2017-8) pelo apoio financeiro em diferentes períodos durante o mestrado.

Sumário

Resumo

A mudança climática antropogênica afeta a estrutura e funcionamento dos ecossistemas. O aquecimento e acidificação global estão entre os principais estressores. Habitats costeiros e estuarinos são dinâmicos e seus organismos são diretamente afetados pelas mudanças abióticas no ambiente. No presente estudo, verificamos como as fases potencialmente vulneráveis de organismos ectotérmicos estuarinos respondem às variações climáticas. Utilizando como modelo os caranguejos violinistas, um grupo-chave na conformação dos ambientes estuarinos, avaliamos respostas morfológicas e fisiológicas frente ao aumento da temperatura e/ou redução do pH. Inicialmente, caracterizamos o ambiente em que se desenvolvem os embriões do caranguejo do Atlântico *Leptuca thayeri*. Temperatura, pH e salinidade no microhabitat (tocas) foram diferentes do ambiente ao entorno. O desenvolvimento embrionário foi afetado por ambos estressores: o aumento da temperatura acelerou o desenvolvimento e diminuiu a sobrevivência independente do pH, sendo a concentração de amônia e volume dos ovos as variáveis resposta que evidenciam o efeito sinérgico entre pH e temperatura. Em uma abordagem interespecífica, demonstramos experimentalmente que a sensibilidade termal de fêmeas ovígeras varia de acordo com a espécie. Fêmeas ovígeras de *Gelasimus borealis* foram mais sensíveis ao aumento de temperatura do que não ovígeras, porém a tolerância termal de *L. thayeri* não variou independente do seu status reprodutivo. A temperatura média mensurada no habitat natural foi abaixo dos limites termais de ambas espécies, porém com extremos acima dos seus limites termais. Desse modo, demonstramos que fases vulneráveis dos caranguejos violinistas são sensíveis às variações abióticas preditas, porém as respostas podem ser individuais e espécie-específicas dependendo do estágio.

Apresentação

A mudança climática é inquestionável, sendo a ação antropogênica uma das suas principais causas nas últimas décadas (IPCC, 2014; Oreskes, 2018). A intensificação no aumento de gases pós era industrial alavancou o aumento da temperatura em escalas regionais e globais (Ahmed et al., 2013; IPCC, 2014). Grande parte do CO_2 emitido é absorvido pelos oceanos, alterando o balanço de carbonato (CO_3^{-2}) e, consequentemente, reduzindo o pH (Feely et al., 2004). A acidificação e aquecimento global estão entre os principais estressores aos ambientes aquáticos, sobretudo os costeiros e estuarinos. Organismos encontrados nesses ambientes dinâmicos são adaptados às variações triviais (escalas diárias e sazonais) dos fatores abióticos e, usualmente, vivem perto aos seus limites fisiológicos, tornando-os suscetíveis a atual e futura variação de temperatura e pH (Helmuth et al., 2006; Vinagre et al., 2018).

Os padrões de fenologia, distribuição e plasticidade fisiológica e comportamental são reconhecidamente afetados pela mudança climática antropogênica (Sunday et al., 2012; Seebacher et al., 2015; Cohen et al., 2018). A variação acelerada dos fatores pode ser aquém a plasticidade fisiológica e comportamental ou adaptação a partir da seleção natural (Pistevos et al., 2011; Seebacher et al., 2015). Revisões demonstram a imensa lacuna no conhecimento acerca das respostas dos organismos frente às mudanças do clima nos trópicos e subtrópicos, sobretudo do hemisfério sul (Sunday et al., 2012; Seebacher et al., 2015; Cohen et al., 2018). Segundo o IPCC (2014), a potência da circulação da corrente do Brasil pode ser reduzida em 44% até 2100, tornando a situação semelhante ao período de degelo. Somado ao aumento na frequência e intensidade de eventos extremos (e.g., ondas de calor, Oliver et al. (2018)), as implicações de tal variação climática aos habitats do Atlântico Sul, principalmente os costeiros e estuarinos, ainda são pouco conhecidas (Bernardino et al., 2015). Dessa forma, faz-se necessário estudos

comparativos e descritivos quanto a sensibilidade dos organismos frente aos futuros cenários climáticos.

Os capítulos desta dissertação visam preencher algumas dessas lacunas em fases reconhecidamente vulneráveis dos organismos, utilizando como modelo os caranguejos violinistas ou chama-maré. O grupo é utilizado em estudos comparativos e ecológicos dada sua importância na conformação dos habitats estuarinos (Cannicci et al., 2008; Natálio et al., 2017) e seus estágios de vida variarem de oceânico (fase larval) a semiterrestre (adultos). Embriões e larvas possuem adaptações fisiológicas e comportamentais aos estressores bióticos e abióticos, porém são tidas como fases potencialmente sensíveis aos estressores, sobretudo temperatura e pH (Przeslawski et al., 2015). Por outro lado, a sensibilidade de fêmeas ovígeras, uma fase de alto custo energético, frente ao aumento da temperatura, ainda não foi avaliada experimentalmente em ectotérmicos. Nesse sentido, no **Capítulo 1** verificamos como o aumento da temperatura e diminuição do pH, em níveis preditos para o final do século, pode afetar o desenvolvimento embrionário do caranguejo violinista *Leptuca thayeri*. No **Capítulo 2** avaliamos se as fêmeas ovígeras são mais sensíveis ao aumento da temperatura do que fêmeas não ovígeras. Nesse estudo, comparamos o limite termal de duas espécies, *L. thayeri* e *Gelasimus borealis*, de gêneros distintos, mas hábitos comportamentais semelhantes durante seu período reprodutivo. Dessa forma, a presente dissertação avaliou como as fases potencialmente vulneráveis de ectotérmicos estuarinos respondem as predições de aquecimento e acidificação global.

CAPÍTULO 1

Multiple stressors effects of warming and acidification in the embryonic development of an estuarine fiddler crab

Pardo, J. C. F[1,2,*] & Costa, T. M[1,2]

[1]Laboratório de Ecologia e Comportamento Animal (LABECOM) - Universidade Estadual Paulista (UNESP), Instituto de Biociências, Campus do Litoral Paulista, Praça Infante Dom Henrique, s/nº, Parque Bitaru, São Vicente, SP, Brazil. PO Box 73601. 11380-972

[2]Programa de Pós-graduação em Ciências Biológicas (Zoologia) - Universidade Estadual Paulista (UNESP), Campus de Botucatu, Instituto de Biociências, Rua Prof. Dr. Antônio Celso Wagner Zanin, 250, Distrito de Rubião Junior, Botucatu, SP, Brazil. 18618-689

* Email address: pardojcf@yahoo.com.br (J.C.F. Pardo)

Manuscript Format in accordance with the guidelines of the Journal of Experimental Marine Biology and Ecology

Abstract

The warming and acidification of coastal areas have been demonstrated to affect habitats' structure and functioning. Predicted effects of anthropogenic climate change on estuarine and coastal organisms are complex and early life-history stages of calcified ectotherms are among the most sensitive group in a changing environment. In this context, we verified the combined effects of temperature increase and pH decrease on the embryonic development of the estuarine fiddler crab *Leptuca thayeri*. Microhabitat (burrows) were characterized and its abiotic parameters (temperature, pH and salinity) differs from the surrounding environment. Embryo development was faster, and survivorship was lower in warmer conditions for either control and low pH. Ammonia concentration and egg volume follows the synergetic effect of pH and temperature through embryo development. Besides the ontogenetic constraints, several eggs developed until late embryonic developmental stages, bringing important insights on the effects of a warmed and acidified environment in early stages of estuarine species.

Key-words: Climate change. Multiple stressors. Synergic effect. Early-life stages. *Leptuca thayeri*.

Introduction

Human mediated environmental changes lead by exploitation and mismanagement entail modifications in ecosystem functioning. Anthropogenic climate change has been recognized as a significant threat to biogeochemical processes and biodiversity (IPCC, 2014; Nagelkerken & Connell, 2015). Global warming and/or acidification, for instance, may drives species distribution and affect behaviour and physiology of many species (Parmesan, 2006; Byrne et al., 2013; Bozinovic & Pörtner, 2015; Lefevre, 2016; Calosi et al., 2017; Cohen et al., 2018). Among global environments, coastal and estuarine habitats are threatened as one of the most vulnerable environments due to their highly restricted areas and complexity of multifaceted biotic (e.g., competition, trophic interactions) and abiotic (e.g., temperature, salinity, pH) factors (Alongi, 2002; Nagelkerken & Connell, 2015; Gunderson et al., 2016).

Estuarine fauna and flora are well-adapted (physiology, morphology and/or behaviour) to daily oscillations in abiotic factors (Duke et al., 1998; Madeira et al., 2012; Fusi et al., 2015); however, severe and rapid changes in abiotic parameters (e.g., temperature increase and pH decrease) may exceed their physiological thresholds resulting in acute and chronic impacts (Crain et al., 2008; Madeira et al., 2012; Byrne & Przeslawski, 2013; Przeslawski, et al., 2015). Consequently, the structure and functioning of estuarine habitats depend on few key-groups which are not necessarily well adapted to climate change.

There is an increasing recognition of the importance to understand how multiple stressors affect biodiversity (Rudd, 2014; Gunderson et al., 2016). Currently, there is a lack of information on the impacts of combined stressors incorporating abiotic parameters obtained by downscaling modelling and field measurements (Bozinovic & Pörtner, 2015; Przeslawski et al., 2015; Gunderson et al., 2016). The combination of stressors may entail

addictive, antagonistic and/or synergic effects, which are, respectively, equal, lower or greater than the sum of their separate effects (Crain et al., 2008; Griffen et al., 2016). The different responses to multiple stressors support the need for studies in groups and species which are still not explored.

Early life-history stages are potentially vulnerable stages in a climate change context, particularly trough effects on survival, excretion, development and calcification (Kroeker et al., 2013; Przeslawski et al., 2015; Navarro et al., 2016). The synergistic effect is the most observed impact in marine species varying among ontogeny stages, stressors and biological responses (Przeslawski et al., 2015 and literature within); however, responses to combined stressors also vary among groups: temperature increase minimizes the effect of low salinity (antagonistic effect) in embryo development of the sea urchin, *Evechinus chloroticus* (Delorme & Sewell, 2014), and pH decrease and temperature increase showed an addictive effect through the embryonic development (i.e. increase deformities) in the Atlantic cod, *Gadus morhua* (Dahlke et a., 2017). Despite their susceptibility to the effects of climate change, studies showing the vulnerability of estuarine early stages are scarce (Przeslawski et al., 2015; Gunderson et al., 2016).

In mangroves, macrofauna is delimited to a few dominant groups which are generally constituted by ectothermic decapod crustaceans and mollusks (Nagelkerken et al. 2008). Among them, fiddler crabs are a key group where their burrowing and feeding behaviour plays an important role in mangrove soil conformation (Kristensen et al., 2008; Citadin et al., 2016; Natálio et al., 2017). Their reproductive strategy varies among species: whereas some female fiddler crabs remain inside burrows during egg development, others behave outside when carrying eggs, experiencing all daily abiotic changes in the surrounding environment (Christy & Wada, 2015). The latter case is represented by *Leptuca thayeri* (previously *Uca thayeri*) Rathbun, 1990, one of the most

widespread fiddler crab species (Costa & Negreiros-Fransozo, 2002; Thurman et al., 2013). At this sense, to better understand the impacts of coastal warming and acidification in the embryonic development of an estuarine species, we first (1) characterized the abiotic factors of the microhabitat of ovigerous *L. thayeri* during the embryo development and then (2) verified the combined effect of temperature increase and pH decrease in their embryonic development using the following response variables: development rate, survivorship, egg volume and ammonia concentration. Based on its biology, we predicted a synergetic negative effect of temperature increase and pH decrease in the embryonic development of *L. thayeri*, hypothesizing lower development rate, higher mortality and lower volume of eggs under extreme conditions with higher excretion of nitrogenous waste (i.e., ammonia concentration) under warmer and more acid treatments.

Material and Methods

Study site

Field samplings of crabs and abiotic factors were conducted in Portinho Mangrove at the Ézio Dall' Aqua City Park, Praia Grande - São Paulo/Brazil (23°59'16.74" S 46°24'26.28" W). The mangrove forest is situated in the `Mar Pequeno´ estuarine system (Santos - São Vicente Estuary Complex) and characterized as a semidiurnal tide cycle with forests colonized by *Avicennia schaueriana*, followed by *Rhizophora mangle* and *Laguncularia racemosa*. Several studies with fiddler crabs were conducted at this area (e.g., Gusmão-Junior et al., 2012; Machado et al., 2013; Checon & Costa, 2017; Príncipe et al., 2018) due the well-established communities of crabs from different species, including *L. thayeri*.

