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Dissertação
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Resumo

A temperatura é, sem dúvida, um dos principais fatores abióticos responsáveis por definir padrões na Natureza, em especial no que toca à distribuição e abundância das espécies. No último século, a temperatura média global subiu 0.6ºC, estando previsto, até ao final deste século, um aumento de 2 a 4ºC. Isto significa que muitos ecossistemas serão sujeitos a uma taxa de aquecimento muito superior àquela a que estiveram sujeitos nos últimos milhares de anos. Na verdade, já são visíveis alterações nos ecossistemas resultantes do recente aquecimento global. Diversos estudos demonstram que o aquecimento nas últimas décadas tem afetado a fenologia dos organismos, a distribuição das espécies e a composição e dinâmica das comunidades, desde as regiões polares às tropicais. Como tal, o aquecimento previsto para as próximas décadas poderá ter graves consequências para os ecossistemas e a biodiversidade, a nível global. Prever os impactos do aquecimento global é, assim, uma tarefa imperativa. As espécies, populações e comunidades não responderão a médias térmicas globais, mas sim a alterações a nível regional. Neste contexto, o Painel Intergovernamental sobre Alterações Climáticas previu, em 2007, uma assimetria na taxa de aquecimento global, com um aquecimento mais rápido nas latitudes mais elevadas do que nas mais baixas. Esta previsão levantou preocupações sobre quais os organismos em maior risco, originando um debate científico acerca da vulnerabilidade das espécies tropicais *versus* temperadas.

Os trópicos e as zonas temperadas albergam a maior parte das espécies do nosso planeta. Alguns estudos preveem que o aquecimento global terá um baixo impacto nos trópicos, já que a taxa de aquecimento nestas regiões será inferior comparativamente às latitudes mais altas. No entanto, o facto das espécies tropicais viverem em ambientes estáveis, sem grandes flutuações térmicas sazonais, poderá fazer com que estas espécies sofram desproporcionalmente com pequenas elevações de temperatura. Uma forma de contribuir para este debate passa por estimar a tolerância térmica e a capacidade de aclimação das espécies. Conhecer os limites térmicos de uma espécie, e a plasticidade desses limites, permite-nos discutir o que poderá acontecer à distribuição e abundância dos organismos no decorrer das alterações climáticas. Atualmente, esta informação está limitada a um número reduzido de espécies.

Um dos ecossistemas ideais para estudo dos impactos das alterações climáticas é o intertidal rochoso. As poças de maré encontradas nesta zona, durante a baixa-mar, são ambientes exigentes para as comunidades que nelas habitam. Estes habitats estão sujeitos às variações térmicas do clima terrestre e marinho, havendo já estudos que indicam que os organismos intertidais estão a viver perto dos seus limites fisiológicos. Isto significa que a elevação de temperatura prevista, assim como o aumento da frequência e intensidade de ondas de calor, poderão ter um enorme impacto nas comunidades da zona intertidal.

Neste contexto, o presente trabalho tem como objetivo estimar a tolerância térmica e a capacidade de aclimação de espécies costeiras, tropicais e temperadas, de forma a discutir e comparar a sua potencial vulnerabilidade face ao aquecimento global. Os locais de estudo foram a costa sudeste Brasileira, tropical, e a costa oeste Portuguesa, temperada. Crustáceos e peixes de diferentes espécies foram recolhidos em poças de maré, com camaroeiros, e testados em laboratório, no Verão de 2014.

Para estimar a tolerância térmica, foram determinados os limites térmicos superiores de 12 espécies tropicais e 23 espécies temperadas. O método usado foi o *Critical Thermal Maximum* (CTMax), no qual os organismos são sujeitos a um incremento de temperatura na ordem do 1^{o}C/h até atingirem o seu máximo térmico crítico, isto é, até perderem o equilíbrio. Baseado na temperatura a que os primeiros sinais de *stress* térmico ocorrem, o CTMax é uma medida conservativa de tolerância térmica, sendo muito utilizado em estudos de *stress* térmico. Este método permitiu determinar que espécies vivem mais perto do seu limite térmico, logo, quais as espécies mais vulneráveis a um aumento de temperatura. As espécies tropicais apresentaram valores de CTMax mais elevados. No entanto, verificou-se que as espécies tropicais vivem mais perto dos seus limites térmicos, sendo mesmo expostas a temperaturas superiores a estes limites durante ondas de calor. Além disso, a variabilidade intraespecífica no CTMax foi mais elevada nas espécies temperadas que nas tropicais, indiciando um potencial evolutivo inferior das espécies tropicais para lidar com um aumento de temperatura. Os resultados obtidos apontam para um maior risco associado às espécies tropicais face a um aumento de temperatura.

Para estimar a capacidade de aclimação, i.e. a capacidade de ajustar os limites térmicos, os organismos foram expostos a temperaturas acima da sua temperatura atual em duas experiências, uma a longo-prazo e outra a curto-prazo. Na experiência a longo-prazo os organismos foram expostos a 3^{o}C acima da temperatura média atual, durante 30 dias, simulando o aumento médio de temperatura previsto até o final deste século. Na experiência a curto-prazo os organismos foram expostos a 6^{o}C acima da temperatura média presente, durante 10 dias, simulando uma onda de calor futura. Os limites térmicos superiores antes e após cada experiência foram determinados, de forma a poder avaliar a capacidade de aclimação dos organismos. Foram testados 4 pares de espécies tropicais-temperadas intertidais, pertencentes às famílias Palaemonidae, Grapsidae, Blenniidae e Gobiidae. Verificou-se que tanto espécies tropicais como temperadas têm a capacidade de aclimar como resposta a elevações de temperatura. No entanto, a capacidade de aclimação das espécies tropicais foi inferior comparativamente à das temperadas, indiciando que as espécies tropicais poderão estar vulneráveis até a pequenas elevações de temperatura ambiente. Como acima mencionado, as espécies tropicais são atualmente expostas a temperaturas superiores aos seus limites térmicos durante ondas de calor, e este *stress* térmico poderá já estar a exercer pressão sobre a tolerância térmica destes organismos. Estes resultados sugerem que a resposta das comunidades intertidais tropicais ao aquecimento global será visível antes que a das comunidades intertidais temperadas.

Provavelmente, as espécies tropicais intertidais irão habitar as mesmas áreas, e refugiar-se em águas mais frias, subtidais, durante elevações de temperatura extremas. No entanto, as interações entre as espécies e os seus competidores, predadores e/ou presas, poderão restringir o uso destes refúgios por parte das espécies intertidais. Tal situação poderá ter graves consequências para as populações do intertidal, conduzindo, no extremo, à sua extinção local caso a adaptação genética destas espécies não seja capaz de acompanhar a taxa de aquecimento prevista.

As conclusões aqui apresentadas corroboram não só estudos científicos que indicam que as comunidades intertidais são sentinelas dos impactos do aquecimento global, como também estudos com anfíbios, répteis e invertebrados que demonstram que espécies com maior tolerância térmica têm menor capacidade de aclimação. Uma das espécies tropicais testadas no presente estudo, o peixe intertidal *Bathygobius soporator*, teve a maior tolerância térmica, de quase 41^{o}C. No entanto, não foi capaz de aclimar em nenhuma das experiências. Esta foi também a temperatura máxima registada em poças de maré durante ondas de calor, onde os organismos foram capturados. Dado o aumento da frequência, intensidade e duração das ondas de calor previsto, é expectável que a temperatura máxima encontrada em poças de maré suba. Assim, caso estes organismos fiquem retidos numa poça durante uma onda de calor futura, poderão morrer, como já foi observado noutras espécies de peixes tropicais nas Ilhas Marshall, no Oceano Pacífico.

O conhecimento dos limites térmicos das espécies, e a plasticidade destes limites, permite-nos inferir quais as alterações na distribuição e abundância das espécies durante o aquecimento global. No entanto, para prever os impactos a nível de interações entre espécies, estrutura das comunidades e dinâmica dos ecossistemas, é necessário testar muitas mais espécies, e aprofundar estudos transgeracionais, já que a variabilidade genética poderá ser decisiva na persistência de populações ameaçadas pelo aquecimento global. Na verdade, a adaptação evolutiva poderá ser a única forma de espécies vulneráveis persistirem caso não sejam capazes de dispersar para locais mais favoráveis. Eventualmente, tal informação poderá ser incorporada na gestão costeira por forma a minimizar a perda de biodiversidade consequente das alterações climáticas.

Em conclusão, os resultados do presente estudo sugerem fortemente que as espécies tropicais intertidais se encontram em maior risco de sofrerem efeitos negativos decorrentes do aquecimento previsto para esta região. Assim, esta tese contribui para o debate científico sobre que espécies são mais vulneráveis ao aquecimento global, tropicais ou temperadas.

Palavras-chave: Aquecimento Global, Tolerância Térmica, Capacidade de Aclimação, Máximo Térmico Crítico, Intertidal Rochoso.

Abstract

Temperature is one of the key abiotic factors responsible for setting ecological patterns in nature. Recent projections indicate that mean global temperature is predicted to increase 2 to 4°C by the end of this century, putting pressure on many ecosystems. Predicting the likely impacts of climate warming is thus imperative. The tropics and temperate zones encompass most of the species found in the planet, and a scientific debate on the vulnerability of tropical *versus* temperate species towards rising temperatures has emerged. One way to answer this debate involves estimating species' thermal tolerance and acclimation capacity, which remains largely unknown. Rocky intertidal species are considered sentinels of climate change. Their physiological limits are close to environmental temperatures, which encourages the investigation of rocky shore species' response to warmer conditions. This was the main goal of the present research. The thermal tolerance and acclimation capacity of tropical and temperate rocky shore species from different taxa were investigated. It was found that tropical species are the ones living closest to their thermal limits. In fact, tropical intertidal species already experience habitat temperatures above their thermal limits during heat waves. It was also found that tropical species have a lower acclimation capacity than their temperate counterparts. This means that tropical species may be vulnerable to even small increases in habitat temperature. Considering future warming trends, these findings suggest that tropical species may be in greater jeopardy than temperate ones. Probably, tropical intertidal species will take refuge in colder, subtidal waters, during extreme thermal events. However, if such refuges are unavailable and/or if genetic adaptation is not able to keep up with the warming rate, intertidal populations may be prone to local extinction. Thus, the assessment of the future impacts of climate warming upon communities' structure and ecosystem dynamics shall include more species and transgenerational studies.

Keywords: Global Climate Change, Thermal Tolerance, Acclimation Capacity, Critical Thermal Maximum, Rocky Shore.

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Chapter 1

General Introduction

General Introduction

1. Thermal tolerance

Angilletta (2009) rightly stated that anyone familiar with physics and chemistry shouldn't be impressed by the discovery that life depends on temperature. The temperature of an organism – a quantitative measure of the kinetic energy of its molecules – constrains the rates of chemical reactions, namely biochemical reactions. As a consequence, temperature affects cellular, systemic, and organismal levels (Rome *et al*., 1992) and potentially limits behavioral and physiological performances linked to development, growth and reproduction (Angilletta *et al*., 2002). The favorable range of temperature or performance breadth of a given species is referred to as thermal tolerance window. Above or below that range the performance is negatively affected and the survival of the species is at stake.

The threat of global climate change has fostered the current interest in understanding species' thermal limits (e.g. Hofmann & Todgham 2010; Somero, 2010; Madeira *et al*., 2012a; Vinagre *et al*., 2013). Understanding the thermal limits of organisms and the plasticity of those limits enables us to argue about what will happen to their distribution and abundance during climate change (e.g. Stillman, 2002; Walther *et al*., 2002; Parmesan, 2006).

Two different experimental approaches are commonly used to determine the thermal tolerance of a species: a) the "static methods", which determine the Lethal Temperature, i.e. the temperature that causes the death of 50% of the individuals in a sample (Stillman & Somero, 2000), and b) the "dynamic methods", in which the temperature that triggers the loss of motor function (Critical Thermal Maximum or Knockdown Temperature) is determined by gradually increasing temperature until a critical point is reached (e.g. loss of the righting response, muscle spasms) (Brattstrom, 1968; Huey et al., 1992; Lutterschmidt & Hutchison, 1997).

The dynamic methods have been more broadly used because they are easier to apply, require fewer animals and provide quick data (Lutterschmidt & Hutchison, 1997). It is generally accepted that the Critical Thermal Maximum (CTMax) is the most efficient index of upper thermal tolerance among ectothermic vertebrates and invertebrates (Becker & Genoway, 1979). Based on the temperature at which the first signs of heat stress occur, CTMax represents the point at which the animal is ecologically or behaviorally dead (Brattstrom, 1968).

Cox (1974) provided a comprehensive definition:

> “The critical thermal maximum (or minimum) is the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death when heated from a previous acclimation temperature at a constant rate just fast enough to allow deep body temperatures to follow environmental temperatures without a significant time lag.”

In this way, the CTMax method provides a conservative index of thermal tolerance, as the organism does not die but is unable to escape from predators and forage due to equilibrium loss. Thus, results are more comparable to natural conditions (e.g. Bennett & Judd, 1992; Mora & Ospina, 2001; Vinagre *et al*., 2013).

2. Thermal acclimation

Assessing the capacity of organisms to acclimate and/or adapt to increased temperatures is crucial to understand the response of populations and communities to global warming. Hofmann & Todgham (2010) pointed out three main response options for organisms facing global climate change: (a) disperse to more hospitable habitats, (b) tolerate the new conditions through phenotypic and physiological plasticity, or (c) adapt to the new environment through genetic change via the process of evolution. All organisms can potentially modify their behavioral, physiological or morphological characteristics in response to environmental temperature (Angilletta, 2009). Phenotypic plasticity as a response is an important mechanism for coping with a changing or fluctuating environment and refers to thermal acclimation, i.e. any phenotypic alteration in physiology in response to environmental temperature that alters performance and plausibly enhances fitness (Huey *et al*., 1999; Angilletta, 2009).

