Journal of Thermal Biology 47 (2015) 19–25

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

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

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
30 October 2014
Accepted 31 October 2014
Available online 6 November 2014

Keywords:
Global change
Thermal tolerance
CTMax
Comparative biology

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.

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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http://dx.doi.org/10.1016/j.jtherbio.2014.10.012
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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 °C min^{-1} to 1 °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 °C h^{-1} towards faster and slower heating rates. Older studies, also with fish, found an increasing thermal tolerance at faster than 1 °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 °C min^{-1}, 1 °C 30 min^{-1} and 1 °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).