Microhabitat characterization

Abiotic parameters were sampled in seven campaigns during spring and summer periods of 2017 (January to March and September to December) (peak of reproduction of *L. thayeri*, Gusmão-Junior et al., 2012). We measured the temperature (thermometer Lutron TM-946 (±0.01°C)), pH (pHmeter mPA2010 MS TECNOPON® (±0.01)) and salinity (Handheld Refractometer ATC®) of water from burrows (microhabitat) of *L. thayeri* ovigerous females (embryos at initial stage of development) and in the mangrove surrounding area (closest water body). Previous observations of ovigerous females of our model species indicate that a vivid red egg mass represents embryos at the first stage of development (as also stated by Christopher et al., 2008). Burrows with chimneys were randomly chosen and sampled in the central region of the mangrove forest. Burrow water temperature were measured with thermocouples (10 cm depth) in situ and then water was collected with syringes with a plastic tube (~10 cm) to analyse pH and salinity in laboratory. All analyses were conducted in less than 30 minutes after field samplings; prior samplings showed that these abiotic factors (pH and salinity) are not significantly changed during this period of time. Abiotic factors were obtained first and then crab carapace width (CW) were measured with calipers. *Leptuca thayeri* ovigerous females build burrows with higher chimneys (Gusmão-Junior et al., 2012); however, not all burrows with chimneys were inhabited by ovigerous crabs at the initial stage of development and those were excluded from our analysis. Air temperature was also measured at each burrow or closest body water measurement. All samplings occurred in daylight spring low tides. Our sampling strategy was taken due to fiddler crabs usually close their burrows in high tide periods (de la Iglesia et al., 1994; Fusi et al., 2015); also, despite recognized activity of some species when covered by water, ovigerous *L. thayeri*

were not observed behaving outside burrows in the study conducted by De Grande et al. (2018).

Crab collection and maintenance

Ovigerous females with embryos at initial stage of development were manually collected during the summer period of 2018 (January to February) by excavating their burrows. Crabs were maintained for one day in aquariums containing PVC pipe connectors (2.5 cm of diameter) to provide shelter and a thin water column [artificial sea salt (Blue Treasure Reef Sea®) dissolved in deionized water (33 PSU – based on microhabitats' mean value)]. A 12h light:dark photoperiod cycle and controlled air temperature (~25°C) were maintained during acclimation.

Experimental design and water chemistry

We verified how predicted coastal warming and acidification affect the embryo development of the fiddler crab *L. thayeri*. Embryos were exposed to 4 factorial combinations (2x2) of temperature increase [control (26ºC), + 4.0 degrees (30ºC)] and pH decrease [control (6.9), - 0.7 units (6.2)]. Control of temperature and pH were based on abiotic field samplings of the microhabitat (burrows with chimneys) of *L. thayeri* ovigerous. Higher temperature is based on 2081-2100 values predicted by general circulation models under the IPCC (2014) scenario RCP 8.5 and lower pH by Caldeira & Wicket (2005) and IPCC (2014) for the late 21st century. Temperature was controlled using thermostats (Aquarium heater HOPAR® H-606) and pH values were reached bubbling CO_2 gas. Abiotic factors were checked each hour until stabilized and three times a day for temperature and pH or day after day for salinity throughout the experiment to maintain their values. As gas bubbling was manually controlled, we had a pH oscillation

which did not exceed ± 0.3 during the experiment with a standardized variation for all treatments. Saltwater temperature was monitored with a thermometer Lutron® TM-946 (±0.01°C) with OMEGA® thermocouples and pH with a pHmeter PG 1400 Gehaka® with an electrode 2A09EBI Analyser® (±0.01). Prior to use, pHmeter calibration was performed with 7.1 and 4.01 buffers (Gehaka®). Desired higher temperature and lower pH values were gradually reached over 1 day, beginning in control values when running the experiment.

Partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3^-), carbonate (CO_3^{2}), aragonite saturation state (ΩArag) and calcite saturation state (ΩCalc) were calculated with pH, total alkalinity (TA) (T50 automatic titrator Mettler toledo®), temperature (T) and salinity obtained at begging and end of the experiment. We calculated with CO2SYS (Robbins et al., 2010) using carbonate dissociation constants K1 and K2 from Mehrbach et al. (1973) refitted by Dickson & Millero (1987) and KSO4 from Dickson (1990) (see Supplementary Table 1 for water chemistry details). Water level was constantly refilled in our microcosms (see details in 'Experimental apparatus' below) with distilled water to maintain controlled salinity values.

The experiment was performed twice for 10 days in January-February/2018. Nearly 10-11 days of development, embryos are expected to present completed eyes, yolk in four lobes and strong heart beat representing an advanced stage of embryonic development (Stage X for *A. lactea* - Yamaguchi, 2001). We used detached embryos in the initial stage of development (blastula stage, based in Yamaguchi, 2001 – see Supplementary Figure 1 for details) from 12 ovigerous females to exclude the maternal effect in egg development and female bias. Roggatz et al. (2016) claimed that ovigerous female crabs, which provide oxygen to embryos during their development, had their clutch ventilation and egg mass care behaviour affected by seawater acidification.

Detached embryos developed when separate from their clutch (e.g., Miller et al., 2016) and females are in charge of controlling their hatching time (De Vries & Forward, 1989). Thus, we eliminated the female crab influence to verify the effects of high temperature and low pH in embryos itself. A portion of the clutches were detached with forceps from the central region of the egg mass. Embryo aggregations (EA) were gently detached from the portion of the clutch under a stereomicroscope (ZEISS Discovery.V8 – Axiocam Erc 5s) with thin hypodermic needles. Each aggregation consisted in 20-40 embryos; most of the embryo filaments detached from the clutch had nearly 20-40 eggs each and to ensure embryo integrity we maintained the EA with their natural egg disposition. Six EA of each female were photographed with the same stereomicroscope mentioned above to posterior analysis. Images were taken at the same magnification and scale (0.2 mm). Embryo aggregations (n = 576/ 144 per treatment) had their eggs counted, transferred using pipets to a well (2 ml vol each) in a 24 Well Cell Culture Plate and then reallocated to the microcosms with saltwater at control treatment parameters. Transfer pipets and needles were constant cleaned during the detaching process with distilled water to avoid contamination. Crabs had measured their carapace width using calipers (mean ± SE: 16.29±0.49 mm).

Experimental apparatus

Microcosms (n = 24/ 6 per treatment) consisted in tanks (39 x 9 x 14) filled with 4.91 liters of artificial saltwater (same mentioned above in 'Crab collection and maintenance'). Each tank had a 24 Well Cell Culture Plate attached with Velcro. Plastic bottom of each well was removed and substituted by a nylon mesh (180 µ) allowing water circulation. Each tank had two connected compartments: one for the culture plates and other for controlling abiotic factors and apparatus setup including the thermostat, plastic

tubes providing CO_2 gas and oxygen supply by air pumps with air stones bubbling at similar intensity (see Supplemental Figure 2 for details). Tanks were checked daily and equally refilled, when necessary, in the setup compartment to prevent heat and pH shock. Photoperiod regime were settled in a 12h light:dark cycle.

Response variables: Stage of development, survivorship, egg volume and ammonia concentration

Day after day until the end of the experiment, EA from three different females (n = 3 EA per microcosm totalizing 9 per treatment) had all eggs (20-40) photographed as mentioned above and embryos scored as dead or alive. Embryos with pale yellow/white coloration and degenerated or relatively underdeveloped eggs were considered dead (Förster & Baeza, 2001; Davis et al., 2013) (see Supplementary Figure 1 for details). Embryonic stages were based in the detailed classification of Yamaguchi et al. (2001) and classified as: (1) blastula (egg capsule with almost undistinguished individual cells with near 24 to 48 hours after ovulation), (2) gastrula (small yolk-free portion with a formed germinal disc), (3) advanced gastrula (yolkfree portion increased), (4) early larvae (differentiation of limb buds, decreasing yolk mass, development of eye placodes and eyes and faint heartbeat) and (5) pre hatching larvae (completed eye, yolk in four lobes, strong heart beat) (see Supplementary Figure 1 for images of all embryonic stages). Stages were assessed according to the specific stage in which most embryos were observed. We assumed the mean stage when two stages were found in similar number (methods based in Davis et al. (2013)). EA were systematically chosen day after day to do not repeat the same pattern of females analysed and prevent female bias over time. None EA returned to the microcosms after manipulation. At the end of experiment, we

247 photographed the remaining EA to posterior analysis and also scored as dead or alive (20-
248 40 embryos).

249 We estimated the differences between initial and final volume of embryos using
250 the formula for oblate spheroids: $V=1/6(\pi \cdot d^2 \cdot D)$ [d= smaller diameter, D = larger
251 diameter] (Simoni et al., 2011). Embryos from the central region of the EA were measured
252 (smaller and larger diameter) with ImageJ. Five embryos of 3 EA from each female clutch
253 were used to estimate the initial embryonic volume (n = 36 EA/ 180 embryos). Since not
254 all EA were alive at the final of experiment, 36 EA (live embryos at final stage of
255 development) of each treatment were chosen randomly using a random sequence
256 generator (n = 144 EA/ 720 embryos). Similar number of EA (n = 36 EA/ 180 embryos)
257 per treatment were analysed for all response variables (stage of development,
258 survivorship and egg volume) at the end of experiment.

259 Ammonia concentration were estimated at the beginning (12h after embryos were
260 inserted in the microcosms, 1st day), middle (5th day) and end of the experiment (10th day)
261 sampling a water aliquot (250ml) of each microcosm (n = 3 per treatment/period).
262 Ammonia concentration of each treatment was determined by colorimetry reading
263 samples at 630 nm in a spectrophotometer (HACH DR/2400) (Koroleff, 1976).

264

265

266

267 **Statistical analysis**

268 Data were checked for normality and homoscedasticity using Shapiro–Wilk's and
269 Levene's test, respectively. Differences between abiotic parameters in microhabitats and
270 the surrounding environment was assessed by an independent t test to compare pH,
271 temperature and salinity between burrows and closest water body. Temperature anomaly
272 (Ta) of air and burrow or closest water body temperature (i.e., Ta = burrow temperature

- air temperature) were compared applying a paired t test. Also, a simple linear regression analysis was applied to estimate the relationship between water inside burrows and air temperature.

Stages of development were analysed with a two-way analysis of variance (ANOVA) using Treatment and Time (days) as fixed factors and Stage of development (1-5) as dependent variables. Additionally, we applied an one-way ANOVA to verify the embryonic stage at the end of experiment (10^{th} day) among treatments. Probability of survival over time was assessed with survival curves using Kaplan-Meier test with Log-Rank and post Chi-Square comparisons; a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used to compare initial and final volume of embryos and survivorship among treatments in the last day of experiment. Since some EA showed dead and live embryos at the same aggregation, we scored each embryo as `dead or alive´. Survival data showed a binomial distribution and were arcsin square root transformed. The assumption of normality was not achieved for volume data and we assumed that ANOVA is robust to deviations from normality in large and balanced data sets (Underwood, 1997; Schmider et al., 2010). A Generalized Linear Model (GLM) for Repeated measures was used to identify differences on ammonia concentration over time and treatments. We applied the Chauvenet's criterion to identify outliers in ammonia data to avoid bias due contamination.

Values throughout the text were reported as mean and standardized error. The critical level (α) was set at 95% of confidence interval (α= 0.05). Analyses were conducted in the software SPSS 25.0 (SPSS Inc., Armonk, NY, U.S.A.).

Results

Microhabitat characterization

We measured the abiotic factors of 39 burrows with chimneys (microhabitat) habited by ovigerous crabs (mean CW: 16.14±0.55) at initial stage of development. All abiotic parameters differed between microhabitat and closest water body. Water inside burrows presented lower temperature and pH and higher salinity than the closest water body (Table 1). Air temperature in mangrove forest during our measurements was 24.44±0.28 °C, being lower (paired t test: t = -6.202, df = 38, p = 0.0000) and positively related (regression: N = 39, df = 38, F = 32.5, R^2 = 0.47, p = 0.0000) with the water inside burrows.

Table 1. Mean (±SE) of temperature, pH and salinity values in the microhabitat (N = 39) and closest water body. Data is presented as t values (t), degrees of freedom (df) and p value from an independent t test.

Abiotic factors	Burrow	Closest water body	t	df	p value
Temperature (°C)	26.16±0.38	28.42±0.36	-5.331	76	0.0000
pH	6.89±0.03	7.75±0.04	-15,284	76	0.0000
Salinity (PSU)	33.51±0.56	25.33±0.65	9,510	76	0.0000

Stage of development

Embryonic development differed among treatments and time (Table 2). Stages among treatments did not differ until the 2nd day (Tukey HSD: p > 0.05). Control temperature treatments showed similar development trend over time (Tukey HSD: p > 0.05); same pattern was observed for the high temperature treatments, which means a non-direct pH effect in their development (Tukey HSD: p > 0.05). Otherwise, the `high

319 temp. and low pH´ treatment showed the more advanced stages at the 4th day among
320 treatments (Tukey HSD: $p < 0.05$).

321 All treatments presented similar embryonic stage of their live embryos at the last
322 day of experiment (10th day) (one way ANOVA: df = 3, F = 1.373, p = 0.254). The
323 majority of embryos were at their latter stage of development (Stage 5: 140/144 embryos).
324

325 **Table 2**. Two-way ANOVA used to evaluate the effects of treatment and time (days) on
326 the embryonic stages of development.

Source of Variation	df	MS	F	p-value
Treatment (T)	3	2.848	10.442	0.0000
Time (TI)	4	31.810	116.620	0.0000
T × TI	12	0.438	1.607	0.109
Residue	71	0.273		

327
328

329 **Survivorship**

330 Control temperature treatments showed similar probability of survival over
331 embryonic development (`control temperature – control pH´: 79.1%; `control temperature
332 – low pH´: 80%) (Kaplan-Meier, Log Rank: $\chi 2$: 0.93, p = 0.33). Higher temperature,
333 independent of pH conditions, affected embryos over time (Kaplan-Meier, Log Rank: p
334 < 0.001), showing the lower percent of survivorship (´high temperature – control pH´:
335 68,8 %; ´high temperature - low pH´ treatment: 72,4%) (Supplemental Table 2 for
336 pairwise comparisons and values) (Figure 1).