Even though both rapid and gradual responses to environmental temperatures can be reversible, some responses remain fixed throughout the life of an organism (Johnston and Wilson, 2006). Following this idea, Angilletta (2009) distinguished between two types of thermal acclimation: (a) developmental acclimation, which encompasses irreversible responses to temperatures undergone throughout ontogeny, and (b) reversible acclimation, which comprises regulated responses to diel or seasonal shifts in temperature, thus offering a greater potential to match physiology to the current environment when compared to developmental acclimation.

Acclimation only benefits an organism when the time needed to acclimate doesn’t surpass the time between thermal switches. Therefore, reversible acclimation should benefit long-lived organisms which experience seasonal changes in temperature during their life and organisms which experience diel variations if acclimation and deacclimation occur rapidly (Angilletta, 2009). Regardless of this, acclimation responses have common properties, particularly the activation of molecules (e.g. genes, enzymes) responsible for a change in the phenotype due to the detection of environmental signals and subsequent transduction into a cellular response (Wilson & Franklin, 2002; Angilletta *et al*., 2006).

> “Do most acclimation responses enhance the fitness of organisms or are these responses merely unavoidable consequences of thermal change?”
>
> (Angilletta, 2009)

Acclimation has traditionally been assumed to be beneficial to organisms by compensating for the impacts of environmental change. This assumption that acclimation responses enhance fitness is known as the Beneficial Acclimation Hypothesis and predicts that a change in the environment of an organism leads to a change in the phenotype that improves performance in the new environment (Leroi *et al*., 1994).

A large body of evidence clearly demonstrates that acclimation enhances performance in some species (e.g. Kinne, 1962; Prosser, 1986; Cossins & Bowler, 1987; Rome *et al*., 1992). Nonetheless, responses to thermal change do not always enhance the performance of an organism and thus acclimation changes cannot just be assumed to be beneficial (e.g. Leroi *et al*., 1994; Huey *et al*., 1999; Wilson & Franklin, 2002). As a matter of fact, beneficial acclimation is only one possibility in a set of phenotypic responses to acclimation (Huey *et al*., 1999), which may in turn be inconsequential or even disadvantageous (Leroi *et al*., 1994). Moreover, the generality of the beneficial acclimation assumption should be rejected and the fact that acclimation imposes costs in terms of survivorship or fecundity to an organism (see Angilletta, 2009; Donelson *et al*., 2012) should be taken into consideration.

3. Overview of thermal studies in ectotherms

Early studies of thermal acclimation have focused on amphibians (e.g. Hutchison, 1961; Brattstrom & Lawrence, 1962; Brattstrom & Regal, 1965; Brattstrom, 1968) and reptiles (e.g. Wilhoft & Anderson, 1960; Hertz *et al*., 1983; Kaufmann & Bennett, 1989). The common ground of these studies was the investigation of animals' ability to modify their functional capacities, particularly locomotor performance, to adjust to the thermal environment. Brattstrom (1968), although focusing on amphibians, stressed some important general conclusions: (a) the rate of thermal acclimation, measured by changes in the critical thermal maximum, is rapid, (b) the acclimation rate and range is a function of the temperature of acclimation, and (c) the inability to make rapid physiological adjustments might have distributional consequences. Corroborating Brattstrom (1968) conclusions, some recent studies with other ectotherms (e.g. Patterson, 1999; Huang *et al*., 2006; Gvoqdík *et al*., 2007) show that thermal acclimation significantly affects critical thermal maximum, i.e. CTMax shifts in accordance with acclimation temperature. Most of the terrestrial ectotherms used in these studies inhabit semi-arid or arid environments, extremely harsh habitats where temperatures and thermal amplitudes are very high.

Extremely thermally stressed environments also exist in the marine environment. Rocky intertidal habitats – regions between the high- and low-tide lines of coastlines – are subject to environmental challenges posed by both aquatic and aerial climatic regimes due to alternating exposures to sharp spatial and temporal gradients in temperature during the tidal cycle. The high thermal conductivity and heat capacity of water cause the body temperature of aquatic organisms to closely follow the temperatures of their aqueous surroundings (Spotila *et al*., 1992). Moreover, the small water volume of tidal pools in the rocky shore means that these environments have low thermal inertia and consequently will be one of the aquatic environments hardest hit by temperature rise, functioning as early indicators of climate warming (see Helmuth *et al*., 2006). Furthermore, a growing awareness that intertidal species are home to diverse biological communities and integral components of nearshore food webs (Horn *et al*., 1999) has led to an increased research on the vulnerability of rocky shore species threatened by climate warming, making the investigation of their thermal limits a pressing need (e.g. Stillman & Somero, 2000; Madeira *et al*., 2012a; Vinagre *et al*., 2013).

Upper thermal limits are already known for a considerable number of temperate marine coastal organisms, which inhabit rocky shores and estuaries (e.g. Cuculescu *et al*., 1998; Madeira *et al*., 2012a, 2012b; Vinagre *et al*., 2013). Madeira *et al*. (2012a) and Vinagre *et al*. (2013) determined the upper thermal limits of various fish and crustaceans of the Northeast Atlantic. Using the CTMax method, Madeira *et al*. (2012a) concluded that species from the intertidal/supratidal zones (e.g. *Gobius niger*, *Pachygrapsus marmoratus* and *Palaemon elegans*) had higher CTMax values in comparison to subtidal and demersal species (e.g. *Diplodus species*, *Lophozozymus incisus* and *Crangon crangon*). Additionally, Vinagre *et al*. (2013) showed that pools in the lower intertidal have temperatures well below the CTMax of the tidal pool species examined (which ranged from 32^{o}C to 35^{o}C), thus being a possible natural refuge during heat waves. As for acclimation capacity, Cuculescu *et al*. (1998) tested the marine crabs *Carcinus maenas* (eurythermal) and *Cancer pagurus* (stenothermal) from the North Sea. The authors found that in spite of the significantly higher CTMax of *C. maenas*, the acclimation ability was greater in *C. pagurus*, suggesting that the ability for acclimation is not directly related to eurythermicity. Notwithstanding, it is relevant that acclimation had a significant effect in both species.

Concerning the investigation of thermal tolerance in tropical marine organisms, Mora & Ospina (2001) investigated the thermal tolerance of 15 reef fishes of the tropical eastern Pacific and found that the CTMax of those species ranged between 34.7^{o}C and 40.8^{o}C. According to the authors, the differences in CTMax among reef-fish species may confer different abilities to colonize warmer habitats, allowing, for instance, tolerant species (e.g. *Mugil curema*, *Bathygobius ramosus* and *Malacoctenus zonifer*) to be common in intertidal pools that could reach 36^{o}C, where other less-tolerant species are infrequent or absent. In light of climate warming scenarios this may have implications in the distribution of reef-fish species. Additionally, Ospina & Mora (2004) studied the effect of body size on the thermal tolerance of seven reef fish species from the tropical eastern Pacific Ocean and verified little variation in CTMax ranging from juveniles to adults. This reduced intra-specific variation in thermal tolerance suggests limited capacity of species to adapt to extreme thermal conditions, thus raising concerns about current global changes in temperature. As regards tropical crustaceans, Stillman & Somero (2000) analyzed the upper thermal tolerance limits of species of porcelain crabs, genus *Petrolisthes*, from intertidal and subtidal habitats throughout the eastern Pacific. During thermal acclimation at elevated temperatures the upper thermal tolerance limits increased, the amount of increase being greater for subtidal than for intertidal species. This result suggests that global warming might impact the distribution limits of intertidal species to a greater extent than that of subtidal species, even though many more species need to be tested.

4. What species might be more vulnerable to climate warming, tropical or temperate?

Ecological forecasting on the likely impacts of climate warming is crucial at a time when several ecosystems seem to be responding to this environmental threat (Walther *et al*., 2002). Explicit hypotheses should be generated and tested. Among the most important questions to be tested are: which are the most vulnerable organisms to climate change and where are they?

A debate has emerged from studies on the effects of warming on ectotherms, arguing about which organisms may face a higher risk from environmental warming, tropical or temperate organisms. The tropics and temperate zones encompass most of the species found in the planet. Some studies predict that climate warming will have a small impact in the tropics (see Root *et al*., 2003), because the rate of warming is predicted to be lower than at higher latitudes (IPCC, 2007). However, it is known that species that live in aseasonal environments may suffer disproportionally from small increases in temperature, which may place tropical organisms at a higher risk than their temperate counterparts that endure larger thermal amplitudes throughout the year and thus presumably have a greater scope for acclimation (see Tewksbury *et al*., 2008).

The vulnerability towards a rise in temperature will depend mostly on the organisms' thermal tolerance and acclimation capacity, which remains unknown for most species. As above mentioned, one of the habitats where climate change impacts may strike first is the intertidal zone. Rocky intertidal habitats exist at the margins between the terrestrial and the marine realms, thus they are not only subject to changes in water temperature, but also to changes in the aerial climatic regime. This way, intertidal communities offer excellent scientific material for studying climate warming impacts.

The works of Mora & Ospina (2001) and Madeira *et al*. (2012a) greatly contributed to the foregoing debate. Mora & Ospina (2001) warned that some tropical reef fishes may be severely threatened in a short-term temperature increase situation, not only by the low intraspecific variability in thermal tolerance, but also because generation time in reef fishes is slower than the time in which ocean is expected to attain higher temperatures. Yet, Madeira *et al*. (2012a) showed that the CTMax of tropical intertidal species is 2 to 5°C higher than the maximum habitat temperature, whereas that of temperate/subtropical species is 1 to 2°C lower. This suggests that maximum habitat temperatures in temperate/subtropical regions may surpass the upper thermal limits of temperate intertidal species, making them particularly vulnerable to further increases in temperature and possibly more vulnerable than tropical species.

Evolutionarily, the foreseen rise in habitat temperature due to climate change is predicted to be rapid and organisms inhabiting ecosystems already subject to local thermal stress may not be able to adapt in pace with the new thermal regime. During the last century the mean global temperature increased by 0.6°C and an increase of 2 to 4°C – by consensus 3°C (Kerr, 2004) – by the end of this century is predicted, which means that many ecosystems are currently warming faster than they have for thousands of years (IPCC, 2007). As such, the projected warming rate for this century of nearly five times the rate of the previous one might have startling consequences for ecosystems and biodiversity. Nevertheless, organisms, populations and ecological communities do not respond to approximated global averages. Regional changes are more relevant in the context of ecological responses to climatic change, and the asymmetry in the warming in many regions will surely lead to different ecological responses (see Walther *et al*., 2002). Therefore, assessing the vulnerability of species to climate warming is an urgent need, as is being able to prioritize conservation efforts based on where species are more vulnerable around the globe.

5. Aim of the thesis

The aim of present study was to estimate the thermal tolerance and acclimation capacity of tropical and temperate rocky shore species from different taxa, so as to infer and compare their vulnerability to climate warming. This research contributes to the international debate on which organisms might face a higher risk from climate change: tropical or temperate.

To this end, sampling and laboratory experiments were carried out in a tropical coastal area, the Brazilian coast, at approximately 20°S, and in a temperate coastal area, the Portuguese coast, at approximately 38°N. An important number of common tropical and temperate species were tested under controlled conditions, allowing a multi-specific study of thermal tolerance and acclimation capacity.

Specifically, the objectives of this thesis were to:

1) Estimate the upper thermal limits (CTMax) of tropical and temperate rocky shore organisms, in order to understand which species are living closest to their thermal limits and to discuss what might happen to the distribution and abundance of those species during climate warming;

2) Compare the intraspecific variability in upper thermal limits of tropical and temperate species to hypothesize which ones have the lowest evolutionary potential to cope with further warming;

3) Test the capacity of tropical and temperate organisms to acclimate their upper thermal limits when exposed to long-term and short-term increases in temperature, so as to understand whether species' tolerance limits can keep in pace with the changing environment.

This thesis is presented in the form of two scientific articles (already submitted to indexed scientific journals), the first one concerning thermal tolerance and the second one concerning acclimation capacity.

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Chapter 2

Vulnerability of tropical and temperate coastal organisms to climate change

Vulnerability of tropical and temperate coastal organisms to climate change

Abstract

The threat of global climate change has fostered the current interest in understanding species' thermal limits. Rocky shores are predicted to be one of the habitats hardest hit by temperature rise, making its inhabiting communities excellent experimental material for climate warming studies. The aim of the present work was to 1) estimate the upper thermal limits (CTMax), 2) the intraspecific variability in upper thermal limits (% coefficient of variation of CTMax), and 3) the warming tolerance (Maximum Habitat Temperature – CTMax) of coastal organisms. Differences in biological groups (decapod crustaceans *vs* fish) were investigated and the effect of region (tropical *vs* temperate) and habitat (intertidal *vs* subtidal) was tested. Specimens were collected and tested during the summer of 2014, in Southeastern Brazil and Western Portugal. No differences were found when comparing decapod crustaceans and fish. CTMax was higher for tropical (34.2^{o}C to 39.7^{o}C) than temperate species (27.4^{o}C to 38^{o}C) and also higher for intertidal than subtidal species. Intraspecific variability was higher in temperate species than in tropical species, but no difference was found between intertidal and subtidal species. Warming tolerance was higher for temperate species than for tropical species and higher for subtidal species than for intertidal species. This study confirms previous reports that stated that the species with the highest thermal limits have the lowest warming tolerance. Our results strongly suggest that tropical intertidal species are the ones in greatest jeopardy considering current climate warming trends. This study contributes to the ongoing scientific debate on which organisms face a higher risk from climate warming: tropical or temperate.

Keywords: Global Climate Change, Upper Thermal Limits, Critical Thermal Maximum, Warming Tolerance, Rocky Shore.

Introduction

Temperature is arguably the most critical abiotic stress that ectothermic organisms experience, affecting biological processes at all organization levels (Rome *et al*., 1992) and limiting behavioral and physiological performances linked to development, growth and reproduction (Angilletta *et al*., 2002). Temperature is thus one of the key factors responsible for setting ecological patterns in nature (Hutchins, 1947).

