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Plasticity and acquisition of the thermal tolerance (upper thermal limit and heat shock response) in the intertidal species *Palaemon elegans*

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ABSTRACT

The marine species sensitivity to climate change will depend on the ways by which these species can adapt to thermal increase and heterogeneity. Here, we present evidence that the intertidal shrimp *Palaemon elegans* acclimates its thermal tolerance, in response to environmental water temperature, through a significant shift of its upper thermal limit with no concomittant acclimation of the heat shock response (*hsp70* stress gene expression threshold). This species is less thermotolerant than its congener *Palaemonetes varians,* and would therefore potentially be more sensitive to an increase in environmental temperature, such as imposed by global warming. In *P. elegans* life cycle, physiological adjustments like the shift of the thermal limit and the acquisition of a significant HSR, occurred during the metamorphosis from larvae to post-larvae. This suggests that this step is a genetically-programmed milestone in the process of thermal tolerance acquisition.

1. Introduction

Predicting the consequences of temperature changes on the species physiology, distribution and survival has become particularly topical in recent years in the context of climate change (e.g. Somero, 2010 and Tomanek, 2010 for marine species). The species relative sensitivity to warming would depend on their thermal tolerance and also on their thermal acclimation potential, defined as the plasticity of their behavioural, physiological or morphological characteristics in response to environmental temperature (Angilletta, 2009). The capacity of marine species to acclimate their upper thermal limit was proposed to be related to their thermal habitat, and more precisely to their maximum habitat temperature (review in Somero et al., 2010; Vinagre et al., 2016). Species encountering the highest temperatures, like the tropical species and the species occuring in the highest part of the intertidal zone, would have high upper thermal limits, but a limited ability to increase their thermal tolerance through acclimation. Recent surveys of marine animals also proposed that the ability of marine species to acclimate their response to thermal stress (the heat-shock response, HSR) depends on the thermal heterogeneity of their habitat (review in Tomanek, 2010). The HSR comprises the cellular induction of the stress protein Hsp70, a chaperone involved in sensing, repairing, and minimizing macromolecular damage (review in Morris et al., 2013). This protein is part of the stress proteome of eukaryotes, a set of evolutionarily conserved proteins that participate to key aspects of the cellular stress response, and is extensively utilized as a bioindicator of environmental stress in many different types of organisms (Kültz, 2005). The onset of stress response (HSR) delineates the limits of normal physiological function and can provide important insights into the capacity and limits of organismal acclimation and adaptive evolution (Kassahn et al., 2009). According to the assumption of Tomanek (2010), the species inhabiting ecosystems with high thermal heterogeneity, like the intertidal zone, induce the HSR frequently and this response is part of their strategy to occupy this thermal niche. These species would not be readily able to modify their thermal range by shifting their upper thermal limit and their threshold for stress gene expression to higher temperatures, and would therefore be vulnerable to temperature changes (Tomanek, 2010; Somero, 2010).

In this context, the present study addresses the capacity for acclimation of the thermal tolerance in the intertidal shrimp *Palaemon elegans* Rathke 1837, by defining the acclimation potential of the upper thermal limit (critical thermal maximum, CTmax) together with the HSR (threshold for induction of *hsp70* gene expression). This work also examined the thermal tolerance (HSR and upper thermal limit) during ontogeny in order to determine the key stages in the acquisition of the