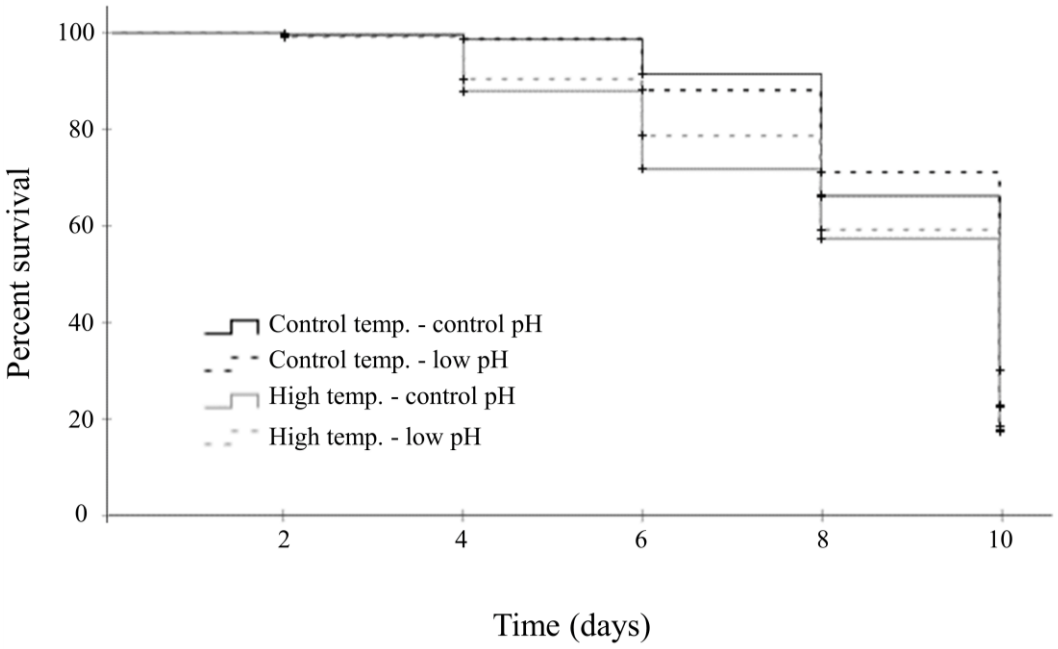


Figure 1. Survival analysis of *Leptuca thayeri* embryos (N = 180 per treatment) in four different combinations of temperature and pH over embryonic development: control temperature – control pH, control temperature – low pH, high temperature – control pH and high temperature – low pH.

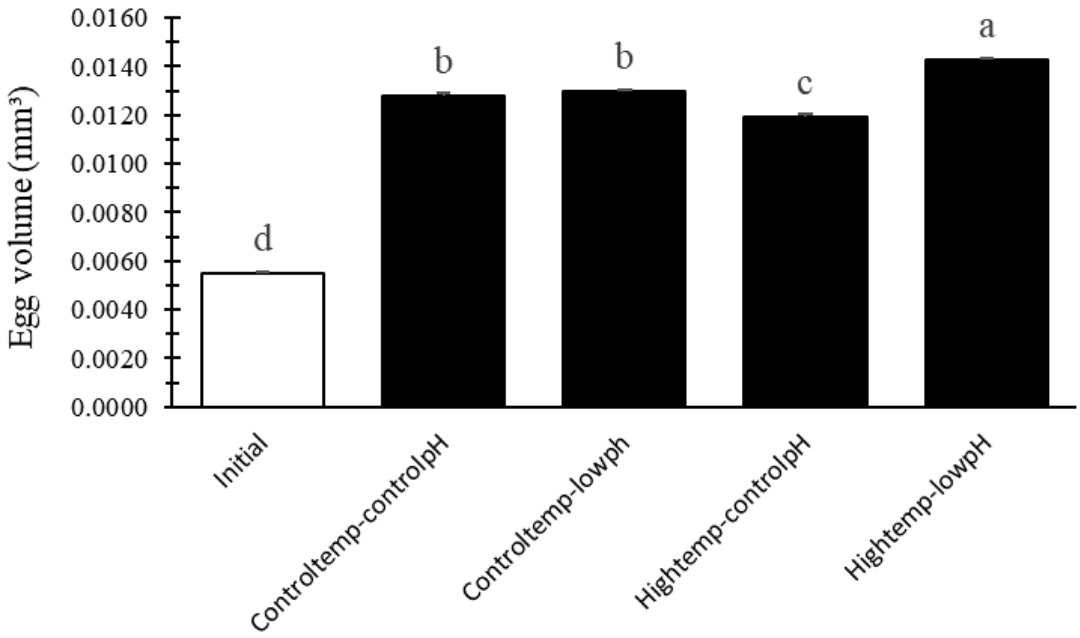
Survivorship rate differs among treatments (one-way ANOVA: df = 3, F = 6.138, p = 0.0003). Similar to the probability of survival results, control temperature treatments showed more live embryos than high temperature groups at their latter stage of development (Tukey HSD: $p < 0.05$) with low pH do not affecting their survivorship rate (Tukey HSD: $p > 0.05$) (Table 3). Some of dead embryos were associated with undefined symbionts, partially or entirely covering their circumference (Table 3).

Table 3. Summary of the survivorship of embryos under the different treatment conditions of temperature and pH highlighting dead, infected and alive embryos at the end of experiment (total num. and %).

Treatment		Embryos		
Temperature	pH	Total	Dead (Infected)	Alive
Control	Control	893	298 (220)	595 (66.63%)
	Low	917	362 (210)	555 (60.52%)
High	Control	943	396 (330)	547 (58.01%)
	Low	804	337 (268)	467 (58.08%)

Egg volume

The initial volume of eggs was lower than final volume for all treatments (ANOVA: $F_{4,895}$ = 1243.927, p = 0.000; Tukey's HSD: p = 0.0000). Except when compared the control treatment and control temperature with low pH (Tukey's HSD: p = 0.858), all other treatments differed in their egg volume values (Tukey's HSD: p = 0.0000). The `high temperature – control pH´ treatment showed the lower and `high temperature – low pH´ the more voluminous eggs (Tukey's HSD: p = 0.0000), highlighting an apparent synergic effect of high temperature and low pH (Figure 2).



365

366 **Figure 2**. Variation in egg volume (Mean ±SE) (N = 180 per treatment) of *Leptuca*
367 *thayeri* at initial and final embryonic development stages for all experimental treatments:
368 control temperature – control pH, control temperature – low pH, high temperature –
369 control pH and high temperature – low pH. Different letters indicate statistical difference
370 between treatments (Tukey's HSD, $p < 0.05$).

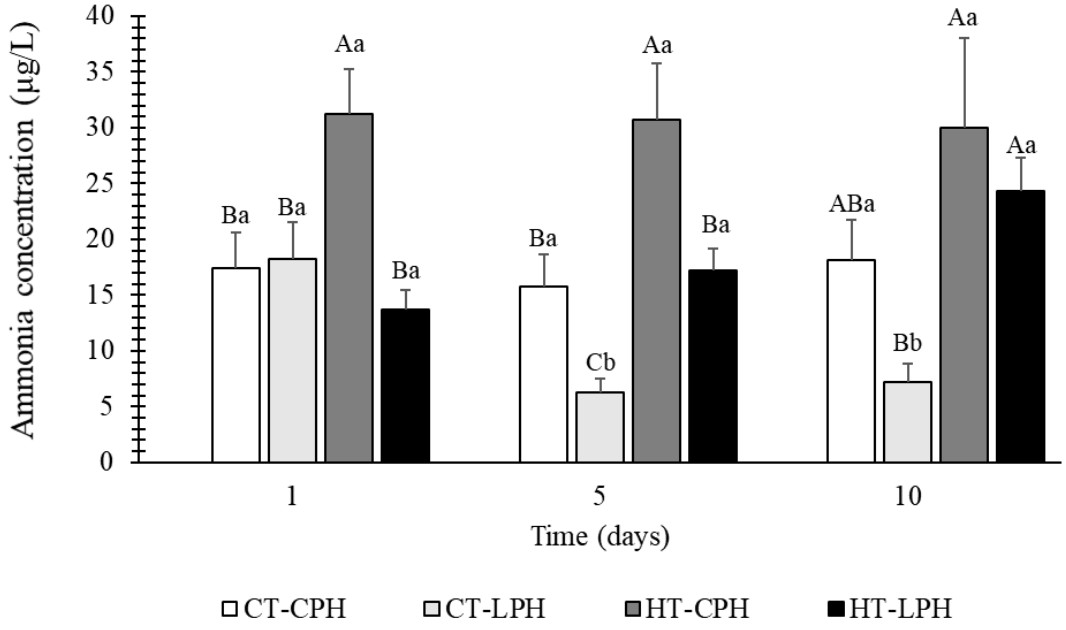
371

372

373 **Ammonia concentration**

374 Ammonia concentration was higher in the `high temperature – control pH´
375 treatment from the beginning until the 5th day of experiment (GLM Repeated measures:
376 df = 3, ms = 0.145, F = 8.359, p = 0.000; *post hoc* LSD: $p < 0.05$) (Figure 3). The 10th
377 day of experiment showed similar ammonia concentration among treatments (*post hoc*
378 LSD: $p > 0.05$), except for the lower values in the control temperature and low pH, being
379 the only treatment which decreased ammonia concentration following the embryo
380 development (*post hoc* LSD: p = 0.011) since 5th day (Figure 3; supplementary Table 4).

381



382

383 **Figure 3.** Mean (±SE) of ammonia concentration at the beginning (1st day), middle (5th
384 day) and end of the experiment (10th day) for the different experimental treatments:
385 control temperature – control pH (CT-CPH), control temperature – low pH (CT-LPH),
386 high temperature – control pH (HT-CPH) and high temperature – low pH (HT-LPH).
387 Different uppercase letters indicate significant differences among treatments at the same
388 day and lowercase letters indicate significant differences among each treatment over time
389 (GLM Repeated measures, post hoc LSD: $p < 0.05$).

390

391 **Discussion**

392 We investigated the effects of predicted costal warming and acidification in the
393 embryonic development of the fiddler crab *L. thayeri*. Microhabitats (burrows with
394 chimneys) of ovigerous females presented distinct abiotic factors (pH, temperature and
395 salinity) values from the surround environment. Our results indicated that high
396 temperature and low pH affected embryos and, despite ontogenic and morphological
397 constraints, several embryos deal with those adverse conditions until their late stages.
398 High temperature accelerated their development and increased mortality, independently

399 of pH conditions. Higher egg volume was observed in a warmer and acidified
400 environment and lower volume in warmer and non-acidified conditions, supporting a
401 synergetic and negative effect of pH and temperature through their embryonic
402 development of *L. thayeri.*

403 Ovigerous females of fiddler crabs use burrows as their microhabitat over the
404 embryo development. Burrows are behaviour- (territorial marking, protection against
405 predators) and physiological (thermal refuge, egg incubation) significant to their builders
406 and considered part of the animals themselves (Laland et al., 2014). From beehives to
407 beavers dams, their microhabitat creates a suitable environment usually differing internal
408 and external abiotic conditions (Hansell, 2007; Laland et al., 2014). Temperature inside
409 crab burrows are usually cooler than outside (e.g., *Leptuca panacea*, Power & Colle,
410 1976). Watson et al. (2018) demonstrated that burrows of the ghost crab *Ocypode*
411 *cordimanus* provide a thermal refugia at different periods of the day: buffers the intense
412 heat of open coastal area during daylight and maintain a constant temperature at night.
413 Our results challenge those inferences as we observed higher temperature inside burrows
414 when compared to air temperature at daylight. However, the mentioned species are from
415 non-vegetated areas and those studies evaluated air temperature inside burrows. *L.*
416 *thayeri*, our model species, lives in vegetated areas and their burrows are constantly
417 flooded, being water a better heat conductor than air. Despite those contrasts, we can
418 expect that burrows of *L. thayeri* ovigerous females also maintain abiotic conditions.
419 Water is a better heat conductor than air and its circulation is reduced inside burrows;
420 burrow internal parameters tend to be distinct than surrounding areas, as we observed for
421 temperature, pH and salinity. Daily temperature fluctuation reduces the optimum and
422 critical temperature and also overall fitness of ectotherms which in turn makes them more
423 sensitive to climate change (Paaijmans et al., 2013). Stable environments are important

424 for embryonic development and their burrows may increase rate and success of healthy
425 embryos, being the sedimentary structure in the shape of chimneys hypothetically an
426 important factor on that dynamic (Gusmão-Júnior et al., 2013). Since we observed that
427 temperature inside burrows are related with air temperature, the tendency of a warming
428 climate may have direct impacts on embryo development.