During the last century temperature increased 0.6^{o}C globally and an increase of 2 to 4^{o}C by the end of this century is predicted, which means that many ecosystems are currently warming faster than they have for thousands of years (IPCC, 2007). In fact, ecological responses to recent climate change are already clearly visible (see reviews by Walther *et al*., 2002; Hofmann & Todgham, 2010). Evidence from polar terrestrial to tropical marine environments indicates that the warming for the past decades has affected the phenology of organisms (e.g. Bairlein & Winkel, 2001; Menzel & Estrella, 2001), the range and distribution of species (e.g. Hughes, 2000; McCarty, 2001; Walther *et al*., 2001), and the composition and dynamics of communities (e.g. Sagarin *et al*., 1999; Walther, 2000). As such, the predicted warming for the coming decades might have startling consequences for ecosystems and biodiversity.

Notwithstanding, organisms, populations and ecological communities do not respond to global averages. Instead, regional changes are more relevant in the context of ecological responses to climatic change (see review by Walther *et al*., 2002). In this regard, the Intergovernmental Panel on Climate Change (2007) predicted an asymmetry in the rate of warming around the globe, with higher latitudes warming faster than lower latitudes. This raises issues on which organisms may face a higher risk from environmental warming, and a scientific debate on the vulnerability of tropical *versus* temperate organisms has arisen (e.g. Tewksbury *et al*., 2008; Deutsch *et al*., 2008; Duarte *et al*., 2012).

The impacts of climate warming on organisms depend primarily on the behavior, morphology, physiology, and ecology of the organisms in question (Kearney and Porter 2004; Helmuth *et al*., 2005; Bradshaw & Holzapfel, 2008), with negative impacts being greatest on individuals physiologically specialized to narrow temperatures and with limited acclimation capacity.

One way to answer this debate involves estimating geographical patterns of warming tolerance, which is the difference between a species' upper thermal limit and its current Maximum

Habitat Temperature (MHT) (Lutterschmidt & Hutchison, 1997; Somero, 2005; Deutsch *et al*., 2008). Low warming tolerance indicates that individuals of a given species may be prone to deleterious and ultimately lethal thermal stress with rising temperatures. Several studies indicate that tropical organisms may face a higher risk than their temperate counterparts since they live at “near-stressful temperatures” and because they have evolved in stable environments, being thermal specialists (see Tewksbury *et al*., 2008). The work of Duarte *et al*. (2012) with tropical tadpoles supports this hypothesis, but only in part. The authors stressed that the rate of warming is predicted to be faster in the temperate zone, which means that a large warming tolerance in the temperate zone may not be as helpful as it would if warming was uniform among different latitudes (Hoffmann, 2010). Supporting this, Madeira *et al*. (2012) showed that maximum habitat temperatures in temperate/subtropical regions may surpass the upper thermal limits of temperate intertidal species, making them particularly vulnerable to further increases in temperature and possibly more vulnerable than tropical ones.

Thus, knowing the upper thermal limits of a given species and the extent to which predicted climate warming will affect these limits is an important endeavor to assess species’ vulnerability to this threat. The Critical Thermal Maximum (CTMax) is a widely used index to quantify upper thermal limits among ectothermic vertebrates and invertebrates (e.g. Becker & Genoway, 1979; Mora & Ospina, 2001; Madeira *et al.,* 2012). Based on the temperature at which the first signs of heat stress occur, CTMax represents the point at which the animal is ecologically or behaviorally dead (Brattstrom, 1968). The CTMax is determined by gradually increasing temperature until a critical point is reached (e.g. loss of the righting response, muscle spasms) (Brattstrom, 1968; Huey *et al*., 1992; Lutterschmidt & Hutchison, 1997; Mora & Ospina, 2001). As such, the CTMax provides a conservative measure of thermal tolerance and allows for an accurate prediction of the responses of organisms to natural conditions (Bennett & Judd, 1992).

The rocky intertidal zone and its inhabiting communities offer excellent experimental material for climate warming studies (see review by Helmuth *et al*., 2006). Hiatt & Strasburg (1960) had previously observed the importance of temperature in tropical rock pools, reporting that in the Marshall Islands they heated to 41^{o}C, when air temperatures reached at most 31^{o}C, leading to the death of some fish inside the pools. These habitats are subject to extreme thermal challenges due to alternating exposures to sharp spatial and temporal gradients during the tidal cycle. Additionally, the small water volume of tidal pools in the rocky shore means that these environments have low thermal inertia and consequently will be one of the aquatic environments hardest hit by temperature rise, functioning as early indicators of climate warming (see reviews by Helmuth *et al*., 2006; Hofmann & Todgham, 2010).

Also, the thermal niche occupied by species seems to be a major determinant of thermal tolerance and cellular response to increasing temperature (Stillman & Somero, 2000; Stillman, 2003; Madeira *et al*., 2012, 2014a). This means that upper thermal tolerance limits reflect microhabitat conditions, with intertidal species showing higher CTMax values than subtidal and demersal species (Madeira *et al*., 2012). However, intertidal species may currently be living closer to their thermal limits and may have reduced ability to increase their thermal tolerance when compared to subtidal species (Stillman & Somero, 2000; Madeira *et al*., 2012; Vinagre *et al*., 2013a). Therefore, further research on thermal tolerance limits of coastal organisms from different thermal niches would greatly improve our understanding of the effects of global warming.

Another important aspect is intraspecific variability. Species with enough genetic variability to generate phenotypes with a wide range of thermal tolerances may become “winners” in a warming world, since exceptionally tough individuals can be selected through successive generations resulting in genetic adaptation (Somero, 2010).

The aim of the present work was to 1) estimate the upper thermal limits (CTMax), 2) the intraspecific variability in upper thermal limits (% coefficient of variation of CTMax), and 3) the warming tolerance (MHT – CTMax) of coastal organisms. Differences in biological groups (crustacean decapods *vs* fish) were investigated and the effect of region (tropical *vs* temperate) and habitat (intertidal *vs* subtidal) was tested.

Materials and methods

Study areas and tested species

Marine decapod crustaceans (shrimps and crabs) and fish were collected in a tropical and a temperate rocky shore, in the summer of 2014 in Southeastern Brazil (23°49’ S; 45°25’ W) and Western Portugal (38°71’ N; 9°48’ W) (Fig. 1). Specimens were collected manually and using hand nets.

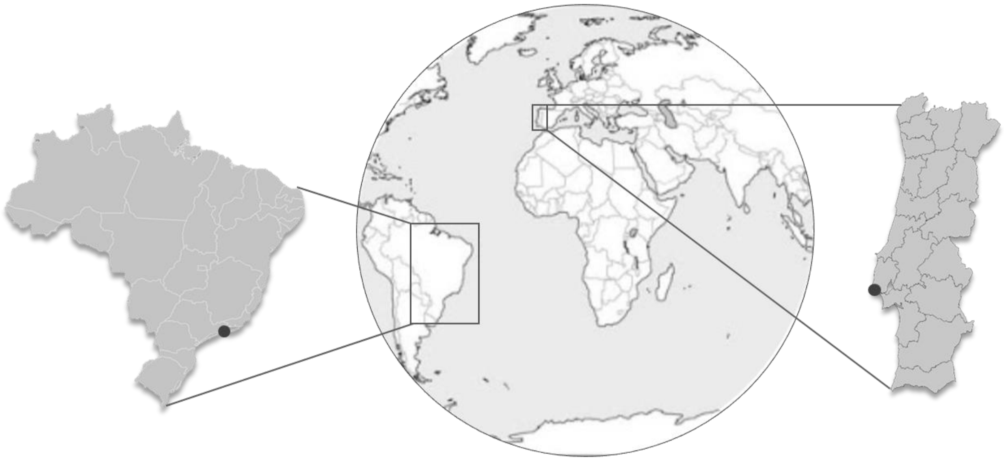


Fig. 1 – Schematic representation of the study areas. Sampling sites' location is illustrated by dot icons.

The tropical area studied has a mean annual sea surface temperature (SST) of 24°C and a mean summer SST of 26°C, while the temperate study area has a mean annual SST of 17°C and a mean summer SST of 19°C (Locarnini *et al.*, 2009). Data on maximum SST was gathered from local meteorological stations (30°C for the tropical area and 22°C for the temperate area). Data on maximum water temperature of tidal pools was registered in the summer of 2014, in both areas, during ebb tides, in 9 tidal pools in the tropical study area and 16 in the temperate study area. The maximum water temperature registered in tropical tidal pools was 41°C, in February 2014, while in the temperate area it was 30°C, in June 2014.

The tropical decapod crustacean species studied can be divided in two groups: the shrimps *Palaemon northropi* (Rankin 1898) and *Hippolyte obliquimanus* Dana 1852, and the crabs *Pachygrapsus transversus* (Gibbes 1850), *Menippe nodifrons* Stimpson 1859 and *Eurypanopeus abbreviatus* (Stimpson 1860). The tropical fish species studied were *Scartella cristata* (Linnaeus 1758), *Eucinostomus melanopterus* (Bleeker 1863), *Bathygobius soporator* (Valenciennes 1837), *Parablennius marmoreus* (Poey 1876), *Stegastes fuscus* (Cuvier 1830), *Sphoeroides testudineus* (Linnaeus 1758) and *Malacoctenus delalandii* (Valenciennes 1836).

The temperate decapod crustacean species studied were the shrimps *Crangon crangon* (Linnaeus 1758) and the crabs *Lophozozymus incisus* (Milne-Edwards 1834) and *Pachygrapsus marmoratus* (Fabricius 1787). The temperate fish species studied were *Lepadogaster lepadogaster* (Bonnaterre 1788) and *Pomatoschistus microps* (Krøyer 1838). Data for temperate species was completed with that published in Madeira *et al*. (2012) (which includes the following species: the shrimps *Palaemon longirostris* (Milne-Edwards 1837) and *Palaemon elegans* (Rathke 1837); the crabs *Carcinus maenas* (Linnaeus 1758) and *Liocarcinus marmoreus* (Leach 1814); and the fish *Dicentrarchus labrax* (Linnaeus 1758), *Diplodus bellottii* (Steindachner 1882), *Diplodus sargus* (Linnaeus 1758), *Diplodus vulgaris* (Geoffroy St. Hilaire 1817), *Gobius cobitis* (Pallas 1814), *Gobius niger* (Linnaeus 1758), *Liza ramada* (Risso 1827), *Paralipophrys trigloides* (Valenciennes 1836) and *Solea lascaris* (Risso 1810)), Vinagre *et al*. (2013a) (which includes the following species: the shrimp *Palaemon serratus* (Pennant 1777), and the fish *Coryphoblennius galerita* (Linnaeus 1758), *Gobius paganellus* Linnaeus, 1758 and *Lipophrys pholis* (Linnaeus 1758)) and Madeira *et al*. (2014b) (which includes data for *Sparus aurata* Linnaeus 1758) for a comprehensive comparison that includes all species ever tested in these study areas, following the same experimental protocols.

This study focused on these species because they are key species in the intertidal/subtidal ecosystems they inhabit.

Acclimation conditions and experimental setup

After collection, organisms were transported to the laboratory facilities and housed in indoor re-circulating aquaria with a constant temperature (the same as the habitat temperature found at the time of capture, 26^{o}C for tropical organisms and 20^{o}C for temperate ones), aerated sea water and salinity 35‰. The water dissolved O_2 level varied between 95% and 100%. The organisms were acclimated for seven days (at 26^{o}C – tropical organisms; at 20^{o}C – temperate organisms) to ensure that all had a similar recent thermal history. They were fed *ad libitum* once a day, with commercial shrimp, and starved 24 hours before the experiments.

The thermal tolerance of each species was determined using the dynamic method described in Mora & Ospina (2001). The parameter measured was the Critical Thermal Maximum (CTMax, given in degrees Celsius), which is defined as the “arithmetic mean of the collective thermal points at which the end-point is reached” (Mora & Ospina, 2001), the end-point being loss of equilibrium. In shrimp and fish, loss of equilibrium was detected when individuals could not coordinate straight swimming and start moving in an angled position. Crabs needed to be stimulated using lab tweezers to force them upside down, and if they were unable to get back upright they would have reached the end-point. This criteria is the same followed by Madeira *et al*. (2012, 2014b) and Vinagre *et al*. (2013a).

To determine the CTMax, the organisms were subjected to a thermostatized bath. During the experiment, animals were exposed to a constant rate of water-temperature increase of 1°C h^{-1}, with constant aeration and observed continuously, until they reached the end-point. The experiments were carried out in shaded day light (14 L; 10D). The temperature at which each animal reached its end-point was measured with a digital thermometer, registered and then CTMax, its standard deviation and coefficient of variation were calculated.

To prevent any additional handling stress, the total length and weight of all individuals were measured at the end of the experiment. Fish were measured with an ichthyometer and shrimp and crabs with a digital slide caliper. The main characteristics of the species studied, and respective sample sizes, are shown in Table 1. Sample sizes were similar to those used by Mora & Ospina (2001), Madeira *et al*. (2012) and Vinagre *et al*. (2013a).

Data analysis

The upper thermal limits for each species were calculated using the equation:

$$\text{CTMax}_{\text{(species)}} = \sum (T_{\text{end-point } n})/n$$

Where $T_{\text{end-point}}$ is the temperature at which the end-point was reached for any given individual, and n stands for sample size.

To determine intraspecific variability of the CTMax, the coefficient of variation (in percentage) was calculated for each species:

$$\%CV = (SD/Mean) \times 100$$

Finally, the warming tolerance, i.e. the difference between CTMax and Maximum Habitat Temperature, provided an estimate on how closer these species may live to their upper thermal limits.

Table 1 – Common name, distribution, environment, sample size and mean total length (mm) for each species, in the present study. This table was constructed based on Fishbase (www.fishbase.com), Encyclopedia of life (www.eol.org) and World Register of Marine Species (www.marinespecies.org).

