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2015

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Effect of warming rate on the critical thermal maxima of crabs, shrimp and fish

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ABSTRACT

The threat of global warming has prompted numerous recent studies on the thermal tolerance of marine species. A widely used method to determine the upper thermal limit has been the Critical Thermal Maximum (CTMax), a dynamic method, meaning that temperature is increased gradually until a critical point is reached. This method presents several advantages over static methods, however, there is one main issue that hinders interpretation and comparison of CTMax results: the rate at which the temperature is increased. This rate varies widely among published protocols. The aim of the present work was to determine the effect of warming rate on CTMax values, using different animal groups. The influence of the thermal niche occupied by each species (intertidal *vs* subtidal) and habitat (intertidal *vs* subtidal) was also investigated. CTMax were estimated at three different rates: 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, in two species of crab, *Eurypanopeus abbreviatus* and *Menippe nodifrons*, shrimp *Palaemon northropi* and *Hippolyte obliquimanus* and fish *Bathygobius soporator* and *Parablennius marmoreus*. While there were significant differences in the effect of warming rates for some species, for other species warming rate produced no significant differences (*H. obliquimanus* and *B. soporator*). While in some species slower warming rates lead to lower CTMax values (*P. northropi* and *P. marmoreus*) in other species the opposite occurred (*E. abbreviatus* and *M. nodifrons*). Biological group has a significant effect with crabs' CTMax increasing at slower warming rates, which did not happen for shrimp and fish. Subtidal species presented lower CTMax, at all warming rates tested. This study highlights the importance of estimating CTMax values at realistic rates that species encounter in their environment and thus have an ecological value.

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1. Introduction

The threat of global warming and its consequences has fueled the recent proliferation of scientific work investigating the vulnerability of species to increased temperatures and consequent distribution shifts (e.g. Walther et al., 2002; Williams et al., 2008; Chown et al., 2010; Somero, 2010; Vinagre et al., 2011; Madeira et al., 2012a). Such vulnerability will depend mostly on each organism's thermal tolerance and upper thermal limits, which remain unknown for most species. This means that experimental tests on species' thermal tolerance are welcome in scientific literature. They are a first step in the understanding of the present and future effects of climate warming.

The Critical Thermal Maximum (CTMax) is one of the most common physiological indices used to quantify the upper thermal tolerance in fish (e.g. Becker and Genoway, 1979; Bennett and Judd, 1992; Fangue et al., 2001; Mora and Ospina, 2001; Rummer et al., 2009; Madeira et al., 2012a; Vinagre et al., 2013). It has also been widely used for other aquatic and non-aquatic organisms, such as shrimp, crabs, amphibians, molluscs and insects (e.g. McMahon 1990, 2001; Terblanche et al., 2005; Deere and Chown, 2006; Hopkin et al., 2006; Duarte et al., 2012; Madeira et al., 2012a; Vinagre et al., 2013). It has also been applied in macrophysiological comparative studies in ectotherms (e.g. Lee and Boulding, 2010; Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997) and in the exploration of upper thermal tolerances across different taxa (Somero, 2005, 2010; Deutsch et al., 2008).

It is a dynamic method, which means that temperature is increased gradually until a critical point is reached, most often loss

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of equilibrium or muscle spasms (e.g. Mora and Ospina, 2001; Duarte et al., 2012; Vinagre et al., 2013; Madeira et al., 2014a, 2014b). The CTMax is quantified as the mean temperature at which individuals reach the critical point. Dynamic methods have many advantages over static methods. They require fewer animals and experiments are faster (Lutterschmidt and Hutchison, 1997), because static metrics, such as the temperature that causes 50% of mortality, or lethal temperature, is determined from a plot of percent mortality at given temperature intervals. Another important advantage of the CTMax method, in particular, is that it is sublethal, rather than lethal, and thus provides a reference for temperature tolerance that takes into account a more conservative thermal limit in which the organism does not die but is unable to escape predators and forage because of equilibrium loss. This means that CTMax results are more comparable to natural conditions, particularly those occurring in tidal pools and temporary ponds (Hiatt and Strasburg, 1960; Bennett and Judd, 1992; Mora and Ospina, 2001; Duarte et al., 2012; Vinagre et al., 2013). Climate change models predict that heat waves will increase in intensity, frequency and duration, this way tidal pools and temporary ponds will present a harder thermal challenge to their inhabitants.