429 Development rate of ectotherms is temperature-dependent. The success of
430 embryonic development was assessed in twenty-one species of British crustaceans
431 showing trends among groups (Wear, 1974 and literature within) and temperature was
432 the main factor observed affecting their early stages. At the present study, embryos were
433 at similar stage until the 2nd day, following a rapidly development in day 04 at the most
434 extreme treatment. As stages were also similar within control and high temperature
435 groups, our results also support that temperature is one of the main drivers in embryonic
436 development, resulting in a faster growth (Wear, 1974; Anger, 2001; Przeslawski et al.,
437 2015). However, faster development, as we observed in a warmer environment, does not
438 mean healthier development. Larval viability, fitness and risk of mortality are usually
439 reduced when embryonic development occurred with temperature ranging outside their
440 trivial variations (Wear, 1974; Kroeker et al., 2013; Harvey et al., 2013). As we observed,
441 high temperature resulted in higher mortality over time in the embryonic development of
442 our model species. In a robust meta-analysis, Harvey et al. (2013) evidenced that
443 survivorship of early-life history stages is negatively and synergistically affect by
444 temperature and pH. Thermal stress and low pH has been recognized as an important
445 threat to oxygen supply, calcification, growth, reproduction, survival and so forth (Pörtner
446 & Knust 2007; Bozinovic & Pörtner, 2015; Griffen et al., 2016) and survivorship is
447 among the most evaluated response variable to those stressors (Crain et al., 2008;
448 Przeslawski et al., 2015).

Low pH environment does not seem to significantly influence egg development or even survivorship. Examples of tolerance to low pH conditions are observed across-taxa where growth, development and survivorship of estuarine and marine early-life stages were not or partially affected (Pansch et al., 2012; Range et al., 2012; Miller et al., 2016; Gravinese, 2017; Jarrold & Munday, 2018). Coastal pH is naturally more variable and complex to predict than open ocean (Hofmann et al., 2011; Carstensen, et al., 2018) and some habitats already experience similar or wider pH variation than predictions for the late 21st century (Dai et al., 2009; Duarte et al., 2013; Carstensen et al., 2018). However, the egg volume may entail a complementary interpretation of the low pH effect on our results. Embryos at the `high temperature and control pH treatment´ may had their metabolic rate increased and exceed their physiological threshold, which explain their lower volume and higher ammonia concentration until their middle development stages. Otherwise, eggs at the most extreme treatment may be not considered more successfully developed despite their higher volume. Their higher volume may be linked to the lack of osmoregulation capacity and ammonia concentration due acid–base (extracellular acidosis), ionic balance and gases exchange due high pCO_2 concentrations (Simoni et al., 2011; Gravinese, 2017), being the decrease of ammonia concentration in low pH treatments an important indicative of that prediction. Those ammonia concentration results also support a non-expressive effect of the occasional difference in the number of embryos among treatments where ammonia values were consistent with our predictions and general pattern. Interestingly, an interactive effect (temperature*pH) resulting in a negative sublethal response in marine biodiversity was not previously reported in Harvey et al.'s (2013) meta-analysis. Summing up the effects on *L. thayeri*'s embryos, temperature increases development rate and pH may disrupts their homeostasis affecting exchanges with surrounding environment.

Symbionts (e.g. bacteria, fungi) were observed in association with embryos and may have also impacted their development. They are commonly found in crustaceans and their clutch in coastal environments (Fisher, 1976; Alda et al., 2011; Silva et al., 2007). Those communities are linked with embryo mortality competing with embryos for oxygen and restricting the gas exchange within egg mass (Fisher, 1976; Silva et al., 2007). Oxygen provision to egg mass by ovigerous females is intensify through the embryo development (Baeza & Fernández, 2002), and crustacean embryos with insufficient oxygen supply by maternal care (lack of grooming) may have their ontogeny development delayed or even die (Förster & Baeza, 2001). Warmer environments favor the symbiont development and further research could be undertaken to investigate this cascade relationship (high temperature - embryo development - symbionts) under the perspective of a warming climate.

Warming and acidification have been heavily discussed as important stressors in marine and costal habitats (Duarte et al., 2013; Rudd, 2014; Nagelkerken & Connell, 2015; Gunderson et al., 2016). In a climate change context, abiotic stressors drive directional selection and define `winners and losers´ at different scales (Somero, 2010). Physiological plasticity plays an important role in how species deal with climate change (Seebacher et al., 2015), and the relevance of individual genetic variability among individuals may trigger species acclimation defining their adaptation by natural selection (Pistevos et al., 2011). It turns more relevant when that responses are observed in an estuarine calcified ectothermic with r-selected reproductive strategy, such as the case of fiddler crabs. In our study, an expressive number of embryos were alive and apparently ready to hatch (morphological perspective) at the end of our experiment in controlled and extreme abiotic conditions. Thus, despite the observed negative impacts of near-future conditions, it would be important to understand the potential adaptation of fiddler crabs

499 in the next life-stages (e.g., larvae) under predicted abiotic factors. Our results gave
500 indicatives that leads to the need of a deeper comprehension of that impacts among
501 ontogeny, populations and generations.

502 **References**

503 Alda, P., La Sala, L., Marcotegui, P. and Martorelli, S.R., 2011. Parasites and
504 epibionts of grapsid crabs in Bahía Blanca estuary, Argentina. *Crustaceana*, *84*(5),
505 pp.559-571.

506 Alongi, D.M., 2002. Present state and future of the world's mangrove
507 forests. *Environmental conservation*, *29*(3), pp.331-349.

508 Anger, K., 2001. *The biology of decapod crustacean larvae* (Vol. 14, pp. 1-420).
509 Lisse: AA Balkema Publishers.

510 Baeza, J.A. and Fernández, M., 2002. Active brood care in *Cancer setosus*
511 (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen
512 consumption and the cost of brooding. *Functional Ecology*, *16*(2), pp.241-251.

513 Bozinovic, F. and Pörtner, H.O., 2015. Physiological ecology meets climate
514 change. *Ecology and evolution*, *5*(5), pp.1025-1030.

515 Byrne, M., Gonzalez-Bernat, M., Doo, S., Foo, S., Soars, N. and Lamare, M.,
516 2013. Effects of ocean warming and acidification on embryos and non-calcifying larvae
517 of the invasive sea star *Patiriella regularis*. *Marine Ecology Progress Series*, *473*,
518 pp.235-246.

519 Byrne, M. and Przeslawski, R., 2013. Multistressor impacts of warming and
520 acidification of the ocean on marine invertebrates' life histories. *Integrative and*
521 *comparative biology*, *53*(4), pp.582-596

Caldeira, K. and Wickett, M.E., 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research: Oceans*, *110*(C9).

Calosi, P., Melatunan, S., Turner, L.M., Artioli, Y., Davidson, R.L., Byrne, J.J., Viant, M.R., Widdicombe, S. and Rundle, S.D., 2017. Regional adaptation defines sensitivity to future ocean acidification. *Nature communications*, *8*, p.13994.

Carstensen, J., Chierici, M., Gustafsson, B.G. and Gustafsson, E., 2018. Long-term and seasonal trends in estuarine and coastal carbonate systems. *Global Biogeochemical Cycles*. DOI: 10.1002/2017GB005781

Checon, H.H. and Costa, T.M., 2017. Fiddler crab (Crustacea: Ocypodidae) distribution and the relationship between habitat occupancy and mouth appendages. *Marine Biology Research*, *13*(6), pp.618–629.

Christopher, C.E., Salmon, M. and Forward Jr, R.B., 2008. Is the hatching clock of fiddler crab larvae (*Uca thayeri*) phenotypically plastic?. *Journal of Crustacean Biology*, *28*(2), pp.328-333.

Christy, J.H. and Wada, K., 2015. *Social ethology in Brachyura*. In: Decapoda: Brachyura, Treatise on Zoology–Anatomy, Taxonomy, Biology, 9, pp.417-468.

Citadin, M., Costa, T.M. and Netto, S.A., 2016. The response of meiofauna and microphytobenthos to engineering effects of fiddler crabs on a subtropical intertidal sandflat. *Austral ecology*, *41*(5), pp.572–579.

Cohen, J.M., Lajeunesse, M.J. and Rohr, J.R., 2018. A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, p.1.

Costa, T.M. and Negreiros-Fransozo, M.L., 2002. Population biology of *Uca thayeri* Rathbun, 1900 (Brachyura, Ocypodidae) in a subtropical South American

546 mangrove area: results from transect and catch-per-unit-effort
547 techniques. *Crustaceana*, *75*(10), pp.1201–1218.

548 Crain, C.M., Kroeker, K. and Halpern, B.S., 2008. Interactive and cumulative
549 effects of multiple human stressors in marine systems. *Ecology letters*, *11*(12), pp.1304–
550 1315.

551 Dahlke, F.T., Leo, E., Mark, F.C., Pörtner, H.O., Bickmeyer, U., Frickenhaus, S.
552 and Storch, D., 2017. Effects of ocean acidification increase embryonic sensitivity to
553 thermal extremes in Atlantic cod, *Gadus morhua*. *Global Change Biology*, *23*(4),
554 pp.1499-1510.

555 Dai, M., Lu, Z., Zhai, W., Chen, B., Cao, Z., Zhou, K., Cai, W.J. and Chenc,
556 C.T.A., 2009. Diurnal variations of surface seawater pCO2 in contrasting coastal
557 environments. *Limnology and Oceanography*, *54*(3), pp.735–745.

558 Davis, A.R., Coleman, D., Broad, A., Byrne, M., Dworjanyn, S.A. and
559 Przeslawski, R., 2013. Complex responses of intertidal molluscan embryos to a warming
560 and acidifying ocean in the presence of UV radiation. *PloS one*, *8*(2), p.e55939

561 De Grande, F.R., Colpo, K.D., Queiroga, H., Cannicci, S. and Costa, T.M., 2018.
562 Contrasting activity patterns at high and low tide in two Brazilian fiddler crabs
563 (Decapoda: Brachyura: Ocypodidae). *Journal of Crustacean Biology*. *38*, pp.407–412.

564 De la Iglesia, H.O., Rodríguez, E.M. and Dezi, R.E., 1994. Burrow plugging in
565 the crab *Uca uruguayensis* and its synchronization with photoperiod and
566 tides. *Physiology & Behavior*, 55(5), pp.913–919.

567 Delorme, N.J. and Sewell, M.A., 2014. Temperature and salinity: two climate
568 change stressors affecting early development of the New Zealand sea urchin *Evechinus*
569 *chloroticus*. *Marine biology*, *161*(9), pp.1999–2009.

De Vries, M.C. and Forward, R.B., 1989. Rhythms in larval release of the sublittoral crab *Neopanope sayi* and the supralittoral crab *Sesarma cinereum* (Decapoda: Brachyura). *Marine Biology*, *100*(2), pp.241-248.

Dickson, A.G., 1990. Standard potential of the reaction AgCls + 1/2 H2 = Ags + HClAq and the standard acidity constant of the ion HSO4-_in synthetic seawater from 273.15-K to 318.15-K. *Journal of Chemical Thermodynamic 22*, 113e127.

Dickson, A.G. and Millero, F.J., 1987. A comparison of the equilibrium-constants for the dissociation of carbonic-acid in seawater media. *Deep Sea Research Part A. Oceanographic Research papers. 34*, 1733–1743.

Duarte, C.M., Hendriks, I.E., Moore, T.S., Olsen, Y.S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J.A. and McCulloch, M., 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts*, *36*(2), pp.221–236.

Duke, N., Ball, M. and Ellison, J., 1998. Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology & Biogeography Letters*, *7*(1), pp.27–47.

Fisher, W.S., 1976. Relationships of epibiotic fouling and mortalities of embryos of the Dungeness crab (*Cancer magister*). *Journal of the Fisheries Research Board of Canada*, *33*, pp.2849–2853

Förster, C. and Baeza, J.A., 2001. Active brood care in the anomuran crab *Petrolisthes violaceus* (Decapoda: Anomura: Porcellanidae): grooming of brooded embryos by the fifth pereiopods. *Journal of Crustacean Biology*, *21*(3), pp.606–615.

592 Fusi, M., Giomi, F., Babbini, S., Daffonchio, D., McQuaid, C.D., Porri, F. and
593 Cannicci, S., 2015. Thermal specialization across large geographical scales predicts the
594 resilience of mangrove crab populations to global warming. *Oikos*, *124*(6), pp.784-795.

595 Gravinese, P.M., 2017. Ocean acidification impacts the embryonic development
596 and hatching success of the Florida stone crab, *Menippe mercenaria*. *Journal of*
597 *Experimental Marine Biology and Ecology*. *500*, pp.-140-146.

598 Griffen, B.D., Belgrad, B.A., Cannizzo, Z.J., Knotts, E.R. and Hancock, E.R.,
599 2016. Rethinking our approach to multiple stressor studies in marine
600 environments. *Marine Ecology Progress Series*, *543*, pp.273-281.

601 Gunderson, A.R., Armstrong, E.J. and Stillman, J.H., 2016. Multiple stressors in
602 a changing world: the need for an improved perspective on physiological responses to the
603 dynamic marine environment. *Annual Review of Marine Science*, *8*, pp.357-378.

604 Gusmão-Junior, J.B., Machado, G.B. and Costa, T.M., 2012. Burrows with
605 chimneys of the fiddler crab *Uca thayeri*: construction, occurrence, and
606 function. *Zoological studies*, *51*(5), pp.598-605.

607 Hansell, M. 2007. *Built by animals: the natural history of animal architecture*.
608 New York: Oxford University Press

609 Harvey, B.P., Gwynn-Jones, D. and Moore, P.J., 2013. Meta-analysis reveals
610 complex marine biological responses to the interactive effects of ocean acidification and
611 warming. *Ecology and evolution*, *3*(4), pp.1016-1030.

612 Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F.,
613 Paytan, A., Price, N.N., Peterson, B., Takeshita, Y. and Matson, P.G., 2011. High-
614 frequency dynamics of ocean pH: a multi-ecosystem comparison. *PloS one*, 6(12),
615 p.e28983.

IPCC, 2014. Climate change 2014: synthesis report. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC Geneva, Switzerland (151 pp).