Tropical Species	**Common name**	**Distribution**	**Environment**	**Sample size**	**Total length (mm) Mean ± SD**
Palaemon northropi	Cross-banded grass shrimp	Western Atlantic	Shallow waters/tide pools	16	30.75 ± 5.23
Hippolyte obliquimanus	Atlantic shrimp	Western Atlantic	Subtidal coastal waters	10	13.09 ± 2.58
Eurypanopeus abbreviatus	Lobate mud crab	Western Atlantic	Shallow waters/tide pools	8	16.50 ± 2.32
Menippe nodifrons	Cuban stone crab	Western and Eastern Atlantic	Shallow waters/tide pools	6	23.66 ± 4.80
Pachygrapsus transversus	Mottled shore crab	Western and Eastern Atlantic	Shallow waters/tide pools	20	12.75 ± 2.31
Bathygobius soporator	Frillfin goby	Western and Eastern Atlantic; Mediterranean Sea	Shallow waters/tide pools	15	54.80 ± 8.49
Scartella cristata	Molly miller	Western and Eastern Atlantic; Northwest Pacific; Mediterranean Sea	Shallow waters/tide pools	8	146.50 ± 31.25
Eucinostomus melanopterus	Flagfin mojarra	Western and Eastern Atlantic	Shallow waters/tide pools	35	10.80 ± 2.01
Parablennius marmoreus	Seaweed blenny	Western Atlantic	Subtidal coastal waters	5	64.20 ± 14.41
Malacoctenus delalandii	Brazilian blenny	Western Atlantic	Subtidal coastal waters	6	65.66 ± 3.20
Stegastes fuscus	Brazilian damsel	Western and Eastern Atlantic	Subtidal coastal waters	6	129.16 ± 16.55
Sphoeroides testudineus	Checkered puffer	Western Atlantic	Subtidal coastal waters	4	122.0 ± 19.64

Temperate Species	**Common name**	**Distribution**	**Environment**	**Sample size**	**Total length (mm) Mean ± SD**
[a]*Palaemon elegans*	Rock pool prawn	North and South Atlantic; Mediterranean Sea; Black Sea; Baltic Sea	Shallow waters/tide pools	25	32.52 ± 7.34
[b]*Palaemon serratus*	Common prawn	North Atlantic; Mediterranean Sea; Baltic Sea	Shallow waters/tide pools	7	41.70 ± 7.00
Crangon crangon	Brown shrimp	North Atlantic; Mediterranean Sea	Shallow waters/tide pools	5	26.80 ± 6.14
[a]*Palaemon longirostris*	Delta prawn	North Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	14	43.79 ± 8.94
[a]*Carcinus maenas*	Green crab	North and South Atlantic; Mediterranean Sea; Indian Ocean; North Pacific	Shallow waters/tide pools	25	28.65 ± 5.80
Pachygrapsus marmoratus	Marbled rock crab	Eastern Atlantic; Mediterranean Sea; Black Sea	Shallow waters/tide pools	10	17.50 ± 2.80
Lophozozymus incisus	Montagu's crab	Eastern Atlantic; South Pacific; Indian Ocean	Shallow waters/tide pools	6	19.33 ± 1.75
[a]*Liocarcinus marmoreus*	Marbled swimming crab	North Atlantic; Mediterranean Sea	Subtidal coastal waters	7	22.35 ± 2.74
[a]*Gobius cobitis*	Giant goby	North Atlantic; Mediterranean Sea; Black Sea	Shallow waters/tide pools	4	46.00 ± 29.41
[a]*Paralipophrys trigloides*		Eastern Atlantic; Mediterranean Sea	Shallow waters/tide pools	9	67.33 ± 28.26
[b]*Lipophrys pholis*	Shanny	Eastern Atlantic; Mediterranean Sea	Shallow waters/tide pools	12	79.50 ± 38.50
[b]*Gobius paganellus*	Rock goby	Eastern Atlantic; Mediterranean Sea; Black Sea; Indian Ocean	Shallow waters/tide pools	8	48.50 ± 4.20
[b]*Coryphoblennius galerita*	Montagu's blenny	Eastern Atlantic; Mediterranean Sea; Black Sea	Shallow waters/tide pools	6	77.80 ± 14.20
Lepadogaster lepadogaster	Shore clingfish	Eastern Atlantic; Mediterranean Sea; Black Sea	Shallow waters/tide pools	6	56.33 ± 11.74
Pomatoschistus microps	Common goby	Eastern Atlantic; Mediterranean Sea; Baltic Sea	Shallow waters/tide pools	6	32.00 ± 3.16
[a]*Gobius niger*	Shadow goby	North Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	9	98.70 ± 6.36
[c]*Sparus aurata*	Gilthead seabream	Eastern Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	6	92.10 ± 8.10
[a]*Liza ramada*	Thin-lipped grey mullet	Eastern Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	6	44.00 ± 3.90

[a]*Solea lascaris*	Sand sole	North and South Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	8	184.38 ± 39.70
[a]*Diplodus sargus*	White seabream	Eastern Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	28	33.89 ± 9.07
[a]*Diplodus bellottii*	Senegal seabream	Eastern Atlantic; Mediterranean Sea	Subtidal coastal waters	17	103.71 ± 8.91
[a]*Diplodus vulgaris*	Two-banded seabream	Eastern Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	13	74.62 ± 7.76
[a]*Dicentrarchus labrax*	European seabass	Eastern Atlantic; Mediterranean Sea; Black Sea	Subtidal coastal waters	7	86.00 ± 6.19

[a] Data adapted from Madeira *et al*. (2012); [b] Data adapted from Vinagre *et al*. (2013a); [c] Data adapted from Madeira *et al*. (2014b)

Statistical analyses first aimed to verify whether these measured parameters (species average CTMax, intraspecific CTMax variation and warming tolerance) vary between major taxonomic groups ('decapod crustaceans' *vs* 'fish'). For that, we used the non-parametric Mann-Whitney procedure, corrected for continuity, to test for ranks over the whole datasets (13 decapods and 22 fish for all variables) for species averages. Because there were no differences between taxonomic groups for these variables (see 'Results'), we then proceeded to a more general approach to examine the effects of fixed factors 'Region' (temperate *vs* tropical) and 'Habitat' (intertidal *vs* subtidal), for the pooled guilds of crustaceans and fish, using an orthogonal two-way analysis of variance. Average values for each species were regarded as replicates. The assumption of homoscedasticity was tested using the Hartley's F_{max} statistic, and met in all cases ($p > 0.05$), which allowed the use of untransformed data. A statistical significance level of 0.05 was considered in all test procedures.

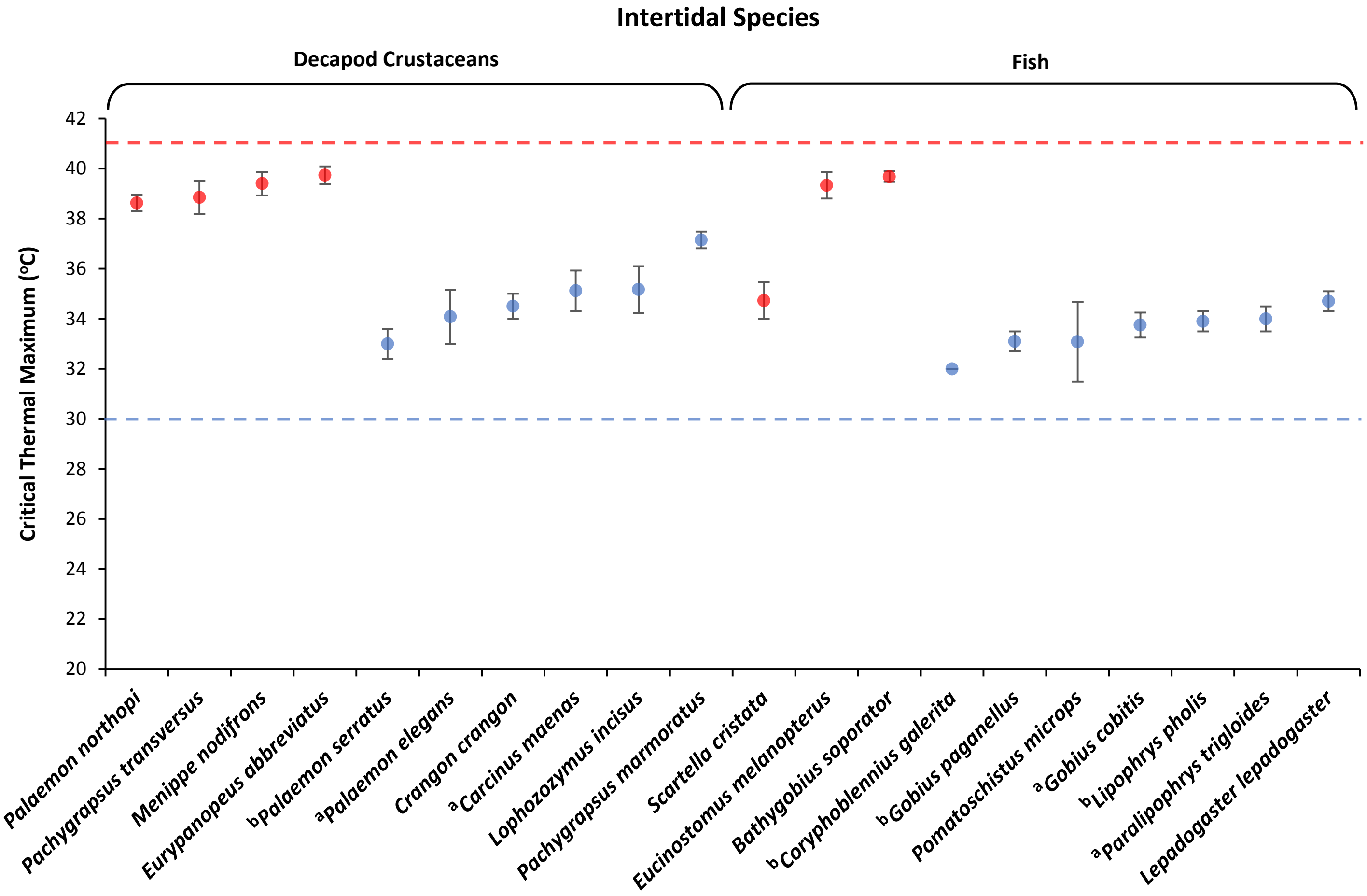
Results

Overall response of decapod crustaceans and fish

Regardless of contrasting physiological mechanisms allowing tolerance to heating (references), there were no significant rank differences between these animal groups for species average CTMax (Figs. 2, 3; $U = 351.5$, $z = 1.50$, $p = 0.13$), intraspecific CTMax variation (Figs. 2, 3; $U = 133.0$, $z = 0.32$, $p = 0.75$) and warming tolerance (Fig. 4; $U = 119.0$, $z = 0.80$, $p = 0.42$), along complete datasets including all regions and habitats. Therefore, we assumed that, on average, decapods and fish responded similarly to experimental heating, and pooled them for all analyses testing for general differences between regions and habitats.

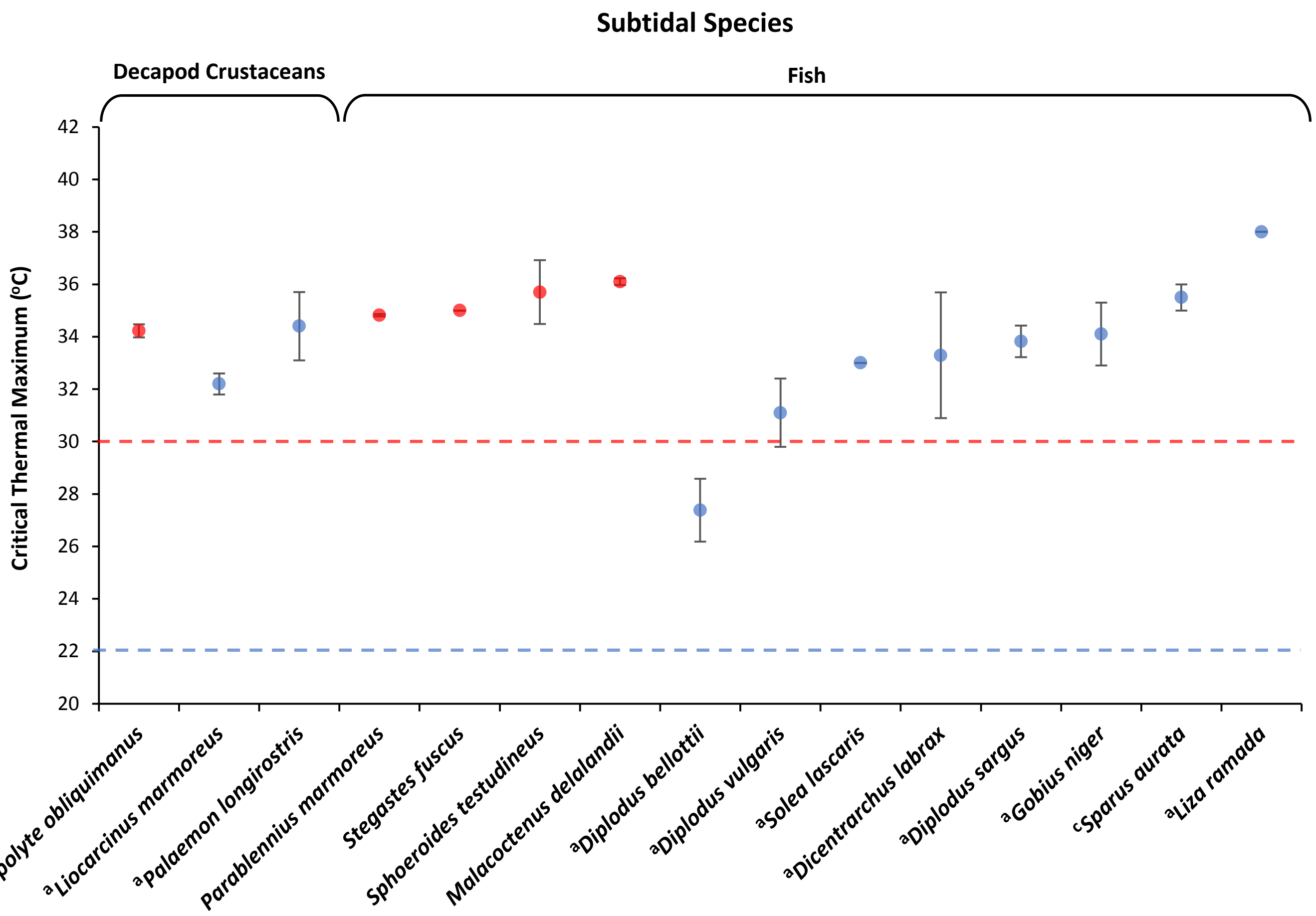
Average CTMax

Figures 2 and 3 show CTMax values for decapods and fish, separately for subtidal and intertidal species. As expected, species average CTMax were higher for tropical species (36.9^{o}C ± 1.3) compared to temperate ones (33.7^{o}C ± 2.0; $F_{1,31} = 25.9$, $p < 0.001$), but also higher for intertidal (36.4^{o}C ± 1.5) compared to subtidal species (34.2^{o}C ± 1.8; $F_{1,31} = 7.7$, $p < 0.01$). There is no factor interaction ($F_{1,31} = 3.7$, $p > 0.05$), suggesting these are independent effects (see Table 2)



[a] Data adapted from Madeira *et al*. (2012); [b] Data adapted from Vinagre *et al*. (2013a)

Fig. 2 – Critical Thermal Maximum of ten decapod crustacean species and ten fish species common in the intertidal zone of the tropical and temperate study areas. Tropical species are presented in red, while temperate species are presented in blue. The dashed lines represent the maximum temperature found in the water of tidal pools in the tropical (41ºC) and temperate (30ºC) study areas.