However, there is one main issue in the dynamic methods that hinders comparative studies: the rate at which the temperature is changed. This rate has varied widely among published protocols, from $10\ ^{\circ}C\ min^{-1}$ to $1\ ^{\circ}C\ 48\ h^{-1}$ (reviews in Lutterschmidt and Hutchison, 1997; Mora and Maya, 2006). Fast warming rates can result in a long lag between the experimental temperature and the internal temperature of the individual, overestimating the upper thermal limit (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997). Slow warming rates may allow the individual to acclimate, also overestimating the upper thermal limit, or allow temperature to exert its lethal effects and, in that case, underestimating the thermal limit (Cocking, 1959; Beitinger et al., 2000).

Very few studies have attempted to test the effect of warming rate on CTMax values. Mora and Maya (2006) tested the effect of five warming rates on the CTMax of a tropical blennid fish, *Acantemblemaria hancocki*, concluding that it decreases significantly from $1\ ^{\circ}C\ h^{-1}$ towards faster and slower heating rates. Older studies, also with fish, found an increasing thermal tolerance at faster than $1\ ^{\circ}C\ h^{-1}$ warming rates (Cocking, 1959; Cox, 1974; Becker and Genoway, 1979). Mora and Maya (2006) attributed these contrasting results to the different species used or to the better quality of equipment used in more recent studies. However, more recent studies with other biological groups have also shown different patterns of thermal limits as an effect of warming rate, in comparison with Mora and Maya (2006). Terblanche et al. (2007) and Faulkner et al. (2014) found that slower warming rates resulted in lower thermal tolerance, in the tsetse fly, *Glossina pallidipes*, and in marine crustaceans, respectively, confirming Cocking (1959), Cox (1974), Becker and Genoway (1979) studies with fish.

Recent physiological studies at the sub-cellular level, using coastal organisms subjected to the CTMax experiment, indicate that the thermal niches of each species are crucial in the thermal response (Madeira et al., 2012b, 2013, 2014a, 2014b). Different patterns of oxidative stress response and heat shock proteins' expression have been detected in crabs, shrimp and fish that occupy different thermal niches in the intertidal–subtidal gradient (Madeira et al., 2012b, 2012c, 2013). Species that occupy colder and more stable thermal niches present peaks of cellular stress biomarkers at lower temperatures than species that occupy warmer and more variable thermal niches. Also, species that are constantly exposed to highly variable environments in terms of temperature, such as in intertidal ecosystems, appear to be always prepared to cope with thermal shock, having high constitutive levels of heat shock proteins and anti-oxidant enzymes (Madeira et al., 2012b, 2013, 2014a, 2014b).

It is reasonable to expect that such physiological mechanisms that result in different sub-cellular response patterns throughout the warming period that precedes the CTMax may also influence the response of each species when CTMax is estimated at different warming rates.

Studies that simultaneously test the effect of warming rate on CTMax over various species and animal groups are still lacking. The present study aims to fill this gap. The aim of the present work was to determine the effect of warming rate on CTMax values estimated at three different rates: $1\ ^{\circ}C\ min^{-1}$, $1\ ^{\circ}C\ 30\ min^{-1}$ and $1\ ^{\circ}C\ h^{-1}$. Two species of crab, shrimp and fish were chosen, in order to assess this effect over different biological groups. Common coastal species were chosen: the crabs *Eurypanopeus abbreviatus* and *Menippe nodifrons*, the shrimp *Palaemon northropi* and *Hippolyte obliquimanus* and the fish *Bathygobius soporator* and *Parablennius marmoreus*. The influence of the thermal niche occupied by each species (intertidal *vs* subtidal) and habitat (intertidal *vs* subtidal) was also investigated, as well as intraspecific variability. This study should bring new insights into the issue of if there is a more appropriate warming rate for the determination of CTMax and if that depends on the biological group or habitat under investigation.

2. Materials and methods

2.1. Specimens collection and acclimation conditions

Specimens of two species of crab, *E. abbreviatus* and *M. nodifrons*, shrimp, *P. northropi* and *H. obliquimanus*, and fish *B. soporator* and *P. marmoreus* were collected in the coast of São Sebastião, São Paulo, Brazil (23°49′S; 45°25′W), in a rocky coastal area, in January of 2014. All species selected have a wide distribution from the northern to the southern hemispheres, mostly in tropical and subtropical waters (Table 1).