Jarrold, M.D. and Munday, P.L., 2018. Diel CO2 cycles do not modify juvenile growth, survival and otolith development in two coral reef fish under ocean acidification. *Marine Biology*, *165*(3), p.49.

Koroleff, F., 1976. Determination of ammonia. In: Grasshoff K, Ehrhardt M, Krem-Ling K (eds) Methods of seawater analysis, vol 2. Verlag Chemie, Weinhein, pp 150–157.

Kristensen, E., 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. *Journal of Sea Research*, *59*(1-2), pp.30-43.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M. and Gattuso, J.P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global change Biology*, *19*(6), pp.1884-1896.

Laland, K., Odling-Smee, J. and Turner, S., 2014. The role of internal and external constructive processes in evolution. *The Journal of physiology*, *592*(11), pp.2413-2422.

Lefevre, S., 2016. Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO2 and their interaction. *Conservation physiology*, *4*(1), p.cow009.

637 Machado, G.B., Gusmão-Junior, J.B. and Costa, T.M., 2013. Burrow morphology
638 of *Uca uruguayensis* and *Uca leptodactylus* (Decapoda: Ocypodidae) from a subtropical
639 mangrove forest in the western Atlantic. *Integrative zoology*, *8*(3), pp.307-314.

640 Madeira, D., Narciso, L., Cabral, H.N. and Vinagre, C., 2012. Thermal tolerance
641 and potential impacts of climate change on coastal and estuarine organisms. *Journal of*
642 *Sea Research*, *70*, pp.32-41.

643 Mehrbach, C., Culberso, C.H., Hawley, J.E., Pytkowic, R.M., 1973. Measurement
644 of apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure.
645 *Limnology Oceanography*, *18*, pp.897-907.

646 Miller, J.J., Maher, M., Bohaboy, E., Friedman, C.S. and McElhany, P., 2016.
647 Exposure to low pH reduces survival and delays development in early life stages of
648 Dungeness crab (*Cancer magister*). *Marine Biology, 163* (5), pp.1–11.

649 Nagelkerken, I.S.J.M., Blaber, S.J.M., Bouillon, S., Green, P., Haywood, M.,
650 Kirton, L.G., Meynecke, J.O., Pawlik, J., Penrose, H.M., Sasekumar, A. and Somerfield,
651 P.J., 2008. The habitat function of mangroves for terrestrial and marine fauna: a
652 review. *Aquatic botany*, *89*(2), pp.155-185.

653 Nagelkerken, I. and Connell, S.D., 2015. Global alteration of ocean ecosystem
654 functioning due to increasing human CO2 emissions. *Proceedings of the National*
655 *Academy of Sciences*, *112*(43), pp.13272-13277.

656 Natálio, L.F., Pardo, J.C., Machado, G.B., Fortuna, M.D., Gallo, D.G. and Costa,
657 T.M., 2017. Potential effect of fiddler crabs on organic matter distribution: A combined
658 laboratory and field experimental approach. *Estuarine, Coastal and Shelf Science*, *184*,
659 pp.158-165.

660 Navarro, J.M., Duarte, C., Manríquez, P.H., Lardies, M.A., Torres, R., Acuna, K.,
661 Vargas, C.A. and Lagos, N.A., 2016. Ocean warming and elevated carbon dioxide:

multiple stressor impacts on juvenile mussels from southern Chile. *ICES Journal of Marine Science*, *73*(3), pp.764-771.

Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C. and Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, *19*(8), pp.2373-2380.

Pansch, C., Nasrolahi, A., Appelhans, Y.S. and Wahl, M., 2012. Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*. *Journal of Experimental Marine Biology and Ecology*, *420*, pp.48-55.

Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics.*, *37*, pp.637-669.

Pistevos, J.C., Calosi, P., Widdicombe, S. and Bishop, J.D., 2011. Will variation among genetic individuals influence species responses to global climate change? *Oikos*, *120*(5), pp.675-689.

Pörtner, H.O. and Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, *315*(5808), pp.95-97.

Powers, L.W. and Cole, J.F., 1976. Temperature variation in fiddler crab microhabitats. *Journal of Experimental Marine Biology and Ecology*, *21*(2), pp.141-157.

Principe, S.C., Augusto, A. and Costa, T.M., 2018. Differential effects of water loss and temperature increase on the physiology of fiddler crabs from distinct habitats. *Journal of Thermal Biology*, *73*, pp.14-23.

Przeslawski, R., Byrne, M. and Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology*, *21*(6), pp.2122-2140.

685 Robbins, L.L., Hansen, M.E., Kleypas, J.A., Meylan, S.C., 2010. *CO2calc - a*
686 *User-friendly Seawater Carbon Calculator for Windows, Max OS X, and IOS (IPhone)*.
687 U.S. Geological Survey Open-File Report 2010-1280 (17 pp).

688 Roggatz, C.C., Lorch, M., Hardege, J.D. and Benoit, D.M., 2016. Ocean
689 acidification affects marine chemical communication by changing structure and function
690 of peptide signalling molecules. *Global Change Biology*, *22*(12), pp.3914-3926.

691 Range, P., Piló, D., Ben-Hamadou, R., Chícharo, M.A., Matias, D., Joaquim, S.,
692 Oliveira, A.P. and Chícharo, L., 2012. Seawater acidification by CO2 in a coastal lagoon
693 environment: effects on life history traits of juvenile mussels *Mytilus galloprovincialis*.
694 *Journal of Experimental Marine Biology and Ecology*, *424*, pp.89-98.

695 Rudd, M.A., 2014. Scientists' perspectives on global ocean research
696 priorities. *Frontiers in Marine Science*, *1*, p.36.

697 Salmon, M., 1987. On the reproductive behavior of the fiddler crab Uca thayeri,
698 with comparisons to *U. pugilator* and *U. vocans*: evidence for behavioral convergence.
699 *Journal of Crustacean Biology, 7*(1), pp.25-44.

700 Schmider, E., Ziegler, M., Danay, E., Beyer, L. and Bühner, M., 2010. Is it really
701 robust? *Methodologym 6*, pp. *147-151*

702 Seebacher, F., White, C.R. and Franklin, C.E., 2015. Physiological plasticity
703 increases resilience of ectothermic animals to climate change. *Nature Climate*
704 *Change*, *5*(1), p.61.

705 Silva, P.V., Luppi, T.A. and Spivak, E.D., 2007. Epibiosis on eggs and brooding
706 care in the burrowing crab *Chasmagnathus granulatus* (Brachyura: Varunidae):
707 comparison between mudflats and salt marshes. *Journal of the Marine Biological*
708 *Association of the United Kingdom*, *87*(4), pp.893-901.

709 Simoni, R., Cannicci, S., Anger, K., Pörtner, H.O. and Giomi, F., 2011. Do
710 amphibious crabs have amphibious eggs? A case study of *Armases miersii*. *Journal of*
711 *Experimental Marine Biology and Ecology*, *409*(1-2), pp.107-113.

712 Somero, G.N., 2010. The physiology of climate change: how potentials for
713 acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of*
714 *Experimental Biology*, *213*(6), pp.912-920.

715 Thurman, C.L., Faria, S.C. and McNamara, J.C., 2013. The distribution of fiddler
716 crabs (*Uca*) along the coast of Brazil: implications for biogeography of the western
717 Atlantic Ocean. *Marine Biodiversity Records*, *6*, pp.1-21.

718 Underwood, A.J., 1997. *Experiments in ecology: their logical design and*
719 *interpretation using analysis of variance*. Cambridge University Press.

720 Watson, G.S., Gregory, E.A., Johnstone, C., Berlino, M., Green, D.W., Peterson,
721 N.R., Watson, J.A. and Schoeman, D.S., 2018. Like night and day: Reversals of thermal
722 gradients across ghost crab burrows and their implications for thermal
723 ecology. *Estuarine, Coastal and Shelf Science*, *203*, pp.127-136.

724 Wear, R.G., 1974. Incubation in British decapod Crustacea, and the effects of
725 temperature on the rate and success of embryonic development. *Journal of the Marine*
726 *Biological Association of the United Kingdom*, *54*(3), pp.745-762.

727 Yamaguchi, T., 2001. Incubation of eggs and embryonic development of the
728 fiddler crab, *Uca lactea* (Decapoda, Brachyura, Ocypodidae). *Crustaceana*, *74*(5),
729 pp.449-458.7.

730

731

732

Acknowledgments

We thank Alessandra Augusto, Camila G. Blanco, Glauco B. O. Machado, Murilo Z. Marochi and Rafael M. Duarte for their valuable comments in the early version of this manuscript and all members of the Laboratório de Ecologia e Comportamento Animal (LABECOM) and Laboratório de Sustentabilidade (LABSUSSA) for their technical support in lab experiments.

Funding

This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): financial aid granted to JCF Pardo (Masters scholarship – FAPESP No 2017/00802-0) and TM Costa (Regular Grant - FAPESP No 2015/50300-6). TM Costa was recipient of the Brazilian Research Council (CNPq) productivity fellowship (No. 303400/2012-9).

SUPPLEMENTARY MATERIAL

Multiple stressors effects of warming and acidification in the embryonic development of an estuarine fiddler crab

Pardo. J. C. F[1.2,*] & Costa. T. M[1.2]

[1]Laboratório de Ecologia e Comportamento Animal (LABECOM) - Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP). Instituto de Biociências, Câmpus do Litoral Paulista. Praça Infante Dom Henrique, s/nº. Parque Bitaru, São Vicente, SP. Brazil. PO Box 73601, 11380-972

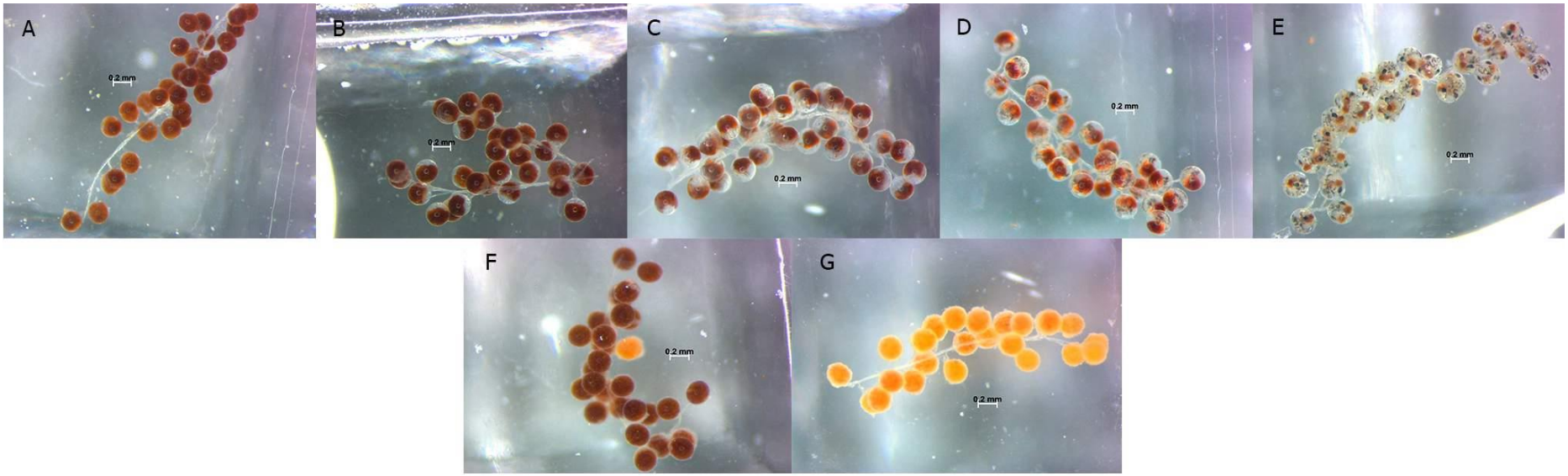
[2]Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP) - Campus de Botucatu. Instituto de biociências. Rua Prof. Dr. Antônio Celso Wagner Zanin, 250. Distrito de Rubião Junior. Botucatu, SP. Brazil. 18618-689

* Email address: pardojcf@yahoo.com.br (J.C.F. Pardo)

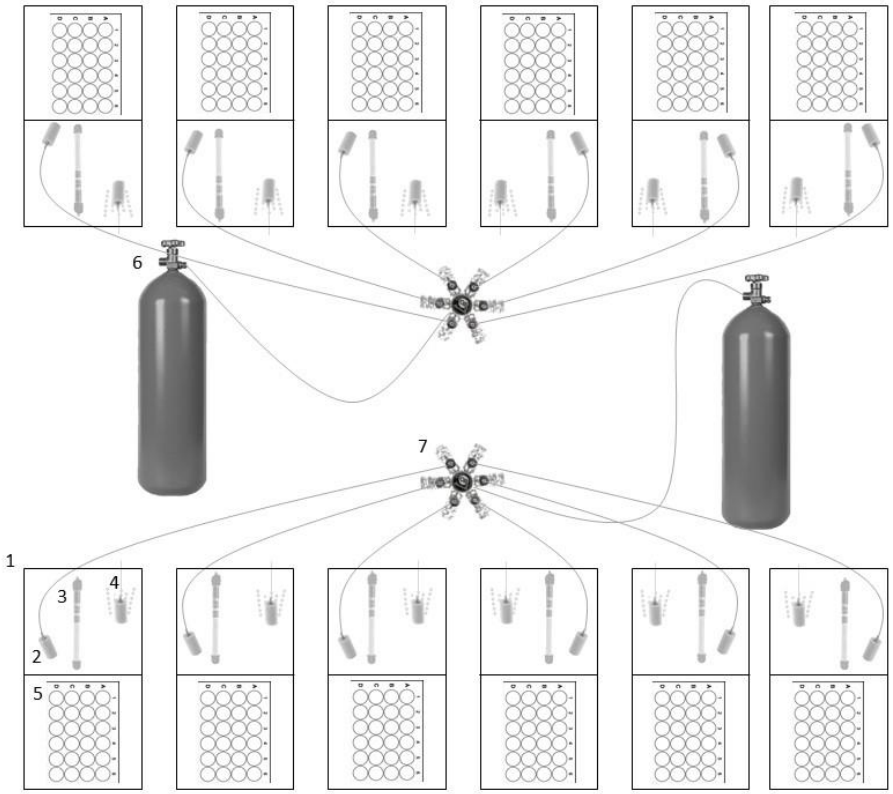
Supplemental Table 1. Mean (±SE) of measured (beginning/final of the experiment) [pH, temperature (T), total alkalinity (TA)] and calculated (CO2SYS) saltwater parameters [partial pressure of carbon dioxide (pCO2), bicarbonate (HCO_3), carbonate (CO_3^{2-}), aragonite saturation state (ΩArag) and calcite saturation state (ΩCalc)] for the experimental treatments.