[a] Data adapted from Madeira *et al*. (2012); [c] Data adapted from Madeira *et al*. (2014b)

Fig. 3 – Critical Thermal Maximum of three decapod crustacean species and twelve fish species common in the subtidal zone of the tropical and temperate study areas. Tropical species are presented in red, while temperate species are presented in blue. The dashed lines represent the maximum coastal waters temperature for the tropical (30ºC) and temperate study areas (22ºC).

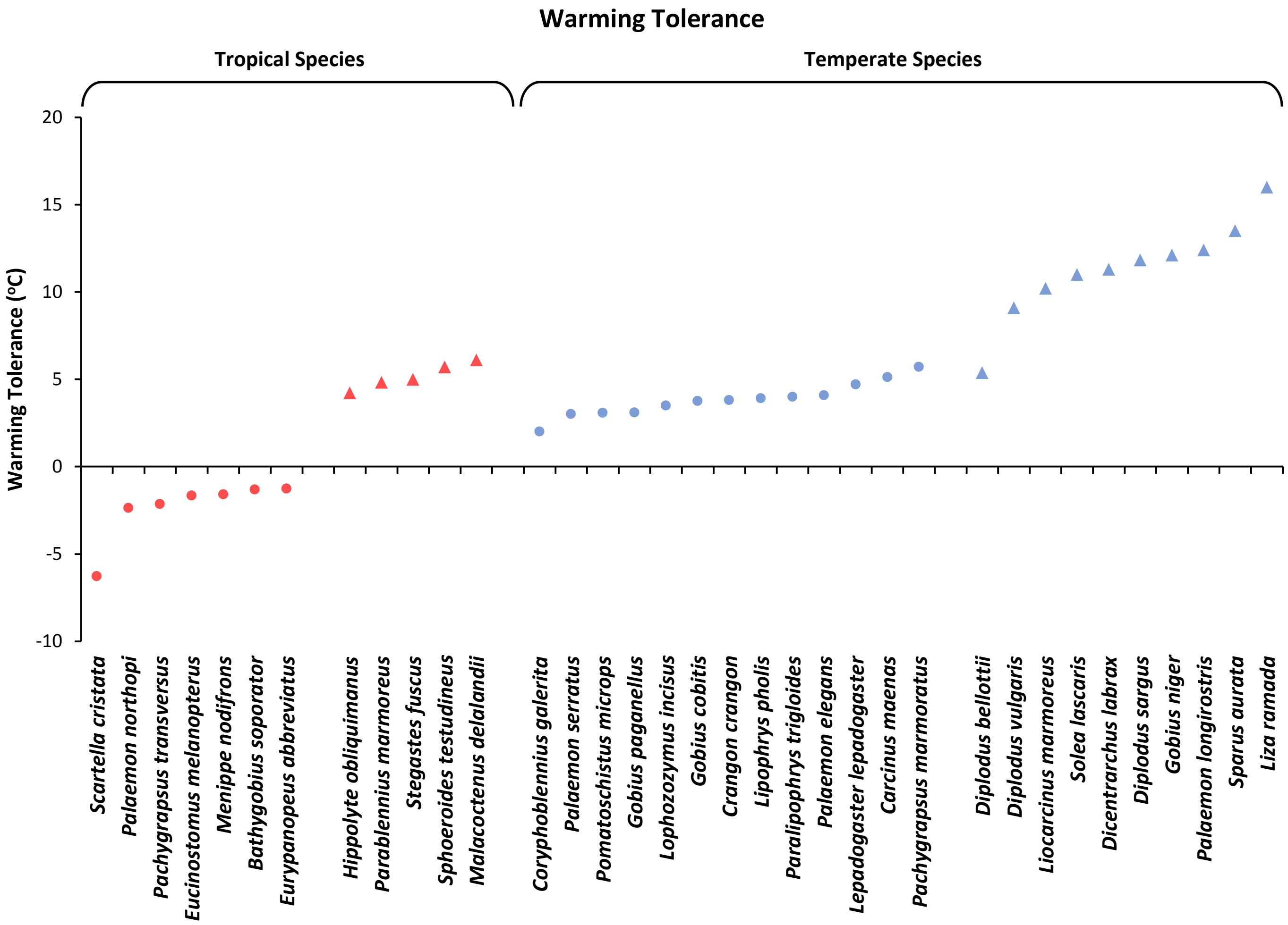


Fig. 4 – Warming tolerance (Critical Thermal Maximum – Maximum Habitat Temperature) of each species in the present study. Tropical species are presented in red, while temperate species are presented in blue. The dot icons refer to intertidal species, whereas triangular icons refer to subtidal species.

Table 2 – Summary results of two-way analyses of variance testing for differences of species average critical thermal maxima (CTMax, average), intraspecific variation of critical thermal maxima (CTMax, %CV) and warming tolerance, between regions (temperate and tropical) and habitats (intertidal and subtidal).

		CTMax (Average)			**CTMax (%CV)**			**Warming tolerance**		
	df	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*
Region	1	92.8	25.9	***	11.2	4.7	*	268.2	53.5	***
Habitat	1	27.3	7.7	**	0.7	0.3	ns	372	74.2	***
Region X Habitat	1	13.2	3.7	ns	3.0	1.2	ns	2.7	0.5	ns
Error	31	3.6								

* p < 0.05; ** p < 0.01; *** p < 0.001

Intraspecific CTMax variation

Intraspecific CTMax variation, as %CV, for the tested decapods and fish was far from homogenous. There were a few species showed marked intraspecific variation, namely the shrimp *Palaemon longirostris* and the fish *Diplodus belloti*, *D. vulgaris* and *Dicentrarchus labrax* within subtidal guilds (Fig. 2), and the shrimp *P. elegans* and the fish *Pomatoschisthus microps* and *Gobius niger*, in the case of intertidal guilds (Fig. 3). All these are temperate species. A more formal analysis of this dataset confirms this trend ($F_{1,31}$ = 4.7, $p < 0.05$), with temperate species (2.3% ± 1.7) showing a higher intraspecific CTMax variation compared to tropical species (1.1 ^{o}C ± 1.0), regardless of habitat (no support for a 'Region' X 'Habitat' interaction; $F_{1,31}$ = 0.3, $p > 0.05$). There were no significant differences between habitats ($F_{1,31}$ = 1.2, $p > 0.05$) (see Table 2).

Warming tolerance

As for the previous parameters, only main effects were detected (non-significant double interaction; $F_{1,31}$ = 0.5, $p > 0.05$), in this case for both 'Region' ($F_{1,31}$ = 53.5, $p < 0.001$) and 'Habitat' ($F_{1,31}$ = 74.2, $p < 0.001$) (see Table 2). These corresponded to very clear differences, either between tropical and temperate species or between intertidal and subtidal species (Fig. 4), with warming tolerance being higher in the temperate than in the tropical species and in the subtidal than in the intertidal species.

The species living farthest from their upper thermal limits are temperate subtidal species, with a mean warming tolerance of 11.3^{o}C, followed by tropical subtidal species, with a mean warming tolerance of 5.2^{o}C (Fig. 4).

Discussion

The current work showed that the warming tolerance of tropical species, in spite of their higher CTMax values, is significantly lower than that of temperate species, in the rocky shore ecosystem. Tropical intertidal species are living closest to their upper thermal limits, in particular tropical intertidal fish, which showed the lowest mean warming tolerance. Our results strongly suggest that tropical intertidal species are the ones in greatest jeopardy considering current climate warming trends.

Similar warming tolerance results have been reported for other taxa, when comparisons between tropical and temperate species are made. In the work of Duarte *et al*. (2012) with temperate and subtropical tadpole communities, the authors also found a trade-off between CTMax and warming tolerance. The tropical community had the highest CTMax values, yet, very low warming tolerance, being prone to future local extinction from acute thermal stress with rising pond temperatures. In addition, Deutsch *et al*. (2008), estimating the direct impact of warming on insect fitness across latitude, showed that warming in the tropics is likely to have the most deleterious effects because tropical insects are currently living very close to their optimal temperature. The authors also pointed that available thermal tolerance data for several vertebrate taxa suggests that these conclusions are general for terrestrial ectotherms. Our work suggests that such conclusions may also be general for coastal marine ectotherms, since no differences in warming tolerance were found between decapod crustaceans and fish. Pörtner (2002) concluded that oxygen limitation of thermal tolerance, visible as limitation in aerobic scope, appears to be a unifying principle among metazoans. The physiological reason being that the borders of the thermal tolerance window are characterized by the onset of internal systemic hypoxia despite fully oxygenated waters, resulting in anaerobic metabolism that cannot be sustained for long periods of time (Pörtner 2001, 2002; Pörtner & Knust 2007).

Mora & Ospina (2001) and Madeira *et al*. (2012) reached a different conclusions from the present work, however they used different reference temperatures. Mora & Ospina (2001) tested the thermal tolerance of 15 reef fishes of the tropical eastern Pacific and found that those species lived far from their upper thermal tolerance limits. The CTMax values ranged from 34.7°C to 40.8°C, similarly to our results. However, these authors used a different MHT reference, 32°C, which led to different warming tolerance results. This reference temperature was for SST, although they observed that shallow intertidal pools could reach temperatures of 36°C, higher than the CTMax of some of the species under study. Madeira *et al*. (2012) concluded that temperate/subtropical species may be more vulnerable to climate warming than tropical species, when comparing results from the same temperate area studied here with the results from Mora & Ospina (2001). The MHT reference used in the work of Madeira *et al*. (2012) for the temperate zone was 35°C, based on atmospheric temperature. In the present study, we used 30°C as MHT, since this is the temperature recorded in rock pools' water during the warmest summer days, when air temperature reaches 35°C.

Our data contribute to the existing body of evidence suggesting that intertidal ecosystems are early warning systems of climate warming impacts (e.g. Southward *et al*., 1995; Stillman & Somero, 2000; Stillman, 2002; Helmuth *et al*., 2006; Tomanek & Zuzow, 2010; Madeira *et al*., 2012; Vinagre *et al*., 2013a). Intertidal habitat conditions reported here are comparable to those found in some similar extreme habitats worldwide (Rummer *et al*., 2009). Due to the especially sharp spatial and temporal gradients in temperature during the tidal cycle, these organisms are exposed to large variations in temperature that subtidal species do not experience (Vernberg & Vernberg 1972; Newell, 1979). Indeed, as early as 1954, Southward & Crisp (1954) proposed the intertidal barnacles *Chthamalus stellatus* and *Balanus balanoides* as indicator organisms of changes in the natural environment, since both species were sensitive to small changes in temperature. Moreover, with current warming trends, intertidal organisms may be exposed to the strongest selection as they already live close to their thermal limits, as showed in the present study.

The latest IPCC models project increases in the duration, intensity and spatial extent of temperature extreme events, i.e. heat waves (IPCC, 2013). The greatest changes in the warmest day of the year are projected for the subtropics and mid-latitudes, but changes in the frequency of warm days and warm nights are largest in the tropics (Sillmann *et al*., 2013). Experiencing thermally stressful conditions for several days will add enormous pressure to tropical organisms in the future, as they already experience MHT above their CTMax during current warmest summer days. This means that organisms will not only be subjected to acute thermal stress, but also to a more chronic or long-term thermal stress. Recent studies on seabass, *Dicentrarchus labrax*, Senegal seabream, *Diplodus bellottii* and Senegal sole, *Solea senegalensis*, have shown that long-term thermal stress (30 days) can occur at temperatures that do not cause any signs of stress in short-term periods (5 to 15 days) (Vinagre *et al*., 2012a, b, c, 2013b, 2014), resulting in increased mortality and decreased growth and condition. This means that long-term stress should be expected at lower temperatures than CTMax.