Individuals were collected using hand nets. Water temperature at the time of capture was 29 °C. Field surface temperature was ~29 °C for the previous month. As thermal history, acclimation and starting temperatures can have an effect on CTMax (e.g. Clarke et al., 2000; Terblanche et al., 2005, 2007), we opted to use the field temperature, as the starting and acclimation temperature in the experiments, ensuring this way that specimens' thermal

Table 1
Species, common name, latitudinal range, distribution area, environment, sample size and size range of the individuals (mm).[a]

Species	Common name	Latitudinal range	Distribution	Environment	sample size	Length (mm)
Eurypanopeus abbreviatus	Lobate mud crab	35°N–33°S	West Atlantic	Shallow waters/tide pools	22	13–24
Menippe nodifrons	Cuban stone crab	23°N–23°S	East and West Atlantic	Shallow waters/tide pools	18	18–32
Palaemon northropi	Cross-banded grass shrimp	32°N–32°S	West Atlantic	Shallow waters/tide pools	27	11–41
Hippolyte obliquimanus	Atlantic shrimp	35°N–33°S	West Atlantic	Subtidal coastal waters	17	8–12
Bathygobius soporator	Frillfin goby	24°N–33°S	East and West Atlantic	Shallow waters/tide pools	15	19–44
Parablennius marmoreus	Seaweed blenny	40°N–33°S	West Atlantic	Subtidal coastal waters	31	20–97

[a] This table was constructed based on fishbase (www.fishbase.com) and Encyclopedia of life (www.eol.org).

history was not disturbed after collection. Temperature was measured with a multi-parameter YSI 600 XLM probe.

After capture, organisms were transported to the laboratory facilities and housed in a closed re-circulating system with 6 aquaria, one species per aquaria, with a total capacity of 72 l, aerated sea water, a constant temperature of 29 °C and salinity 35‰. Temperature, salinity and dissolved oxygen were measured with a multi-parameter YSI 600 XLM probe, several times a day. The water dissolved O_2 level varied between 95% and 100%. The organisms were acclimated for 7 days, thus ensuring that all had a similar recent thermal history. They were fed *ad libitum* with commercial food pellets, twice a day, and starved 24 h before the experiments.

2.2. Experimental setup

The thermal tolerance of these species was determined by the dynamic method described in Mora and Ospina (2001). The aim was to determine the Critical Thermal Maximum (CTMax), which is defined as the "arithmetic mean of the collective thermal points at which the end-point is reached" (Mora and Ospina, 2001). The end-point was equilibrium loss. To determine the CTMax animals were exposed to a constant rate of water-temperature increase, in a thermostatized bath, MultiTemp III Pharmacia Biotech, with constant aeration, and observed continuously, until they reached the end-point. CTMax were estimated at three different rates: 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, for all species.

In shrimp and fish, loss of equilibrium was observable when they could not coordinate to swim straight and started swimming in an angled position. Crabs needed to be stimulated with a lab tweezer 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 Vinagre et al. (2013) and Madeira et al. (2012a, 2012b, 2012c, 2014b).

The temperature at which each animal reached its end-point was measured with a YSI 600 XLM probe and recorded. The CTMax average and its standard deviation were calculated for each species ($15 \leq n \leq 31$). All experiments were carried out in shaded day light (14L; 10D). To prevent any additional handling stress, the total length of all individuals was measured (to the nearest mm) at the end of each trial. Fish were measured with an ichthyometer, shrimp and crabs with a digital slide calliper.

The main characteristics and the thermal niche occupied by the species studied were summarized in Table 1. Sample sizes were similar to those used by Mora and Ospina (2001) and Madeira et al. (2012a, 2012b, 2012c).

This work was authorized by ethical committees in Portugal (reference 021941) and Brazil (reference 13.1.981.53.7), and is thus in accordance with the ethical guidelines of both countries.

2.3. Data analyses

The CTMax for each species, at each warming rate, was calculated using the following equation:

$$CTMax = \frac{\sum_{i=1}^{n}(T_{end-point})}{n}$$

where $T_{end-point}$ is the temperature at which a given individual reached its end-point and n is the sample size. To determine intraspecific variability of the CTMax, the coefficient of variation (in percentage) was calculated for each species.

A one-way ANOVA was performed for each species, to test the effect of warming rate on the CTMax of each species. For main effects ($p < 0.05$), the Tukey *post-hoc* test was performed.