		Measured parameters			Calculated parameters
Treatment		pH	T (ºC)	A_t (μmol.Kg^{-1})	pCO_2 (μatm)
Control temp.	Control pH	6.99±0.02	26.13±0.10	2324.11±169.32	5721.62±517.68
	Low pH	6.20±0.05	26.43±0.13	2105.99±165.83	31713.16±7705.09
High temp.	Control pH	6.95±0.02	29.88±0.12	2537.12±28.36	7031.64±305.71
	Low pH	6.21±0.05	29.95±0.16	2376.15±35.30	37964.41±4579.74

Sup. Table I cont.		Calculated parameters			
Treatment		HCO_3^- (μmol.Kg^{-1})	CO_3^{2-} (μmol.Kg^{-1})	Ω_{Arag}	Ω_{Calc}
Control temp.	Control pH	2273.87 ±165.78	24.85±2.25	0.40±0.04	0.61±0.06
	Low pH	2096.07±166.38	5.19±3.80	0.08±0.03	0.13±0.04
High temp.	Control pH	2480.54±26.95	27.94±1.37	0.46±0.02	0.69±0.03
	Low pH	10983.44±8627.22	4.95±0.45	0.08±0.01	0.12±0.01



Supplemental Figure 1. Classification scheme of the development of *Leptuca thayeri* embryos based on yolk content, eyes presence and heart beating: (A) blastula (egg capsule with almost undistinguished individual cells with near 24 to 48 hours after ovulation), (B) gastrula (small yolk-free portion with a formed germinal disc), (C) advanced gastrula (yolkfree portion increased), (D) early larvae (differentiation of limb buds, decreasing yolk mass, development of eye placodes and eyes and faint heartbeat) and (E) pre hatching larvae (completed eye, yolk in four lobes, strong heart beat). Description were based in Yamaguchi (2001) for the embryonic development of the fiddler crab *Austruca lactea*. Additional examples of degenerated or relatively underdeveloped eggs (F) and infested with symbionts and dead embryos (G).



Supplemental Figure 2. Schematic design of the microcosms used in the laboratory experiment. Tank overview (1) with two connected compartments, one for the apparatus setup compartment including air stone bubbling O_2 (2), thermostat (3) and air stone (4) for CO_2 bubbling and other for the 24 Well Cell Culture Plate (5). CO_2 system consisted in gas cylinders (6) with aquarium CO_2 6-way Splitters (7).

Supplemental Table 2. Result summary of the Log Rank test's pairwise comparisons among experimental treatments. P-values in bold mean statistical significance.

Treatment		Control temp. - control pH		Control temp. - low pH		High temp. - control pH		High temp. - low pH	
Temp.	pH	Chi-Square	p-value	Chi-Square	p-value	Chi-Square	p-value	Chi-Square	p-value
Control	Control	-	-	0.93	0.33	29.53	**0.0000**	10.17	**0.001**
	Low	0.93	0.33	-	-	19.89	**0.0000**	5.38	**0.02**
High	Control	29.53	**0.0000**	19.89	**0.0000**	-	-	5.11	0.23
	Low	10.17	**0.001**	5.38	**0.02**	5.11	0.23	-	-

CAPÍTULO 2

Maternal status elicits species-specific responses to warming in fiddler crabs

Pardo, J. C. F.[1,2,*], Cannicci, S.[3], Jimenez, P. J.[3], Granado, P.[4] & Costa, T. M[1,2]

[1]Laboratório de Ecologia e Comportamento Animal (LABECOM) - Universidade Estadual Paulista (UNESP), Instituto de Biociências, Campus do Litoral Paulista, Praça Infante Dom Henrique, s/nº, Parque Bitaru, São Vicente, SP, Brazil. PO Box 73601. 11380-972

[2]Programa de Pós-graduação em Ciências Biológicas (Zoologia) - Universidade Estadual Paulista (UNESP), Campus de Botucatu, Instituto de Biociências, Rua Prof. Dr. Antônio Celso Wagner Zanin, 250, Distrito de Rubião Junior, Botucatu, SP, Brazil. 18618-689

[3]The Swire Institute of Marine Science, School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China.

[4]Instituto do Mar, Universidade Federal de São Paulo (IMar/UNIFESP), Rua Dr. Carvalho de Mendonça, 144, Santos, SP 11070-100, Brazil

* Email address: pardojcf@yahoo.com.br (J.C.F. Pardo)

Manuscript Format in accordance with the guidelines of Biology Letters

Abstract

Coastal and estuarine ectotherms are potentially vulnerable to temperature instability. Thermal tolerance varies among groups and life-stages. However, the influence of maternal status in a climate warming scenario it is still unexplored. The fiddler crabs *Leptuca thayeri* and *Gelasimus borealis* were used as model species to verify if ovigerous females are more sensitive to warming than non-ovigerous. Environmental characterization showed that both species experienced temperatures way above their thermal limit (TL). Ovigerous *G. borealis* had lower TL than non-ovigerous; otherwise, *L. thayeri* had similar TL despite their reproductive status. The lighter and narrower body *G. borealis* had higher TL possibly due their living environmental and evolutionary history. Habitat and reproductive patterns may explain disparities between species' thermal tolerances. Beyond theoretical approaches, we present an experimental support on maternal status' TLs showing a non-general pattern for an ectotherm group.

Key-words: Ectotherms. Ovigerous status. Thermal limit. Thermal tolerance. *Leptuca thayeri. Gelasimus borealis.*

Introduction

Thermal variability imposes direct and indirect impacts to ecosystems and their organisms [1]. Slow and rapid (extreme heatwaves) temperature variations lead changes in phenology [2], shifts in species location [3] and individual-level effects on physiology and behaviour [4]. In this context, the temperature-dependent group of ectotherms are particularly more sensitive [5]. Coastal and estuarine ectotherms are even more vulnerable to temperature increase, usually living close to their critical thermal limits [6]. Species may have different thermal limits according to their environment and life-history stages [7; 8]. Marine embryos and larvae are recognized as potentially vulnerable stages in a warming context [9]. However, at our knowledge, there are no experimental studies showing the warming sensitivity on ovigerous females. Parental care is an energy-costly behaviour presented in many taxa [10]. Most ovigerous brachyurans change their physiology and behaviour reducing their time exposed and activity to care for embryos during their development [11]. Energy is invested in pleonal pumping which provides oxygen to their egg mass, mainly in the later embryo development stages [11, 12]. In this sense, these physio-behavioural changes in females with extruded eggs may carry consequences for the ovigerous female itself in a warming context.

At this context, we verified the habitat range temperature and thermal limits of ovigerous and non-ovigerous females of two estuarine key-stone species, *Leptuca thayeri* and *Gelasimus borealis*. Despite their geographical distance and distinct clades, both fiddler crab species live in low to middle intertidal soils and have similar reproductive habits, behaving (i.e., feeding on soil surface) outside their burrows even when ovigerous [13, 14]. We assessed the following questions: (1) Are ovigerous females more sensitive to temperature increase than non-ovigerous females? (2) Do ovigerous females from distinct genus with similar reproductive behaviour have different thermal limits? Physio-

behavioural traits leads to predict that ovigerous of *L. thayeri* and *G. borealis* are more sensitive to warming than non-ovigerous. We hypothesized (H0) that ovigerous females have lower thermal limit than non-ovigerous females for both species.

Material and methods

Environmental characterization and crab collection (see supplementary online material for details) of *L. thayeri* were conducted in Portinho Mangrove, Praia Grande - São Paulo, Brazil (23°59'16.74" S 46°24'26.28" W) and *G. borealis* samplings at the Tung Chung mangrove, Hong Kong, Republic of China (22°16'56.7"N 113°55'43.3"E). We measured the habitat temperature experienced by the ovigerous fiddler crabs during their embryo development installing thermo-loggers [Maxim® iButtons (±0.1°C)] at the following three zones: soil surface, below (20 cm) and above ground (attached to branches, 20 cm). Fiddler crabs usually takes 13-14 days of embryo development until release their larvae [15, 16]. Thus, iButton loggers stayed at least 15 days (spring and neap tide) logging the temperature with 5-minute interval.

We used heart rate (beats per minute) and thermal limit as our response variable [17] for ovigerous and non-ovigerous female crabs (*L. thayeri*: n = 10 per reproductive status; *G. borealis*: n = 20 per reproductive status) subjected to a thermal ramp (see the electronic supplementary material for details). The experiment procedures were conducted in both countries with same methodology and equipment.

Data were checked for normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test). Unequal sample size and non-normality data leads to compare the thermal limit with a factorial permutational analyses of variance (PERMANOVA) (9999 permutations) using Species (*L. thayeri* and *G. borealis*) and Reproductive status

(ovigerous and non-ovigerous) as fixed factors. A *post hoc* PERMANOVA analyses were conducted to verify the significant difference in reproductive status. Carapace width (CW), body (with and without egg mass) weight and body/egg mass ratio among ovigerous and non-ovigerous females of both species were analysed with a Welch's t test for unequal sample sizes. Differences were considered significant if $p < 0.05$. Analyses were conducted in SPSS 25.0 (SPSS Inc., Armonk, NY, U.S.A.) and PAST v3.20.

Results

The mean measured temperature experienced by non-ovigerous and ovigerous female crabs was below their thermal limits with some extremes above their thermal limit (Figure 1; electronic supplementary material, Table S1). Ovigerous *G. borealis* were more sensitive to warming than non-ovigerous, being the latter with higher thermal limit than non-ovigerous and ovigerous *L. thayeri* (PERMANOVA, $F = 1.313$, $DF = 3$, $p = 0.02$; *post hocs*: $p < 0.01$) (Figure 2; electronic supplementary material, Table S2, S3). Ovigerous and non-ovigerous *L. thayeri* showed similar lethal temperatures (*post hoc* PERMANOVA: $p = 0.42$) (Figure 2; electronic supplementary material, Table S3) and were heavier and wider with heavier egg mass than *G. borealis* (summary of Welch's t test results in Table 1).

Discussion

Our study highlights that ovigerous females, a high energy-costly life stages, have species-specific responses to warming in fiddler crabs. Theoretical models predict that early-stages and spawners of fishes have the narrow thermal window widths across their life stages [18]. Our results give the first experimental support to a non-general pattern in

ectotherms. Although their thermal limits are below the mean environmental temperature, several field measured temperatures surpassed their thermal limit and direct warming may affect species thermal windows at individual level [7, 18]. In ectothermic biology, their metabolic and behavioural processes, such as fecundity, foraging and growing, are known to be affected by thermal variability [7, 8]. Therefore, invertebrates evolved specific mechanisms to cope with extreme temperatures [18]. Fiddler crabs use burrows as thermal refuges [19] and males major claws act as heat sinks [20], for example. However, as extreme thermal events are becoming longer and more frequent (e.g., marine heatwaves, 21), ectothermic species, mainly the ones living in exposed habitats and warming-sensitive life-stages, encounter direct and severe constraints.

Both species live in estuarine areas with distinct tree cover, which explain the higher temperatures on *G. borealis* exposed habitat (Tung Chung mangrove). Adapted to warmer conditions, non-ovigerous *G. borealis* had higher TL than *L. thayeri* showing a potential wider aerobic performance window, despite its lower weight and carapace width. Following the general trend, species living in more exposed areas tend to present lower mortality rates in high temperatures, as observed for other fiddler crab species [22]. Our model species have similar reproductive behaviour but not the same reproductive pattern: *L. thayeri* has a seasonal reproduction period [23] and *G. borealis* an annual pattern, breeding during most part of the year [24]. As observed for the fiddler crab *Minuca pugnax*¸ ovigerous females have low hepatosomatic index (i.e., indirect index of energy status) due to reproduction [25]. Energy costs involved in continuous reproduction strategies may explain the contrast ovigerous TL results between both species. Also, ovigerous *L. thayeri* have heavier egg mass than *G. borealis.* Broods accumulate water and larger egg masses may help as heat sink in species with high fecundity.

Species are extending their range limits due to climate change [3]. The Atlantic marsh fiddler crabs *Gelasimus pugnax*, as an example, northern range expanded their distribution [26]. Based on our environment characterization and TL results, fiddler crabs living inside coastal forests may have favourable environmental temperature conditions to expand their distributions, besides other abiotic (e.g., salinity, pH) and biotic factors (e.g., niche competition). Considering carrying-egg females a crucial life-stage and a bottleneck on population dynamics, complementary physiological and behavioural information are essential to clarify how ovigerous females from different groups deal with warming in the context of climate change.