The intraspecific variability was higher for temperate than for tropical species. The lower phenotypic variation in rocky shore tropical species suggests a low evolutionary potential for species to cope with further warming. Still, all organisms can potentially modify their behavioral, physiological or morphological characteristics in response to habitat temperature (Angilletta, 2009) and accordingly can: (a) disperse to more hospitable habitats, (b) tolerate the new conditions through phenotypic and physiological plasticity, or (c) adapt to the new environment through genetic change via the process of evolution (see review by Hofmann & Todgham, 2010). Our data shows that among tropical intertidal species, *Scartella cristata* has the lowest warming tolerance (Fig. 4), yet, it has the highest variability in the response to temperature among the species of the same habitat, which may compensate its perceived vulnerability.

Additionally, non-genetic parental effects or epigenetic inheritance may result in transgenerational acclimation to increased temperature. Such effects have been documented in marine fish by Donelson *et al*. (2012) who found that the tropical damselfish *Acanthochromis polyacanthus*, although highly sensitive to small increases in water temperature, could rapidly acclimate over multiple generations. Such discovery indicates that tropical marine species are more capable of coping with global warming than previously suggested and illustrates a potential limitation of short-term trials in predicting the long-term impacts of climate change.

Probably, tropical intertidal species will inhabit the same areas and take refuge in colder, subtidal waters during extreme thermal events. However, the interactions between species and its competitors, predators and/or prey, may restrain intertidal species' use of such refuges. This adverse scenario may have serious consequences for intertidal populations and ultimately lead to their local extinction if genetic and/or epigenetic adaptation is not able to keep up with the warming rate.

In summary, tropical rocky shore species are potentially more vulnerable to climate warming than their temperate counterparts, particularly intertidal ones. This conclusion places the tropics, the world's biodiversity hotspots, at the greatest risk for climate change impacts on rocky shore ecosystems.

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Chapter 3

Acclimation capacity of tropical and temperate coastal organisms

Acclimation capacity of tropical and temperate coastal organisms

Abstract

Understanding the impact of global warming on biodiversity is one of the most important challenges faced by mankind. Equally important is the identification of which ecosystems and species are more vulnerable to this threat. Recently, there has been a debate on whether the tropics or temperate zones are more vulnerable to warming. The rate of climate warming is predicted to be lower in the tropics than in temperate zones, however, species that live in aseasonal environments may suffer disproportionally from small increases in temperature. Vulnerability towards higher temperatures will depend mostly on the organisms' acclimation capacity, which remains largely unknown for most species. The present study aimed to compare the acclimation capacity of tropical and temperate rocky shore organisms. The species chosen were the tropical shrimp *Palaemon northropi* and the temperate shrimp *Palaemon elegans*; the tropical crab *Pachygrapsus transversus* and the temperate crab *Pachygrapsus marmoratus*; the tropical Blenniidae fish *Parablennius marmoreus* and the temperate Blenniidae fish *Coryphoblennius galerita*; and the tropical Gobiidae fish *Bathygobius soporator* and the temperate Gobiidae fish *Pomatoschistus microps*. The critical thermal maximum (CTMax) was estimated at a control temperature and 1) after 30 days at "control +3^{o}C", followed by 2) "10 days at control +6^{o}C". The long term trial represented the future summer temperature, while the short term trial represented a future heat-wave. All species tested have some acclimation capacity ($CTMax_{Trial}$ - $CTMax_{Control}$), with the exception of the fish from the Gobiidae family, which did not acclimate. The tropical species tested showed a lower acclimation capacity than their temperate counterparts. Given that tropical rocky shore organisms are already living very close to their thermal limits and that their acclimation capacity is limited, it is likely that the impacts of climate warming will be evident sooner in the tropics than in the temperate zone.

Keywords: Global Climate Change, Upper Thermal Limits, Critical Thermal Maximum, Rocky Shore, Intertidal

Introduction

Global climate warming is unequivocal (IPCC, 2007) and its fingerprint on Earth's ecological systems is already clearly visible (Walther *et al*., 2002). Impacts upon species distribution, phenology and physiology have been predicted and demonstrated by numerous publications (see reviews by Walther *et al*., 2002; Parmesan, 2006). Species responses at the, so far, relatively low average warming rates, are evident, thus the predicted further warming of 3^{o}C, by the end of this century (Kerr, 2004), raises concerns about future climate change impacts upon ecosystems and biodiversity.

The identification of which ecosystems and species are more vulnerable to climate change is a crucial step in the investigation of this issue. Recently, there has been a debate on whether the tropics or temperate zones are more vulnerable to warming (Ghalambor *et al*., 2006; Tewksbury *et al*., 2008). This is important since the tropics and temperate zones encompass most of the species found in the planet.

The tropics are predicted to warm at lower rates than the temperate zones (IPCC, 2007), however tropical species may be particularly vulnerable to such a temperature increase. Organisms inhabiting thermally stable habitats, such as the tropics, may be less tolerant to environmental change, i.e. have a narrower tolerance range (see Hoffmann & Todgham, 2010; Pörtner & Peck, 2010). This way, tropical species may be more vulnerable to further warming compared to their temperate counterparts. In fact, thermal studies regarding different taxa from different latitudes, such as terrestrial insects, amphibians and marine invertebrates, have shown that tropical organisms are living quite close to their thermal limits (e.g. Stillman, 2003; Deutsch *et al*., 2008; Tewksbury *et al*., 2008; Duarte *et al*., 2012). More recently, Leal *et al*. (unpublished) found that tropical intertidal organisms are already experiencing maximum habitat temperatures above their upper thermal limits during the warmest summer days, while their temperate counterparts are not. However, the vulnerability towards a rise in temperature will depend mostly on the organisms' acclimation capacity, which remains unknown for most species.

Acclimation can be described as "any phenotypic response to environmental temperature that alters performance and plausibly enhances fitness" (Angilletta, 2009). It implies the detection of an environmental signal, the transduction of this signal into a cellular response, and the activation of molecules (e.g. genes, ribosomes, enzymes) that cause a change in the phenotype (Wilson &

Franklin, 2002; Angilletta *et al*., 2006). Thus, thermal acclimation comprises regulated responses to diel or seasonal changes in temperature, so as to match physiology to the current environment (Angilletta, 2009).

The ability to acclimate results from existing phenotypic plasticity in populations and it's an important mechanism for coping with environmental temperature changes (Wilson & Franklin, 2002; Lucassen *et al*., 2006). Through acclimation, ectotherms are able to maintain physiological functions and performance across a wide thermal range. This is a common attribute in species that experience pronounced seasonal variations in temperature, such as the ones inhabiting temperate mid-latitudes (Huey & Hertz, 1984; Guderley & St-Pierre, 2002). The organisms at greatest risk from climate warming impacts will be the ones with narrow thermal tolerance ranges, limited acclimation capacity, long generation times and reduced dispersal (see Pörtner & Farrell, 2008).

The increases in frequency, duration, intensity and spatial extent of heat waves (IPCC, 2013) mean that tropical and temperate organisms will be subjected to both long-term and short-term thermal stress. Both have the potential to illicit an acclimation response.

Stillman (2003) explored the interspecific variability in response to warming in porcelain crabs (genus *Petrolisthes*) and found that species with the greatest tolerance to high temperatures displayed the smallest acclimation capacity. Additionally, Rezende *et al*. (2014) demonstrated that the temperature range that an organism can tolerate is expected to narrow down with the duration of the thermal challenge, suggesting that a trade-off exists between tolerance to acute and chronic exposition to thermal stress. These findings suggests that species with the higher thermal limits may be the most vulnerable to small sustained increases in temperature (but see Calosi *et al*., 2007). This way, tropical species appear as the most vulnerable to climate warming (see review by Somero, 2010). However, besides thermal limits *per se*, intraspecific variability must also be considered. Species with enough genetic variability to generate phenotypes with a wide range of thermal tolerances may become "winners" in a warming world, since exceptionally tough individuals can be selected through successive generations resulting in genetic adaptation (Somero, 2010).

One of the habitats where climate change impacts are likely to strike first is the intertidal zone. Rocky shore habitats exist at the margins between the terrestrial and the marine realms, this way they are not only subject to the changes in water temperature, but also the aerial climatic regime, functioning as early warning systems for climate change impacts (Helmuth *et al*., 2006).

Scientists have long used rocky shore ecosystems as natural laboratories for studying thermal stress and are now seeing it as an interesting model system for the investigation of climate warming impacts (Helmuth *et al*., 2006).

The present study aimed to test and compare the capacity of tropical and temperate coastal organisms to acclimate their upper thermal limits when exposed to long-term and short-term increases in temperature. Coastal shrimps, crabs and fish were tested. An effort was made to collect tropical species that had a temperate con-generic counterpart, for a more direct comparison. When this was not possible species from the same family were chosen. In addition, the species chosen are key species in the coastal rocky shore ecosystems they inhabit.

The Critical Thermal Maximum (CTMax) of each species was estimated at a control temperature (26°C for tropical organisms and 20°C for temperate organisms) and after a 1) long-term trial (30 days at "control temperature +3°C"), representing the future summer temperature, and a 2) short-term trial (10 days at "control temperature +6°C"), representing future heat waves.

Differences in CTMax were investigated, for each species, between the control, the long-term and the short-term trials. Differences of acclimation capacity ($\text{CTMax}_{\text{Trial}}$ - $\text{CTMax}_{\text{Control}}$) and in the coefficient of variation of CTMax were investigated between tropical and temperate organisms.

Materials and methods

Study areas and tested species

Coastal shrimps, crabs and fish were collected in a tropical and a temperate rocky shore, in the summer of 2014 in Southeastern Brazil (23°49' S; 45°25' W) and Western Portugal (38°71' N; 9°48' W). The tropical area studied has an annual mean sea surface temperature (SST) of 24°C and a mean summer SST of 26°C, while the temperate study area has an annual mean SST of 17°C and a mean summer SST of 19°C (Locarnini *et al*., 2009).

The species selected for this study were the tropical shrimp *Palaemon northropi* and the temperate shrimp *Palaemon elegans*; the tropical crab *Pachygrapsus transversus* and the temperate crab *Pachygrapsus marmoratus*; the tropical Blenniidae fish *Parablennius marmoreus* and the temperate Blenniidae fish *Coryphoblennius galerita*; and the tropical Gobiidae fish *Bathygobius soporator* and the temperate Gobiidae fish *Pomatoschistus microps*.

Acclimation conditions and experimental setup

After collection, organisms were transported to the laboratory facilities and housed in indoor re-circulating aquaria with constant temperature, aerated sea water and salinity 35‰. Organisms were distributed among 8 aquaria, with only a species *per* aquarium, so as to mitigate agonistic interactions. The number of individuals in each aquarium ranged between 7 and 17. The water dissolved O_2 level varied between 95% and 100%. Organisms were fed *ad libitum* once a day, with commercial shrimp, and starved 24 h before testing their thermal limits.

Organisms were acclimated for seven days at the same temperature as the habitat temperature found in the natural environment at the time of capture, 26°C for tropical organisms and 20°C for temperate ones, to ensure that all had a similar recent thermal history. CTMax was determined for a subset of the organisms to determine control values of CTMax. Afterwards, two acclimation trials were carried out as follows: 1) long-term trial, in which organisms were acclimated for 30 days at 3°C above the control temperature, i.e. 29°C for tropical organisms and 23°C for temperate ones, followed by a 2) short-term trial, in which organisms were acclimated for 10 days at 3°C above the previous trial temperature, i.e. 32°C for tropical organisms and 26°C for temperate ones. After each trial, the CTMax were determined. It is important to mention that different organisms of each species were tested in each CTMax trial, meaning that no organism was exposed to more than one CTMax trial.

The CTMax method is widely used to quantify upper thermal limits among ectothermic vertebrates and invertebrates (e.g. Becker & Genoway, 1979; Cuculescu *et al*., 1998; Mora & Ospina, 2001; Madeira *et al*., 2012). The CTMax is determined by gradually increasing temperature until a critical point is reached (e.g. loss of the righting response, muscle spasms) (Brattstrom, 1968; Huey *et al*., 1992; Lutterschmidt & Hutchison, 1997). Mora & Ospina (2001) defined CTMax as the "arithmetic mean of the collective thermal points at which the end-point is reached", the end-point being loss of equilibrium. In shrimp and fish, loss of equilibrium was detected when individuals could not coordinate straight swimming and start moving in an angled position. Crabs needed to be stimulated with lab tweezers to force them upside down, and if they were unable to get back upright they would have reached the end-point. This criteria is the same followed by Madeira *et al.* (2012) and Vinagre *et al*. (2013).

To determine the CTMax, the organisms were subjected to a thermostatized bath. During the experiment, animals were exposed to a constant rate of water-temperature increase of 1°C h^{-1}, with constant aeration and observed continuously, until they reached the end-point. The experiments were carried out in shaded day light (14 L; 10D). The temperature at which each animal reached its end-point was measured with a digital thermometer, registered and then CTMax, its standard deviation and coefficient of variation were calculated.

To prevent any additional handling stress, the total length of all individuals were measured at the end of the CTMax experiment. Fish were measured with an ichthyometer and shrimps and crabs with a digital slide caliper. The main characteristics of the species studied, and respective sample sizes on each trial, are shown in Table 1. Sample sizes were similar to those used by Mora & Ospina (2001), Madeira *et al.* (2012) and Vinagre *et al*. (2013).

Data analysis

The upper thermal limits for each species were calculated using the equation:

$$\mathrm{CTMax}_{\,(\mathrm{species})} = \sum (T_{\mathrm{end-point}\ n})/n$$

Where $T_{\mathrm{end\text{-}point}}$ is the temperature at which the end-point was reached for any given individual, and n stands for sample size.

The CTMax values after each acclimation trial (control, long-term and short-term) as well as the acclimation capacity (the difference between the CTMax estimated in the long-term and short-term trials and the CTMax control value: $\mathrm{CTMax}_{\mathrm{Trial}}$ - $\mathrm{CTMax}_{\mathrm{Control}}$) of all species were compared. A one-way ANOVA or Kruskal–Wallis test was performed, depending on the normality (Shapiro–Wilk's test) and/or homocedasticity (Levene's test) of the data. For significant differences, *post-hoc* Tukey HSD test (parametric) or Dunn test (non-parametric) were performed. A significant level of 0.05 was considered in all test procedures.