Two additional factorial ANOVAs were performed for all species simultaneously, to test (a) the effect of warming rate and biological group (crabs *vs* shrimp *vs* fish) on the CTMax and (b) the effect of the habitat (intertidal *vs* subtidal) on the CTMax. For significant main effects ($p < 0.05$), the Tukey *post-hoc* test was performed. The effect of warming rate was not simultaneously tested for biological group (crabs *vs* shrimp *vs* fish) and habitat (intertidal *vs* subtidal) because the design was incomplete, no subtidal species of crabs was tested. This way, we opted to perform two separate factorial ANOVAs.

3. Results

The CTMax values for *E. abbreviatus* were 38.2 °C ($n=6$; sd$=0.8$), 39.5 °C ($n=8$; sd$=0.4$) and 39.7 °C ($n=8$; sd$=0.4$), estimated at warming rates of 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, respectively. For *M. nodifrons* these values were 39.0 °C ($n=6$; sd$=0.6$), 39.8 °C ($n=6$; sd$=0.4$), 39.4 °C ($n=6$; sd$=0.5$), estimated at warming rates of 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, respectively. For *P. northropi* these values were 39.7 °C ($n=10$; sd$=0.5$), 39.7 °C ($n=10$; sd$=0.1$), 38.8 °C ($n=7$; sd$=0.2$), estimated at warming rates of 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, respectively. For *H. obliquimanus* these values were 34.7 °C ($n=6$; sd$=0.8$), 34.8 °C ($n=6$; sd$=0.3$), 34.8 °C ($n=5$; 0.0), estimated at warming rates of 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, respectively. For *B. soporator* these values were 39.3 °C ($n=6$; sd$=0.8$), 39.8 °C ($n=4$; sd$=0.1$), 39.2 °C ($n=5$; sd$=0.6$), estimated at warming rates of 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, respectively. For *P. marmoreus* these values were 36.6 °C ($n=11$; sd$=0.7$), 35.8 °C ($n=12$; sd$=0.5$), 35.6 °C ($n=7$; sd$=0.7$), estimated at warming rates of 1 °C min^{-1}, 1 °C 30 min^{-1} and 1 °C h^{-1}, respectively. Standard deviations around the CTMax values were relatively low (Fig. 1). The coefficient of variation ranged from 0% to 2.4% (Table 2).

There were significant differences in the effect of warming rates in *E. abbreviatus*, *M. nodifrons*, *P. northropi* and *P. marmoreus*, but not for *H. obliquimanus* and *B. soporator* (Table 3). While in *E. abbreviatus* and *M. nodifrons* slower warming rates lead to lower CTMax values, in *P. northropi* and *P. marmoreus* the opposite occurred (Fig. 1).

The factorial ANOVA that investigated the simultaneous effect of biological group (crabs *vs* shrimp *vs* fish) and warming rate revealed differences among the biological groups but not among warming rates, nor any combined effect of biological group and warming rate (Table 4), with Tukey tests showing differences between fish and crabs, and crabs and shrimp, but not between fish and shrimp. CTMax was higher for crabs than for shrimp and fish.

The factorial ANOVA that investigated the simultaneous effect of habitat (intertidal *vs* subtidal) and warming rate revealed differences among the habitats but not among warming rates. However, a combined effect of habitat and warming rate was detected (Table 5), with Tukey tests showing differences between intertidal and subtidal for all warming rates tested. CTMax was lower for subtidal species at all warming rates tested.