References

1. Harley CD. 2011. Climate change, keystone predation, and biodiversity loss. *Science* **334**, 1124-7 (doi: 10.1126/science.1210199).

2. Cohen JM, Lajeunesse MJ, Rohr JR. 2018. A global synthesis of animal phenological responses to climate change. *Nat. Clim. Change* **8**, 224 (doi: 10.1038/s41558-018-0067-3).

3. Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**, 637-69 (doi: 10.1146/annurev.ecolsys.37.091305.110100).

4. Bozinovic F, Pörtner HO. 2015. Physiological ecology meets climate change. *Ecol. Evol*. **5**, 1025-30 (doi: 10.1002/ece3.1403).

5. Paaijmans KP, Heinig RL, Seliga RA, Blanford JI, Blanford S, Murdock CC, Thomas MB. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.* **19**, 2373-80 (doi: 10.1111/gcb.12240).

171 6. Stillman JH. 2003. Acclimation capacity underlies susceptibility to climate
172 change. *Science* **301**, 65 (doi: 10.1126/science.1083073).
173 7. Madeira D, Narciso L, Cabral HN, Vinagre C. 2012. Thermal tolerance and
174 potential impacts of climate change on coastal and estuarine organisms. *J. Sea Res.* **70**,
175 32-41 (doi: 10.1016/j.seares.2012.03.002).
176 8. Fusi M, Babbini S, Giomi F, Fratini S, Dahdouh-Guebas F, Daffonchio D,
177 McQuaid CD, Porri F, Cannicci S. 2017 Thermal sensitivity of the crab *Neosarmatium*
178 *africanum* in tropical and temperate mangroves on the east coast of Africa. *Hydrobiologia*
179 **803**, 251-63 (doi: 10.1007/s10750-017-3151-1).
180 9. Przeslawski R, Byrne M, Mellin C. 2015. A review and meta-analysis of the
181 effects of multiple abiotic stressors on marine embryos and larvae. *Glob. Change Biol*.
182 **21,** 2122-40 (doi: 10.1111/gcb.12833).
183 10. Clutton-Brock TH. The evolution of parental care. Princeton University Press;
184 1991.
185 11. Christy JH, Wada K. 2015. Social ethology in Brachyura. Treatise on Zoology-
186 Anatomy, Taxonomy, Biology. The Crustacea, Volume 9 Part C (2 vols): Brachyura,
187 p.417.
188 12. Baeza JA, Fernández M. 2002. Active brood care in *Cancer setosus* (Crustacea:
189 Decapoda): the relationship between female behaviour, embryo oxygen consumption and
190 the cost of brooding. *Funct. Ecol*. **16**, 241-51 (doi: 10.1046/j.1365-2435.2002.00616.x).
191 13. Salmon, M. 1987. On the reproductive behavior of the fiddler crab *Uca thayeri*,
192 with comparisons to *U. pugilator* and *U. vocans*: evidence for behavioral convergence. *J.*
193 *Crustacean Bio.* **7**, 25-44 (doi: 10.1163/193724087X00027).
194 14. Kwok WPW, Tang W. 2006. *Fiddler crabs in Hong Kong – An overview*. Hong
195 Kong Biodiversity, 1–7.

15. DeCoursey PJ. 1979. Egg-hatching rhythms in three species of fiddler crabs. In *Cyclic phenomena in marine plants and animals* 399-406 (doi: 10.1016/B978-0-08-023217-1.50058-8).

16. Christy JH. 1982. Adaptive significance of semilunar cycles of larval release in fiddler crabs (Genus *Uca*): test of an hypothesis. *Biol. Bul.* **163,** 251-263 (doi: 10.2307/1541264).

17. Somero GN. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits and cost of living. *Integr. Comp. Biol*. **42**, 780–789 (doi: 10.1093/icb/42.4.780).

18. Pörtner HO, Farrell AP. 2008. Physiology and climate change. *Science* **322**, 690-692 (doi: 10.1126/science.1163156).

19. Powers LW, Cole JF. 1976. Temperature variation in fiddler crab microhabitats. *J. Exp. Mar. Bio Ecol.* **21**, 141-157 (doi: 10.1016/0022-0981(76)90035-6).

20. Darnell MZ, Munguia P. 2011. Thermoregulation as an alternate function of the sexually dimorphic fiddler crab claw. *Am. Nat.* **178**, 419-428 (doi: 10.1086/661239).

21. Oliver EC, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander LV, Benthuysen JA, Feng M, Gupta AS, Hobday AJ, Holbrook NJ. 2018. Longer and more frequent marine heatwaves over the past century. *Nat, commun,* **9**, 1324 (doi: 10.1038/s41467-018-03732-9).

22. Principe SC, Augusto A, Costa TM. 2018. Differential effects of water loss and temperature increase on the physiology of fiddler crabs from distinct habitats. *J. Therm. Biol.* **73**, 14-23 (doi: 10.1016/j.jtherbio.2018.02.004).

23. Costa TM, Silva SM, Negreiros-Fransozo ML. 2006. Reproductive pattern comparison of *Uca thayeri* Rathbun, 1900 and *U. uruguayensis* Nobili, 1901 (Crustacea,

220 Decapoda, Ocypodidae). *Braz. Arch. Biol. Tech*. 49, 117-23 (doi: 10.1590/S1516-
221 89132006000100014).
222 24. Shih JT, Tseng SS. 1999. Progesterone-like substance in ovary and
223 hepatopancreas of *Uca vocans borealis*. *Zool. Stud.* **38,** 458-65.
224 25. Brodie RJ, Roberts B, Espinosa JI, Heilman K, Borgianini SA, Welch JM, Reinsel
225 KA. 2017. Seasonal and latitudinal variations in the energy reserves of the mud fiddler
226 crab *Uca pugnax*: implications for the response to climate change. *Aquatic Biol.* 26, 113-
227 23 (doi: 10.3354/ab00683).
228 26. Johnson DS. 2014. Fiddler on the roof: a northern range extension for the marsh
229 fiddler crab *Uca pugnax*. *J. Crustacean Bio.* **34**, 671-3 (doi: 10.1163/1937240X-
230 00002268).

231

232

233 **Acknowledges**

234 We thank all members of the Laboratory of Ecology and Animal Behaviour
235 (LABECOM) (UNESP) and Mangrove Ecology Lab (HKU) - Swire Institute of Marine
236 Science (SWIMS). We also thank Cecily for the technical support.

237

238 **Ethics**. Experiments were conducted in agreement with Brazil and Hong Kong laws.

239 **Data accessibility**. Data are available as the electronic supplementary material.

240 **Authors' contributions**. J.C.F.P., T.M.C. and S.C. conceived the study; J.C.F.P.
241 developed experiments, carried out analyses and drafted the manuscript. J.C.F.P., P.J.J.
242 and P.G. designed the experiments.

243 **Competing interests**. We have no competing interests.

Funding. J.C.F.P. was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) via Masters scholarship (FAPESP No 2017/00802-0, 2018/00577-0). T.M.C. was supported by FAPESP (Regular Grant - FAPESP No 2015/50300-6) and recipient of the Brazilian Research Council (CNPq) productivity fellowship (No. 303400/2012-9).

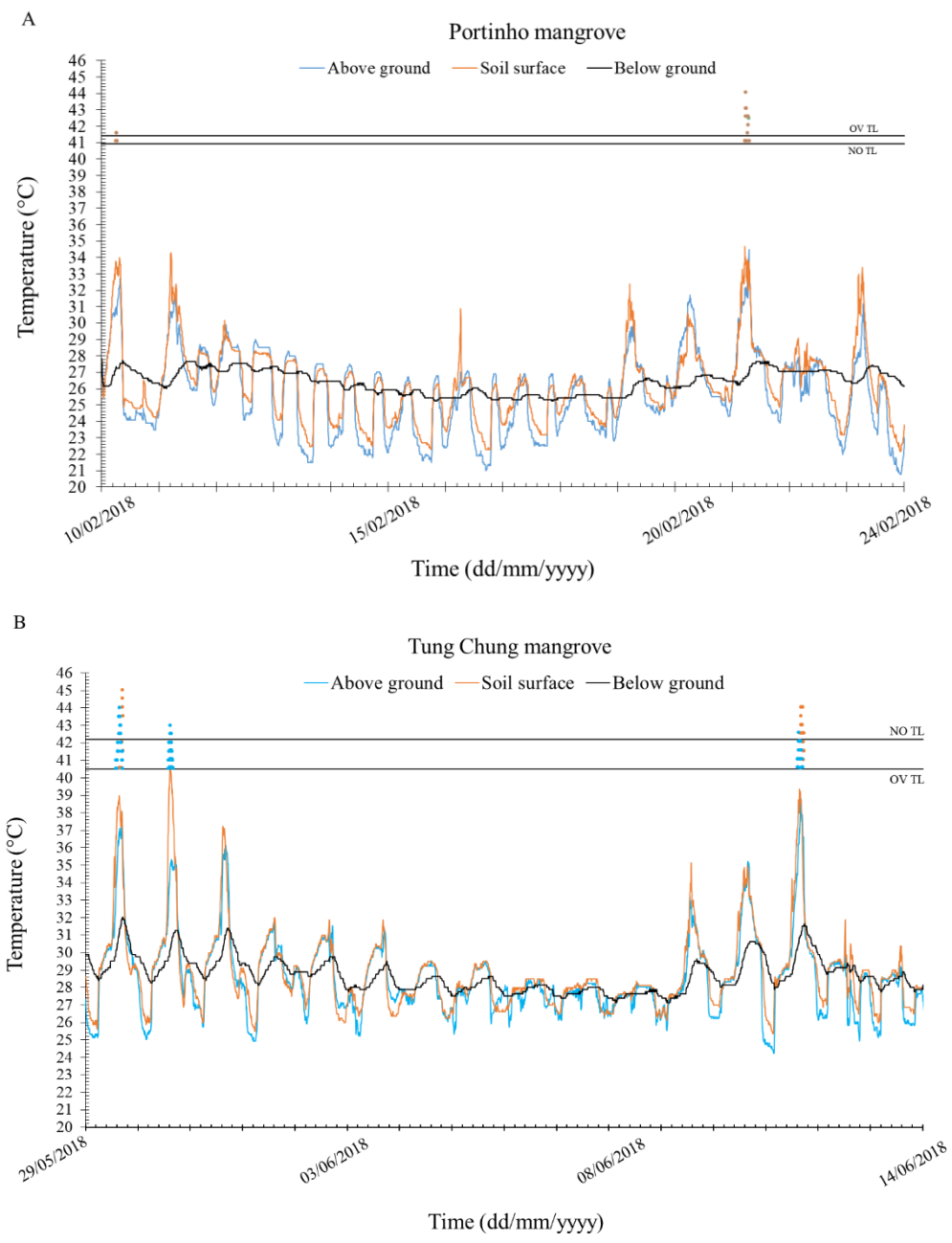
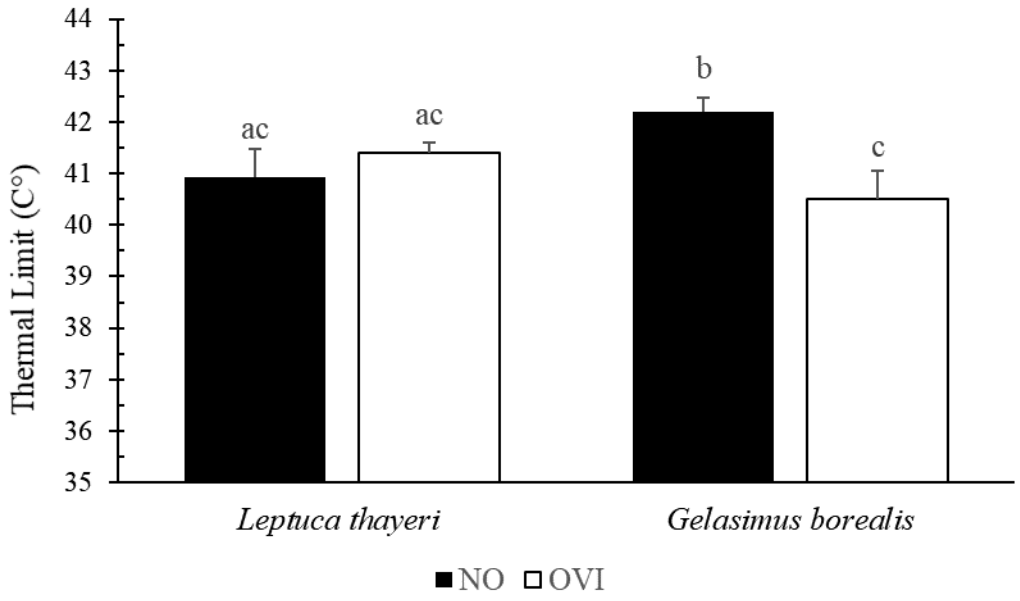


Figure 1: Mean field environment temperature of the fiddler crab habitats on (A) Portinho mangrove, Brazil, (10/02/2018-24/02/2018) and (B) Tung Chung mangrove, Hong Kong, (29/05/2018-14/06/2018) at the different zones: soil surface and above and below ground. Black lines represent thermal limits (TLs) of each species and reproductive status and dots are the extremes values above species' TLs observed in all loggers from each zone

277 and day. Estimations were made with five loggers at each zone, except for soil surface
278 and below ground in Tung Chung mangrove (four loggers/each).