Differences in acclimation capacity were also tested between the tropical-temperate species pairs, paired according to the shortest phylogenetic distance (*P. northropi* vs *P. elegans*; P. *transversus* vs *P. marmoratus*; *P. marmoreus* vs *C. galerita*; *B. soporator* vs *P. microps*) through Student's t-tests or Mann–Whitney tests depending on the normality (Shapiro–Wilk's test) and homocedasticity (Levene's test) of the data.

Table 1 – Main characteristics of shrimp, crab and fish species used in the present study, together with respective sample sizes used for (1) control, (2) long-term, and (3) short-term trials. The length range for each species is also shown, cephalothorax length for shrimps, carapace width for crabs and total length for fih. Sources: Fishbase (www.fishbase.com), Encyclopedia of life (www.eol.org) and World Register of Marine Species (www.marinespecies.org).

Tropical Species	**Common name**	**Family**	**Distribution**	**Environment**	**Sample size**			**Length (mm)**
Palaemon northropi	Cross-banded grass shrimp	Palaemonidae	Western Atlantic	Intertidal	(1) 16	(2) 7	(3) 49	20–42
Pachygrapsus transversus	Mottled shore crab	Grapsidae	Western and Eastern Atlantic; Mediterranean; Eastern Pacific	Intertidal/ Supratidal	(1) 20	(2) 14	(3) 18	8–18
Parablennius marmoreus	Seaweed blenny	Blenniidae	Western Atlantic	Intertidal/ Subtidal; Demersal	(1) 5	(2) 7	(3) 16	38–97
Bathygobius soporator	Frillfin goby	Gobiidae	Western and Eastern Atlantic; Mediterranean Sea	Intertidal; Demersal	(1) 15	(2) 16	(3) 20	29–77
Temperate Species								
Palaemon elegans	Rock pool prawn	Palaemonidae	North and South Atlantic; Mediterranean Sea; Black Sea; Baltic Sea	Intertidal	(1) 9	(2) 20	(3) 17	29–40
Pachygrapsus marmoratus	Marbled rock crab	Grapsidae	Eastern Atlantic; Mediterranean Sea; Black Sea	Intertidal/ Supratidal	(1) 10	(2) 10	(3) 10	12–25
Coryphoblennius galerita	Montagu's blenny	Blenniidae	Eastern Atlantic; Mediterranean Sea; Black Sea	Intertidal; Demersal	(1) 6	(2) 8	(3) 7	30–53
Pomatoschistus microps	Common goby	Gobiidae	Eastern Atlantic; Mediterranean Sea; Baltic Sea	Intertidal; Demersal	(1) 6	(2) 6	(3) 18	25–42

To determine intraspecific variability of the CTMax, the coefficient of variation (in percentage) was calculated for each species at each acclimation temperature:

$$\%CV = (SD/Mean) \times 100$$

Differences in intraspecific variability among latitudinal groups (tropical *vs* temperate) were tested through Student's *t*-tests or Mann–Whitney tests depending on the normality (Shapiro–Wilk's test) and homocedasticity (Levene's test) of the data.

Results

CTMax of tropical species did not change after the long-term trial, but it increased after the short-term trial, for all species except the Gobiidae fish, *Bathygobius soporator* (Fig. 1; Table 2). CTMax of temperate species increased after both the long-term and short-term trials for all species except the Gobiidae fish *Pomatoschistus microps* (Fig. 1; Table 2). No variation in CTMax values was observed in the temperate fish *Coryphoblennius galerita*, so no statistical procedures were carried out to test differences in CTMax values after each acclimation regime.

Table 2 – Kruskal-Wallis test results for CTMax values after different acclimation conditions in tropical and temperate species. Comparisons were made among all acclimation temperatures, i.e. 26°C, 29°C and 32°C for tropical species, and 20°C, 23°C and 26°C for temperate ones. Significant differences are presented in bold ($p < 0.05$). Sample sizes are given in Table 1.

Tropical Species	**Acclimation Capacity**		
	df	H	*p*
Palaemon northropi	2	43.76	**0.000**
Pachygrapsus transversus	2	28.43	**0.000**
Parablennius marmoreus	2	9.31	**0.009**
Bathygobius soporator	2	4.99	>0.05
Temperate Species			
Palaemon elegans	2	32.45	**0.000**
Pachygrapsus marmoratus	2	14.14	**0.001**
Pomatoschistus microps	2	6.26	>0.05

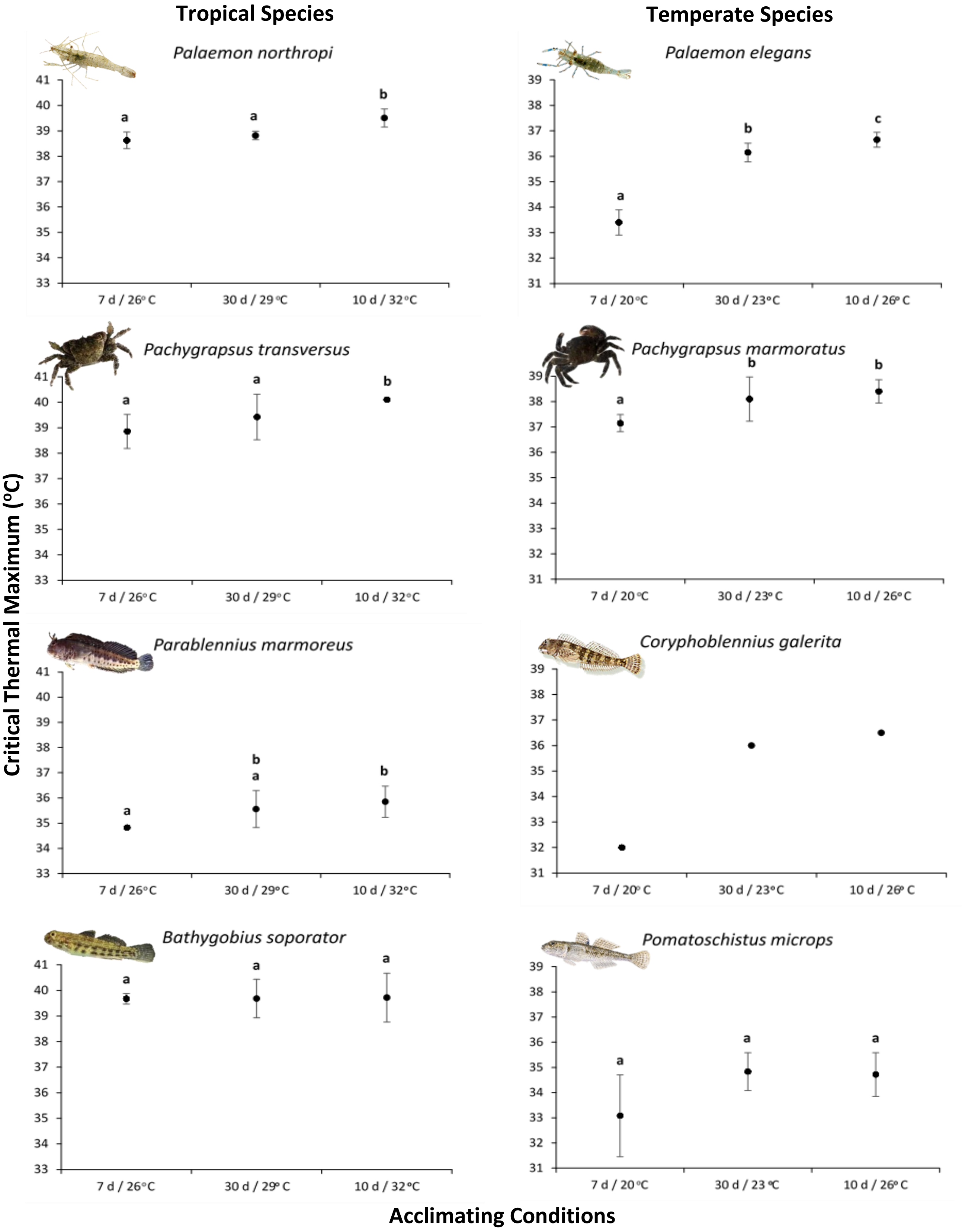

Fig. 1 – Critical thermal maxima for tropical (left) and temperate (right) species, after three different acclimating conditions (see text). Bars stand for standard deviation. Values sharing a common letter are not significantly different ($p > 0.05$).

In what concerns acclimation capacity, in the long-term trial, the temperate shrimp *Palaemon elegans* and the temperate fish *Coryphoblennius galerita* had the highest acclimation capacity considering all species ($p < 0.05$; df = 5; H = 52.22) (Fig. 2a). In the short-term trial, *P. elegans* and *C. galerita* had also a higher acclimation capacity than the majority of the species ($p < 0.05$; df = 5; H = 77.32) (Fig. 2b), except for the tropical crab *Pachygrapsus transversus*.

Acclimation capacity: tropical *versus* temperate species

Among the shrimps, the temperate species *P. elegans* had a significantly higher acclimation capacity in both trials compared to its tropical con-generic *P. northropi*, i.e. *P. elegans* acclimated 2.8°C and 3.2°C in the long-term and short-term trials, respectively, while *P. northropi* only acclimated 0.2°C and 0.9°C, respectively (Table 3). Yet, among the crab species of the genus *Pachygrapsus* no significant differences were found (Table 3). Among the Blenniidae fishes, the tropical species *P. marmoreus* showed significantly lower acclimation values than the temperate blenny, only acclimating 0.8°C and 1.3°C, respectively, while *C. galerita* acclimated 4.0°C and 4.5°C in the long-term and short-term trials, respectively (Table 3). Gobiidae fishes, both tropical and temperate, did not acclimate (Table 2).

Table 3 – Mann-Whitney tests results for differences in acclimation capacity ($CTMax_{Trial}$ - $CTMax_{Control}$) between ecologically equivalent tropical and temperate coastal species. Differences were analyzed for species acclimating to both long-term and short-term trials. Significant differences are presented in bold ($p < 0.05$).

	Acclimation capacity			
	Tropical species' acclimation	Temperate species' acclimation	U	*p*
Long-term Trial				
P. northropi vs P. elegans	0.2	2.8	140.0	**0.00**
P. transversus vs P. marmoratus	0.6	1.0	80.0	0.52
P. marmoreus vs C. galerita	0.7	4.0	49.0	**0.00**
Short-term Trial				
P. northropi vs P. elegans	0.9	3.2	833.0	**0.00**
P. transversus vs P. marmoratus	1.2	1.3	90.00	1.00
P. marmoreus vs C. galerita	1.0	4.5	112.0	**0.00**

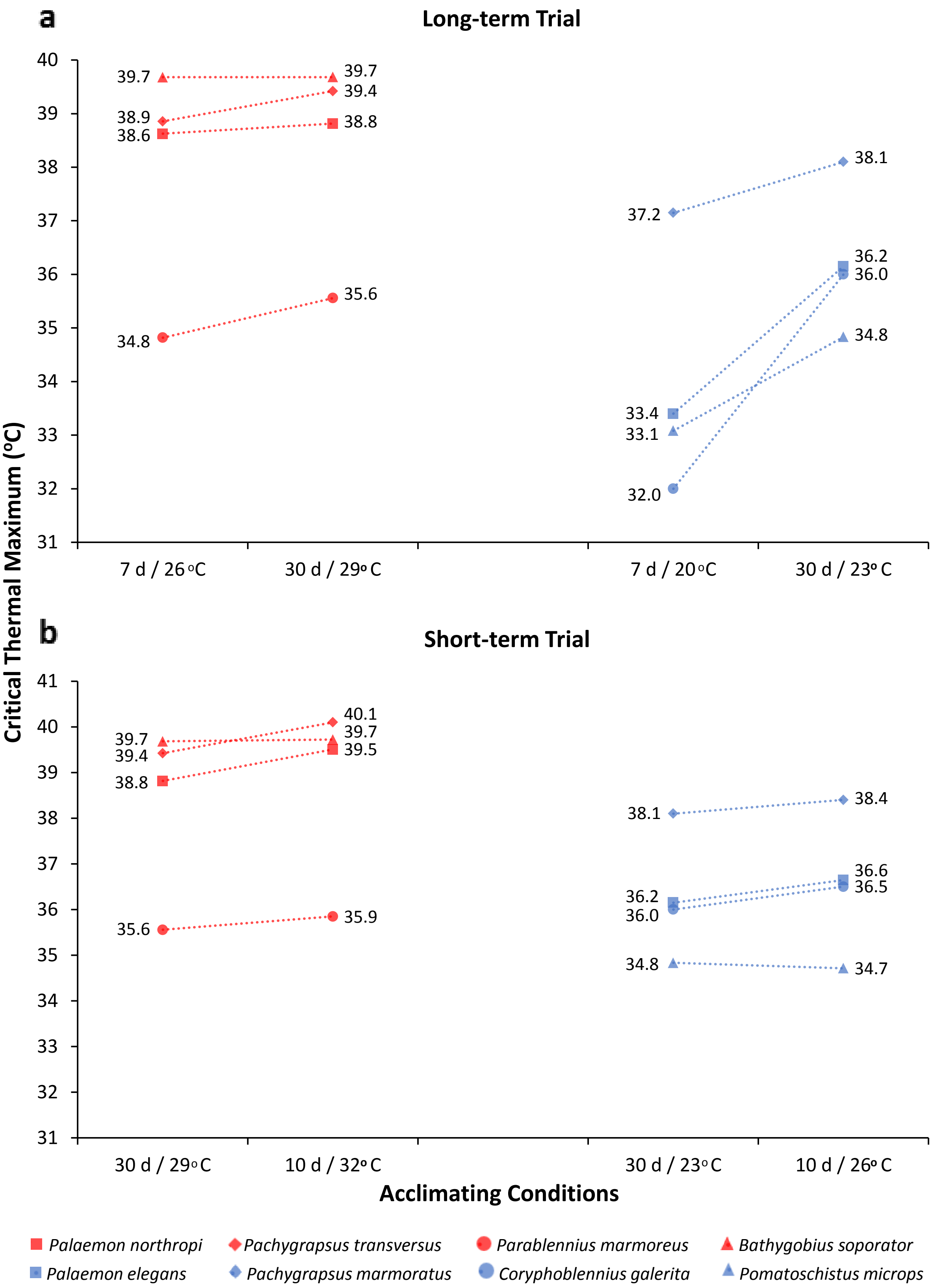

Fig. 2 – Thermal acclimation capacity of the tropical and temperate species after the long-term trial (**a**) and short-term trial (**b**). Tropical species are presented in red, while temperate species are presented in blue.