4. Discussion

Significant differences in the effect of warming rates were found for some species, while for other species warming rate produced no significant effect. While in some species slower warming rates lead to lower CTMax values, in other species the opposite occurred. There were differences on the CTMax values according to the biological group, with crabs having higher values than shrimp and fish. Differences were also detected according to
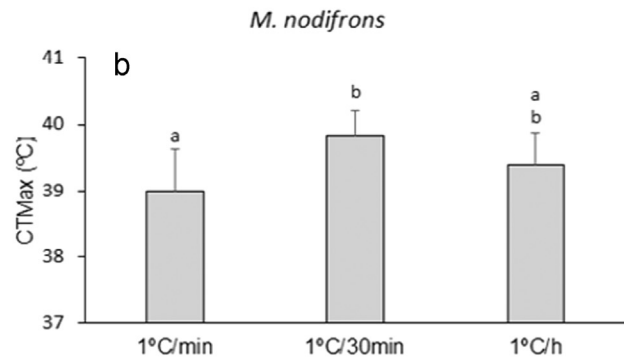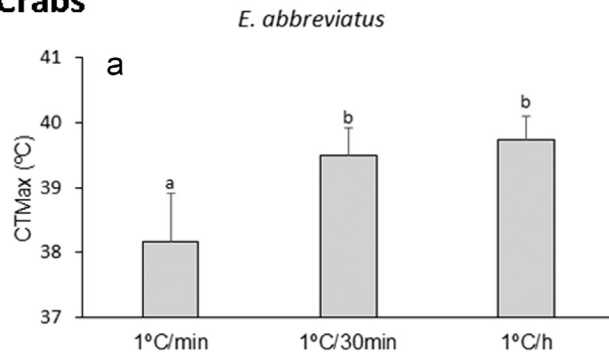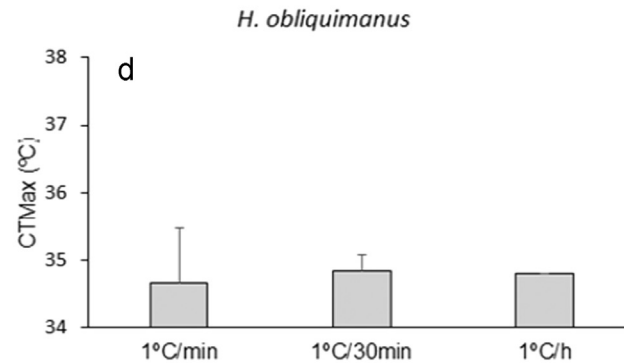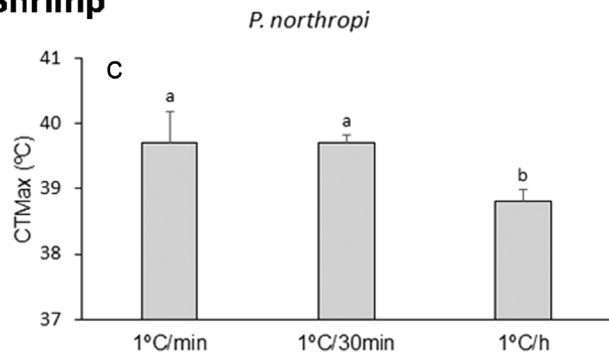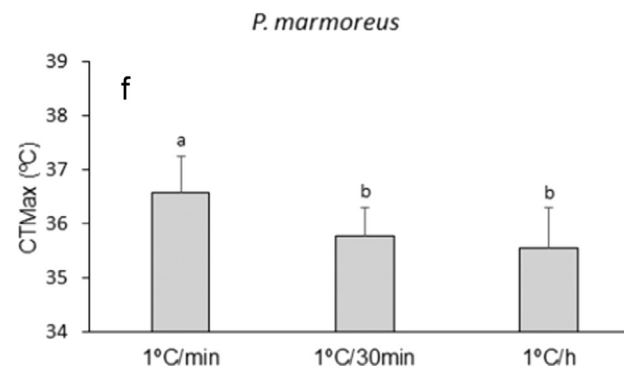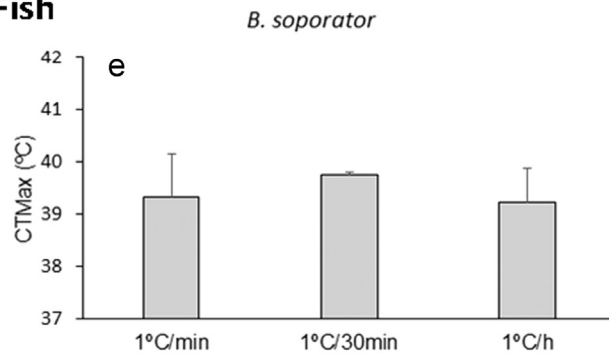
Fig. 1. Critical Thermal Maxima of *Eurypanopeus abbreviatus* (a), *Menippe nodifrons* (b), *Palaemon northropi* (c), *Hippolyte obliquimanus* (d), *Bathygobius soporator* (e) and *Parablennius marmoreus* (f), estimated at $1\ ^\circ C\ min^{-1}$, $1\ ^\circ C\ 30\ min^{-1}$ and $1\ ^\circ C\ h^{-1}$. Different letters indicate significantly different values in the *post hoc* tests. Bars represent the standard deviation.