279



280

281 **Figure 2:** Thermal limit (TL) (±SE) of the non-ovigerous (NO) and ovigerous (OVI)
282 fiddler crabs *Leptuca thayeri* and *Gelasimus borealis*. Different letters indicate statistical
283 difference between treatments (PERMANOVA: $p < 0.05$).

284

285 **Table 1:** Welch's t test results among carapace width (CW), body (with and without egg
286 mass) weight and egg mass. Values are expressed as mean±SE. Degrees of freedom, df1:
287 numerator, df2: denominator.

	Species		Welch's test			
	Leptuca thayeri	*Gelasimus borealis*				
	CW (mm)		Statistic	df1	df2	p-value
NO	18.72±0.32	13.29±0.23	174.13	1	19.29	0.000
OVI	18.3±0.34	13.41±0.19	158.13	1	14.61	0.000
	Weight (g)					

NO	2.65±0.43	0.62±0.03	205.78	1	10.21	0.000
OV (with egg mass)	2.96±0.17	0.61±0.03	176.5	1	9.58	0.000
OV (without egg mass)	2.52±0.15	0.55±0.03	167.15	1	9.54	0.000
Egg mass*	0.44±0.04	0.06±0.01	5.22	1	27.31	0.03

*****comparison using body/egg mass ratio

Electronic Supplementary Material

Maternal status elicits species-specific responses to warming in fiddler crabs

Pardo, J. C. F[1,2,*], Cannicci, S.[3], Jimenez, P. J.[3], Granado, P.[4] & Costa, T. M[1,2]

[1]Laboratório de Ecologia e Comportamento Animal (LABECOM) - Universidade Estadual Paulista (UNESP), Instituto de Biociências, Campus do Litoral Paulista, Praça Infante Dom Henrique, s/nº, Parque Bitaru, São Vicente, SP, Brazil. PO Box 73601. 11380-972

[2]Programa de Pós-graduação em Ciências Biológicas (Zoologia) - Universidade Estadual Paulista (UNESP), Campus de Botucatu, Instituto de Biociências, Rua Prof. Dr. Antônio Celso Wagner Zanin, 250, Distrito de Rubião Junior, Botucatu, SP, Brazil. 18618-689

[3]The Swire Institute of Marine Science, School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China.

[4]Instituto do Mar, Universidade Federal de São Paulo (IMar/UNIFESP), Rua Dr. Carvalho de Mendonça, 144, Santos, SP 11070-100, Brazil

* Email address: pardojcf@yahoo.com.br (J.C.F. Pardo)

Legends of supplementary files

* Supplementary material and methods:

a) Crab collection and maintenance

b) Upper thermal limits of heart function

*Supplementary results

Table S1

Table S2

Table S3

Material and Methods

a) Crab collection and maintenance

Non-ovigerous and ovigerous female crabs (embryos in advanced stage of development) of *L. thayeri* (carapace width (CW): 17-20 mm)) and *G. borealis* (CW: 12-15 mm) were collected at the same areas of the environmental temperature characterization. Carapace width range was based on the most frequent width of collected ovigerous crabs in the sampling area. Prior observations showed that black egg mass represented embryos at late stages of development. We manually sampled all individuals by excavating their burrows and transferred them to the laboratory. Only undamaged crabs in the intermold were collected. Crabs were maintained for two (minimum) to five (maximum) days in aquariums with a thin saltwater layer (artificial sea salt dissolved in distilled water (S33-35)), containing PVC pipe connectors (2.5 cm of diameter) to provide dry shelter. Saltwater was partially changed daily in all aquariums. Crabs were fed a*d libitum* daily after the second day with fish food for marine carnivores. A 12h light:dark photoperiod cycle and controlled air and water temperature (25°C) were maintained during acclimatization.

b) Thermal limits - heart function

After the period of acclimatization, specimens were individually transferred to glass containers (covered with a black contact paper) with 1 ml of saltwater to avoid dehydration. Containers were placed in a water bath and subjected to a thermal ramp starting at 25°C increasing the temperature by 1°C every 20 min until 50°C (general average temperature rise in mangroves). We used a non-invasive method to estimate heart

rates which consists in carefully attaching an infrared sensor (Vishay Semiconductors, CNY70) to the cardiac region of the crabs using blue tack adhesive and cyanoacrylate instant glue [1]. Signals were filtered and recorded with a Picoscope® oscilloscope connected to a computer. The lethal temperature was estimated using the endpoint for the heart function (flat-line temperature (FLT)) [2].

We evaluated and estimated the relationship between the crabs' body temperature and the water during the thermal ramp. K-type thermocouples were inserted into the branchial chambers of ovigerous and non-ovigerous crabs allocated in the water bath (as mentioned above). Temperature (±0.01°C) of both crabs and water were logged with a Lutron TM-947SD thermometer. We applied a linear regression analysis generating an equation [3] for each species and reproductive status and estimated the real body temperature and TL for specimens from the heart function experiments (Simple Linear Regression: *L. thayeri*: non ovigerous: N_{crabs}: 5; N = 2552, df = 2551, F = 53215.21, r2 = 0.95, p = 0.000/ ovigerous: N_{crabs}: 7; N = 3614, df = 3613 , F = 65913.34, r2 = 0.94, p = 0.000; *G. borealis*: non ovigerous: N_{crabs}: 20; N = 10303, df = 10302, F = 658071.9, r2 = 0.98, p = 0.000/ ovigerous: N_{crabs}: 20; N = 10296, df = 10295, F = 868740.3, r2 = 0.98, p = 0.000).

All crabs had measured their carapace width (± 0.1 mm) and weight (± 0.001 g) with and without eggs after trials.

Results

Table S1: Mean (±SE), minimum and maximum temperature on Portinho mangrove, Brazil, and Tung Chung mangrove, Hong Kong, of three different zones: above ground, soil surface and below ground. Min and max values referred to the extremes observed in all loggers from each zone.

	Temperature								
	Above ground			Soil surface			Below ground		
Study site	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Portinho mangrove	25.63 ±0.04	20.8	34.5	26.23 ±0.04	22.18	34.67	26.4 ±0.01	25.24	27.84
Tung Chung mangrove	28.45 ±0.03	24.10	45.08	29.82 ±0.03	24.58	44.05	28.66 ±0.01	26.67	33.61

Table S2: PERMANOVA test on differences in CTmax across Species (*Leptuca thayeri* and *Gelasimus borealis*) and Reproductive Status (ovigerous and non-ovigerous) (fixed factors). Sum of squares, Sum of sqrs; Degrees of freedom, df; Mean Square, MS; pseudo-F-statistic value, F; p value, p.

Source	Sum of sqrs	df	MS	F	p
Species	0.4788	1	0.4788	0.062787	0.6855
Reprod. Status	**30.038**	**3**	**10.013**	**1.313**	**0.0259**
Interaction	-243.32	3	-81.105	-10.636	0.8066
Residual	396.54	52	76.258		
Total	183.74	59			

Table S3: P values of *post hoc* PERMANOVA analyses on CTmax between Reproductive Status (ovigerous and non-ovigerous) (fixed factor). *Leptuca thayeri* non-ovigerous (LTNO); *L. thayeri* ovigerous (LTOVI); *Gelasimus borealis* non-ovigerous (GBNO); *G. borealis* ovigerous (GBOVI).

	LTNO	LTOVI	GBNO	GBOVI
LTNO		0.4284	**0.0071**	0.6448
LTOVI	0.4284		**0.0048**	0.2769
GBNO	**0.0071**	**0.0048**		**0.005**
GBOVI	0.6448	0.2769	**0.005**	

References

1. Burnett NP, Seabra R, Pirro M de, Wethey DS, Woodin SA, Helmuth B, Zippay ML, Sara G, Monaco C, Lima FP. 2013. An improved noninvasive method for measuring heartbeat of intertidal animals. *Limnol. Oceanogr. Methods*. **11**:91–100 (doi: 10.4319/lom.2013.11.91).

2. Marshall DJ, McQuaid CD, Williams GA. 2010. Non-climatic thermal adaptation: implications for species' responses to climate warming. *Biol. Lett.* **6**:669–673 (doi: 10.1098/rsbl.2010.0233).

3. Stillman JH, Somero GN. 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. *J. Exp. Biol.* **1855**:1845–1855.

Considerações finais

> "For centuries, scientists thought that earth processes were so large and powerful that nothing we could do would change them. This was a basic tenet of geological science: that human chronologies were insignificant compared with the vastness of geological time; that human activities were insignificant compared with the force of geological processes. And once they were. But no more. There are now so many of us cutting down so many trees and burning so many billions of tons of fossil fuels that we have become geological agents. We have changed the chemistry of our atmosphere, causing sea level to rise, ice to melt, and climate to change. There is no reason to think otherwise. And, in my view, there is, at this point in history, no excuse for not taking action to prevent the very significant losses that are likely to ensue— indeed, losses that are already becoming evident—if we sit around denying the reality that science has made clear."
>
> Oreskers, 2018
>
> E.A. Lloyd, E. Winsberg (eds.), *Climate Modelling*

Nossos resultados demonstram que as fases tidas como vulneráveis de caranguejos violinistas, ectotérmicos estuarinos de grande importância na conformação dos seus habitats, demonstram sensibilidade às variações abióticas atreladas à mudança climática antropogênica. Respostas fisiológicas e morfológicas revelam que o aumento da temperatura e redução do pH, preditos para o final do século, afetam negativamente e individualmente os organismos, podendo acarretar uma cascata de efeitos na dinâmica dos ecossistemas. Apesar de adaptados à constante variação abiótica, os organismos estuarinos, sobretudo as fases vulneráveis (embriões, larvas e fêmeas ovígeras), vivem perto dos seus limites fisiológicos com curtas janelas termais, resultando em uma tolerância limitada e sensível as variações preditas. Contudo, as respostas não ocorrem de forma absoluta e/ou são espécie-específicas, o que conduz a perguntas mais específicas quanto a tolerância e consequências nessas fases determinantes da ontogenia dos organismos. Apesar dos recentes esforços da comunidade científica, a literatura ainda

carece de informações mais realísticas sobre os efeitos das atuais e futuras mudanças nas condicionantes abióticas e suas interações nos ecossistemas. Estudos abordando estressores múltiplos ou isolados e seus efeitos a curto e longo prazo ao longo de gerações e interações tróficas são alguns exemplos de áreas a serem exploradas.

Referências

Ahmed, M., Anchukaitis, K.J., Asrat, A., Borgaonkar, H.P., Braida, M., Buckley, B.M., Büntgen, U., Chase, B.M., Christie, D.A., Cook, E.R. and Curran, M.A., 2013. Continental-scale temperature variability during the past two millennia. *Nature Geoscience*, 6(5), p.339.

Bernardino, A.F., Netto, S.A., Pagliosa, P.R., Barros, F., Christofoletti, R.A., Rosa Filho, J.S., Colling, A. and Lana, P.C., 2015. Predicting ecological changes on benthic estuarine assemblages through decadal climate trends along Brazilian Marine Ecoregions. *Estuarine, Coastal and Shelf Science*, 166, pp.74-82.

Cannicci, S., Burrows, D., Fratini, S., Smith, T.J., Offenberg, J., Dahdouh-Guebas, F., 2008. Faunal impact on vegetation structure and ecosystem function in mangrove forests: a review. *Aquatic Botany* 89, 186–200.

Cohen, J.M., Lajeunesse, M.J. and Rohr, J.R., 2018. A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, 8(3), p.224.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J. and Millero, F.J., 2004. Impact of anthropogenic CO2 on the CaCO3 system in the oceans. *Science*, 305(5682), pp.362-366.

Helmuth, B., Mieszkowska, N., Moore, P. and Hawkins, S.J., 2006. Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, pp.373-404.

IPCC, 2014. Climate change 2014: synthesis report. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC Geneva, Switzerland (151 pp).

Natálio, L.F., Pardo, J.C., Machado, G.B., Fortuna, M.D., Gallo, D.G. and Costa, T.M., 2017. Potential effect of fiddler crabs on organic matter distribution: A combined laboratory and field experimental approach. *Estuarine, Coastal and Shelf Science*, 184, pp.158-165.

Oliver, E.C., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L.V., Benthuysen, J.A., Feng, M., Gupta, A.S., Hobday, A.J. and Holbrook, N.J., 2018. Longer and more frequent marine heatwaves over the past century. *Nature Communications*, 9(1), p.1324.

Oreskes, N., 2018. The scientific consensus on climate change: How do we know we're not wrong?. In Climate Modelling (pp. 31-64). Palgrave Macmillan, Cham.

Pistevos, J.C., Calosi, P., Widdicombe, S. and Bishop, J.D., 2011. Will variation among genetic individuals influence species responses to global climate change? *Oikos*, 120(5), pp.675-689.

Przeslawski, R., Byrne, M. and Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology*, 21(6), pp.2122-2140.

Seebacher, F., White, C.R. and Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5(1), p.61.

Sunday, J.M., Bates, A.E. and Dulvy, N.K., 2012. Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, 2(9), p.686.

Vinagre, C., Mendonça, V., Cereja, R., Abreu-Afonso, F., Dias, M., Mizrahi, D. and Flores, A.A., 2018. Ecological traps in shallow coastal waters—Potential effect of heat-waves in tropical and temperate organisms. *PloS one*, 13(2), p.e0192700.