Intraspecific variability

Tropical species' intraspecific variability ranged from 0.1% to 1.7% in the control trial; from 0.4% to 2.3% in the long-term trial; and from 0% to 1.7% in the short-term trial (Table 4). In temperate species, intraspecific variability ranged from 0% to 4.9% in the control trial; from 0% to 2.3% in the long-term trial; and from 0% to 2.5% in the short-term trial (Table 4). No significant differences among latitudinal groups (tropical *vs* temperate) were found for the control trial, neither for the long-term nor the short-term trials (Table 4).

Table 4 – CTMax intraspecific variability, after three different acclimation regimes (see text), given by the coefficient of variation in percentage (%CV) for tropical and temperate species. Student's *t* test results for differences in the coefficient of variation between tropical and temperate species in the three acclimation regimes are also shown. Significant differences are presented in bold ($p < 0.05$).

	%CV		
Tropical Species	26°C	29°C	32°C
Palaemon northropi	0.9	0.4	0.9
Pachygrapsus transversus	1.7	2.3	0.0
Parablennius marmoreus	0.1	2.1	1.7
Bathygobius soporator	0.5	2.3	0.0
Temperate Species	20°C	23°C	26°C
Palaemon elegans	1.5	1.0	0.8
Pachygrapsus marmoratus	0.9	2.3	1.2
Coryphoblennius galerita	0.0	0.0	0.0
Pomatoschistus microps	4.9	2.2	2.5
Tropical *vs* Temperate	Control Trial	Long-term Trial	Short-term Trial
t-value	0.91	0.44	0.18
p	0.39	0.68	0.86

Discussion

The present study revealed that all species tested have some acclimation capacity, with the exception of the fish from the Gobiidae family, which did not acclimate. The tropical rocky shore species tested showed a lower acclimation capacity than their temperate counterparts. Since acclimation will play a major role in determining whether tolerance limits can keep in pace with the changing environment, tropical species appear to be more vulnerable to further warming.

The present work corroborates Stillman & Somero (2000) and Stillman (2002, 2003) conclusions that species with the greatest tolerance to high temperatures display the smallest acclimation capacity. The authors tested congeneric species of porcelain crabs, genus *Petrolisthes*, from intertidal and subtidal habitats throughout the eastern Pacific. In the eastern Pacific, *Petrolisthes* species live throughout temperate and tropical regions, and Stillman & Somero (2000) and Stillman (2002, 2003) found that the species with the greatest tolerance to high temperatures have done so at the expense of acclimation capacity, and that those would be the most susceptible species to the smallest increases in habitat temperatures. Our work adds support to this assumption.

In the present study, when subjected to 3^{o}C above their current habitat temperature, tropical species were unable to significantly acclimate their thermal limits as opposed to temperate ones. This points to a limited acclimation capacity for tropical species, considering the future rise in temperature of 3^{o}C expected from climate change. Yet, when subjected to 6^{o}C above their current habitat temperature, simulating a future heat wave, the majority of tropical species was able to acclimate significantly their thermal limits, on average by 0.8^{o}C. This acclimation capacity, although limited, may be a means of coping with future climate warming.

The temperate shrimp *Palaemon elegans*, alongside the fish *Coryphoblennius galerita*, were the only species able to acclimate its thermal limits after all acclimation regimes. These species had also the highest acclimation capacity in the long-term trial, and were among the highest acclimations in the short-term trial. Curiously, *P. elegans* has a broad distribution, being widespread along the western Baltic, the North Sea, the Atlantic coast of Europe and the Mediterranean (Campbell, 1994), and is known to make seasonal migrations, moving offshore during winter, and being even observed at depths of 30 meters (Bilgin *et al*., 2008). This wide distribution range probably favors genetic exchange and genetic diversity within its populations. On a different perspective, it may have been its high acclimation capacity that allowed this species to disperse and colonize such thermally different habitats. As for *C. galerita*, its distribution range is not as wide as in *P. elegans*. However, this intertidal fish has a peculiar behavior, being able to remain out of water under rocks or seaweeds during ebb tides (Martin & Bridges, 1999). This means that this species may often experience extreme temperature fluctuations due to the greater influence of the aerial climate.

On the opposite side of the acclimation capacity spectrum are the Gobiidae fishes analyzed in the present work, which did not acclimate to any of the temperatures tested. *B. soporator*, the tropical goby tested, is a resident intertidal species with homing behavior (Gibson, 1999) and with limited dispersal capability (Lima *et al*., 2005). Similarly, *P. microps* is limited to very shallow tidal pools and estuaries, and differences in migratory behavior between populations are known to restrict genetic exchange in this species (Gysels *et al*., 2004). As pointed by Brattstrom (1968), species with restrictive geographic ranges have less ability to adjust physiologically, i.e. acclimate, when compared to species with a broad geographic range. Species with limited dispersal will have less genetic exchange, and therefore a potential for lower genetic variability, which may be the underlying cause of these species inability to acclimate. Bearing in mind that genetically depauperate species seem most destined to be “losers” in a warming world (Somero, 2010), *B. soporator* and *P. microps* may be in jeopardy from future environmental warming.

B. soporator corroborates studies with other species, such as amphibians, lizards and crabs, that indicate that the species with the highest upper thermal limits have the lowest acclimation capacity, in this case none (e.g. Feder, 1978, 1982; Tsuji, 1988; Stillman & Somero 2000; Stillman 2002, 2003; Rezende *et al.*, 2014). Its’ CTMax of nearly 41^{o}C is the highest reported here, however this is the same temperature recorded in tide pools during heat waves, in southeastern Brazil (personal observation), where specimens were captured. Given the more frequent, longer and intense heat waves that are predicted by climate change models (IPCC, 2013), it is reasonable to predict that the maximum tide pool temperature will also increase, meaning that, in the future, if this fish are trapped in a tide pool they may die, as observed for other tropical fish species by Hiatt & Strasburg (1960) in the Marshall Islands.

Despite what was mentioned above on the limited dispersal of *P. microps*, in the present study this species shows one of the highest intraspecific variability in terms of its upper thermal limit, which is indicative of genetic diversity within its population. This means that, despite the apparent lack of acclimation capacity of this species, environmental pressure is likely to select the most thermally resistant individuals leading to an increase in the upper thermal levels throughout the coming generations due to genetic adaptation.

The differential vulnerability of tropical and temperate organisms lies not only on their different capacity to acclimate to higher temperatures, but also on the future warming rate. The Intergovernmental Panel on Climate Change (2007) predicts an asymmetry in the rate of warming around the globe, with higher latitudes warming faster than lower latitudes. This way, the higher acclimation capacity in temperate species may actually be crucial for the maintenance of temperate populations.

On the other hand, tropical coastal species although exposed to lower warming rates may be vulnerable to even small increases in temperature, due to their low acclimation capacity, as reported in the present study. In fact, tropical coastal species are already experiencing habitat temperatures above their thermal limits during heat waves (Leal *et al*., unpublished), and this thermal stress may already be pressuring this organisms towards higher thermal tolerance limits. This way, tropical intertidal communities' responses to recent climate change will most probably be observed sooner than those of temperate ones.

In addition, one should not overlook the fact that acclimation must impose some cost to an organism (Hoffmann, 1995). Acclimation requires energy that an organism might otherwise use for a different function, and may impose costs in terms of survivorship or fecundity (see Angilletta, 2009). These energetic costs are difficult to quantify, but qualitative assessments suggest the costs are substantial (Somero, 2002). Munday *et al*. (2008) reported restricted growth in coral reef fish, *Acanthochromis polyacanthus*, Nilsson *et al.* (2009) observed a reduction in the respiratory scope of several coral reef fishes and Donelson *et al*. (2012) revealed that fish acclimated to a higher temperatures were on average smaller and in poorer condition than fish kept at present day temperatures. Smaller size and poorer condition mean that fewer fish will potentially survive to maturity at elevated temperatures, with fewer and smaller offspring being produced compared with good condition counterparts, raising concerns about communities' future structure and composition (Donelson *et al*., 2012). Similar costs of acclimation to higher temperatures were also observed in temperate fish. Lower growth rates, poorer condition and higher mortality were reported for European sea bass, *Dicentrarchus labrax* (Vinagre *et al.*, 2012a, b), higher mortality and lower growth rates were also reported for the Senegal sole, *Solea senegalensis* (Vinagre *et al*., 2013b) and higher mortality was reported for Senegal sea bream, *Diplodus bellottii* (Vinagre *et al*., 2014).

Ultimately, the short-term temperature extremes that an organism can tolerate will depend on its phenotypic plasticity, but in the long run, evolutionary shifts in thermal limits will depend on the presence of additive genetic variance, with the selection of thermally tolerant genotypes over multiple generations being decisive (Rezende *et al*., 2011; Donelson *et al*., 2012). The crucial issue is whether the rate of evolutionary adaptation will be fast enough to keep up with the rate of environmental warming.

In summary, the present work shows that acclimation as a means of coping with rising environmental temperatures is an attribute of both tropical and temperate intertidal organisms, and shall play a key role in the persistence of such communities in the face of global warming. Temperate coastal organisms seem to have a higher acclimation capacity, when compared to their tropical counterparts, which may be crucial given that it is at the mid-latitudes that warming will be faster. Given that tropical rocky shore organisms are already living very close to their thermal limits and that their acclimation capacity is low, it is likely that the impacts of climate warming will be evident sooner in the tropics than in the temperate zone. More species need to be tested to confirm this findings and to allow more complex future studies that also take into account species' interactions, community structure and ecosystem function.

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Chapter 4

Concluding Remarks

Concluding Remarks

Rocky intertidal habitats are predicted to be one of the habitats hardest hit by temperature rise, making its inhabiting communities excellent experimental material for studying climate warming impacts. These habitats are integral components of nearshore food webs and home to diverse biological communities, which encourages the investigation of how will intertidal organisms fare in the face of such threat.

Many intertidal organisms are currently living close to their thermal limits. This means that intertidal communities may be especially vulnerable to further increases in habitat temperature, as well as more frequent and intense summer extreme temperature events. Such vulnerability may vary across latitude and will depend mainly on an organism's thermal tolerance and acclimation capacity. Such knowledge remains unknown for most species.

The aim of this thesis was to compare the vulnerability to climate warming of rocky shore organisms from different latitudes, tropical and temperate. Firstly, it was determined which species were currently living closest to their thermal tolerance limits. Secondly, the acclimation capacity as a means of coping with rising habitat temperatures was tested.

With respect to thermal tolerance, the upper thermal limits of 12 tropical species from different taxa (shrimps, crabs and fish) were compared to those of 23 temperate species. Tropical species showed higher upper thermal limits than their temperate counterparts. Yet, it was found that tropical intertidal species are the ones living closest to their upper thermal limits, experiencing habitat temperatures above their thermal limits during current summer extreme events. Moreover, the intraspecific variability was found to be higher for temperate than for tropical species, suggesting a lower evolutionary potential for tropical species to cope with further warming. Considering future warming scenarios, these findings place the tropics, the world’s biodiversity hotspot, at greatest risk from environmental warming.

As for acclimation capacity, comparisons were made among 4 tropical-temperate species pairs, paired according to the shortest phylogenetic distance, and belonging to the Palaemonidae, Grapsidae, Blenniidae and Gobiidae families. It was found that both tropical and temperate organisms have the ability to acclimate as a means of coping with rising environmental temperatures. However, tropical species showed a lower acclimation capacity than their temperate counterparts. This means that tropical species may be vulnerable to even small increases in habitat temperature. As noted above, tropical intertidal species are already experiencing habitat temperatures above their thermal limits during heat waves, and this thermal stress may already be pressuring organisms towards higher thermal tolerance limits. This suggests that tropical intertidal communities' responses to climate change will be observed sooner than those of temperate ones. It is likely that tropical intertidal species will inhabit the same areas and take refuge in colder, subtidal waters during extreme thermal events. However, if such refuges are unavailable and/or if genetic adaptation is not able to keep up with the warming rate, intertidal populations may be prone to local extinction.

The conclusions here presented contribute to the existing body of knowledge suggesting that intertidal ecosystems are sentinels of climate warming impacts, and corroborate previous works reporting that species with the greatest tolerance to high temperatures display the smallest acclimation capacity. The understanding of species' thermal limits, and the ability to adjust those limits, enables us to argue about what will happen to the distribution and abundance of organisms during global climate change. Yet, in order to assess climate warming impacts upon species' interactions, community structure and ecosystem dynamics, many more species need to be tested, and an effort to deepen transgenerational studies should be made, since genetic variability may be decisive in the persistence of populations in a warming world. In fact, evolutionary adaptation may be the only way that vulnerable species can persist if they are unable to disperse to climatically favorable habitats. Above all, such information may someday be incorporated into management programs designed to minimize biodiversity loss under rapid climate change.

In conclusion, the results here presented strongly suggest that tropical intertidal species are the ones in greatest jeopardy considering climate warming trends. As such, this thesis greatly contributes to the ongoing scientific debate on which organisms face a higher risk from climate warming: tropical or temperate.