Table 2
CTMax intraspecific variability given by the coefficient of variation (CV%).

	$1\ ^\circ C\ min^{-1}$	$1\ ^\circ C\ 30\ min^{-1}$	$1\ ^\circ C\ h^{-1}$
Eurypanopeus abbreviatus	2.0	1.1	0.9
Menippe nodifrons	1.6	1.0	1.2
Palaemon northropi	1.2	0.3	0.4
Hippolyte obliquimanus	2.4	0.7	0.0
Bathygobius soporator	2.1	0.1	1.6
Parablennius marmoreus	1.8	1.5	2.1

Table 3
One-way ANOVA results for the effect of warming rate on CTMax values. Significant results are presented in bold.

	df	F	p-Value
Eurypanopeus abbreviatus	2	18.1	**0.00**
Menippe nodifrons	2	4.1	**0.04**
Palaemon northropi	2	20.6	**0.00**
Hippolyte obliquimanus	2	0.2	0.84
Bathygobius soporator	2	0.8	0.47
Parablennius marmoreus	2	7.4	**0.00**

Table 4
Factorial ANOVA results for the effect of biological group (crabs, shrimp and fish) and warming rate on CTMax values. Significant results are presented in bold.

	df	F	p-Value
Biological group	2	16.71	**0.00**
Warming rate	2	0.10	0.90
Biological group × warming rate	4	1.39	0.24
Error	121		

the habitat, with subtidal species having lower CTMax, at all warming rates tested.

Only the shrimp *P. northropi* and the fish *P. marmoreus* exhibited lower CTMax values with slower warming, like previously reported for insects (Terblanche et al., 2007), marine crustaceans (Faulkner et al., 2014), and marine fish (Cocking, 1959; Cox, 1974; Becker and Genoway, 1979). Crabs showed the opposite effect, and the shrimp *H. obliquimanus* and the fish *B. soporator* showed no effect of warming rates on CTMax values. We conclude that there may be patterns in the response to climate warming, according to

Table 5
Factorial ANOVA results for the effect of habitat (intertidal and subtidal) and warming rate on CTMax values. Significant results are presented in bold.

	df	F	p-Value
Habitat	1	841.5	**0.00**
Warming rate	2	1.9	0.16
Habitat × warming rate	2	6.3	**0.00**
Error	124		

biological group, habitat or other factors not tested in this work, however, more species need to be tested for a definite conclusion.

Since the beginning of thermal experimentation using dynamic methods, authors have called for a standardization of warming rates in the determination of thermal limits. This would be of great value for inter-specific comparisons. Becker and Genoway (1979) recommended $0.3\,°C\,min^{-1}$, Lutterschmidt and Hutchison (1997) recommended $1\,°C\,min^{-1}$ and, more recently, Mora and Maya (2006) suggested $1\,°C\,h^{-1}$.

These authors argue that the more appropriate warming rate is the one that is fast enough to avoid acclimation, and the lethal effects of high temperatures, but slow enough to prevent a lag between water temperature and internal body temperature of the animal. However, all of these important phenomena are potentially species-specific. The results from the present work seems to confirm this. Previous studies have shown that acclimation time varies among species (e.g. Lutterschmidt and Hutchison, 1997; Chung, 2001), while the lag between water temperature and the internal temperature of the individual is dependent on the area/volume ratio, which, in term, depends on the species and may also depend on size and on the ontogenic stage of individuals (Stevens and Fry, 1974). The dependence of the most suitable warming rate for CTMax experiments on these phenomena, which are dependent on the species and even on developmental stage, had previously been noted by Mora and Maya (2006), who concluded that establishing standard warming rates for interspecific comparisons may be premature. The results from the present study agree with this conclusion, since organisms with different body shapes and surface/volume ratios, such as crabs and shrimp/fish were tested, and crab's response was different from that of shrimp/fish.

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 authors suggest that the use of thermal tolerance landscapes, which include both the time of exposure and temperature, will be more adequate in providing an index of thermal tolerance that is ecologically relevant.

The present study shows that thermal niche has an effect on the CTMax in the species tested in the present study, with subtidal species having lower CTMax values at all warming rates. This had been previously shown in coastal crabs, shrimp and fish. The subtidal is a much more thermally stable environment, than the intertidal. Species inhabiting the intertidal have evolved specific adaptations that allow them to cope with environmental stress due to periodical exposure to terrestrial conditions (Stillman, 2002). A higher CTMax is believed to be a necessary evolutionary adaptation of intertidal organisms in contrast to the lower CTMax exhibited by subtidal organisms that do not suffer this level of environmental pressure (Mora and Ospina 2001; Madeira et al., 2012a, 2012b, 2014a, 2014b).

However, in what concerns the effect of warming rate, *H. obliquimanus* and *P. marmoreus*, the two subtidal organisms tested, showed different response patterns. While the CTMax of *H. obliquimanus* was not affected by the warming rate, the CTMax of *P.*

marmoreus was. This means that other factors are probably at play, possibly the evolutionary history of these species. Different responses from species from the same thermal niche have also been detected at the sub-cellular level, even in congeneric species. Vinagre et al. (2014) found that *Palaemon elegans* and *Palaemon serratus*, two shrimp species that occur in intertidal pools, present different oxidative stress response patterns when subjected to the CTMax experiment. Since both species occupy the same thermal niche, and were acclimated to the same temperature, the authors concluded that although congeneric, these species may have different evolutionary histories influencing their sub-cellular response to thermal stress. Judge et al. (2011) had previously reported subcellular responses to thermal stress in tropical gastropods, which were not fully concordant with the micro-habitat and temperature that they endured, concluding that detailed information on the specimens' physiological state and prior conditions was needed.

To the best of our knowledge, the CTMax values presented here are new to literature, since they had never been estimated for any of these species. The only exception being *B. soporator*, previously tested in the Florida Keyes (Rummer et al., 2009). The CTMax value estimated for this species was $40.9\,°C$, at a warming rate of $0.39\,°C\,min^{-1}$, slightly higher than the CTMax values estimated in the present study, $39.3\,°C$, $39.8\,°C$ and $39.2\,°C$, estimated at warming rates of $1\,°C\,min^{-1}$, $1\,°C\,30\,min^{-1}$ and $1\,°C\,h^{-1}$, respectively.

Information on the upper thermal limits of tropical animals is particularly welcome to scientific literature because it contributes to the ongoing discussion on the vulnerability of tropical animals to climate warming (Deutsch et al., 2008; Tewksbury et al., 2008). Species that evolved in non-seasonal environments, like the tropics, are less likely to have broad thermal intervals and to acclimate to different temperatures. Thus, tropical species may be more vulnerable to alterations in temperature because their thermal limits may be closer to their optimal temperature (Stillman, 2003; Ghalambor et al., 2006; Deutsch et al., 2008). For this theory to be tested, the thermal limits of a much wider range of animals need to be estimated experimentally. So far, the species tested in the present study have CTMax values above the maximum water temperature for this area, which is $30.1\,°C$ (data from the CEBIMar-USP meteorological station), however tidal pools can reach $41\,°C$, during heat waves.

Intraspecific variability was low for all species, at all warming rates tested, which is in accordance with Mora and Ospina (2001), Madeira et al. (2012a) and Vinagre et al. (2013). It is generally accepted that thermal tolerance varies within a genetically fixed range that is subjected to phenotypic alteration (Cuculescu et al., 1998). Thermal history at the individual level and parental effects are generally considered the most important factors determining phenotypic plasticity (Cossins and Bowler, 1987; Shaefer and Ryan, 2006). The methodology followed in the present study, which encompasses seven days at the same acclimation temperature for all the individuals studied, aims to prevent the interference of thermal history and to establish a similar thermal baseline for all individuals. This probably explains the low variability in the thermal response found in the present study. However, site fidelity and low dispersal from each group of individuals, possibly fidelity to the tide pool or rocky beach, may also be at play.

The present study highlights the importance of testing each species response to warming rates, when using dynamic methods for the assessment of thermal tolerance. It is possible that when a large number of species, representative of different thermal niches, biological groups, and evolutionary histories, are tested, some patterns concerning the most appropriate warming rate for CTMax studies in some groups may be found, allowing a standardization of protocols. Future research must also take into

account that the most appropriate warming rate may be specific to the habitat under study (e.g. tidal pools, temporary ponds). Knowledge on natural warming rates during extreme events, such as heat-waves, may be crucial to define the most realistic conditions that organisms will face, thus leading to the estimation of habitat-specific CTMax values concerning a real thermal challenge that may produce evolutionary adaptation or local extinction.

Acknowledgements

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