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adult thermal tolerance. The marine rockpool shrimp *Palaemon elegans* (Decapoda, Caridea) is native to European coastal waters, and mainly occurs in the Eastern Atlantic intertidal zone (Gabrowski, 2006). This species is harvested for human consumption, used as a fishing bait, and is also an important food source for relevant commercial fish species such as the Moronidae and Sparidae families (Grabowski, 2006). This shrimp is thus a common temperate intertidal species, and recent studies have defined the upper thermal limit and the stress protein levels of warm-acclimated adult specimens of *P. elegans* (24 °C-acclimated, Madeira et al., 2012 ; 20 °C-acclimated, Madeira et al., 2015), and also showed that the acclimation capacity of the CTmax is higher in *P. elegans* than in a tropical related species of shrimp (acclimation to 23 °C and 26 °C , Vinagre et al., 2016). Here, the ability of adults *P. elegans* to acclimate their heat shock response, in parallel to their upper thermal limit, is assessed through long-term acclimation (4 to 8 months) at either 10 °C or 20 °C temperature conditions. This temperature difference coincides with the seasonnal difference between the mean winter (10 °C) and summer (20 °C) temperatures, as well as with the daily amplitude of variation in temperate marine rockpools (Madeira et al., 2015 ; Vinagre et al., 2016). Assessing the acclimation ability upon a 10 °C-variation would therefore approximate the maximum potential of acclimation for this species. Finally, the thermal tolerance was examined for the first time during *P. elegans* ontogeny, by determining the upper thermal limit and *hsp70* expression of the different developmental stages that occupy distinct thermal niches.

2. Materials and Methods

2.1. Sampling and acclimation

Specimens of *P. elegans* were collected in October 2011, using a shrimp net, from the Bay of Saint-Malo (France ; 48°64′N, −2°00′W). They were transported to the laboratory and transferred to aerated aquaria filled with artificial seawater (salinity of 35 g l^{-1}), and submitted to a 12 h:12 h light:dark cycle. The water temperature was gradually decreased from 17 °C (field temperature at the time of collection) to 10 °C, or increased to 20 °C, at a rate of 1 °C per week. The shrimp were regularly fed with granules (JBL Novo Prawn) *ad libitum,* and were allowed to acclimate for 8 months at 10 °C or 20 °C prior to the experiments (except for the CTmax experiments on 20 °C-acclimated shrimp that were conducted after 4 months of acclimation). The 10 °C and 20 °C acclimation temperatures were chosen to correspond to sea surface mean temperatures in winter and summer season in this region (Mounier and Gouery, 1992).

In May, several females acclimated at 20 °C developed eggs, which hatched in the laboratory by early June. Only one female developped eggs in the 10 °C-acclimated batch, and these eggs were not used for further experiments. The larvae were reared in large beakers at 20 °C under a 12 h:12 h light:dark cycle (optimal conditions for larval survival as determined according to Rochanaburanon and Williamson, 1976 and Dalley, 1980), and the water was changed every day. The larvae and early post-larvae were fed with newly hatched *Artemia franciscana* nauplii every two days. The larval stages were identified following Fincham (1977). The identification of instars beyond zoea 5 until metamorphosis is uncertain, since the number of stages can vary according to the rearing conditions with the insertion of extra moults between zoea 5 and 6 with no clear morphological distinction between the moults (Fincham, 1977). All those stages beyond zoea 5 were therefore named zoea 5+. The changes which occur from the final zoea to the first post-larval stage (PL) are easily identifiable, the most noteworthy change upon metamorphosis being the acquisition of an abdominal propulsion with the pleopods, while the larvae employed a propulsion with the thoracic appendages. This results in a major behavioural difference since the larvae swim upside down and backwards, while the post-larvae swim in an upright position and in a forward direction. The metamorphosis occurred between 23 and 26 days in the different batches of larvae.

2.2. Critical thermal maximum (CTmax) determination

The thermal limit was determined by the dynamic method as previously used for *Palaemonetes varians* (for details, see Ravaux et al., 2012). The temperature was increased at a constant rate until the first occurrence of spasms and loss of equilibrium, i.e. when shrimp lose the ability to escape the conditions which may ultimately lead to death (review in Lutterschmidt and Hutchinson, 1997a, 1997b). Specimens of similar size (cephalothorax length 11.4 ± 1.6 mm, n = 20) were submitted to a temperature increase at a constant rate of 0.9 °C min^{-1} (for both 10 °C- and 20 °C-acclimated specimens). The CTmax was defined as the arithmetic mean of the collective thermal points at which the end-point is reached (see Madeira et al., 2012), by using the equation : $CTmax = \Sigma\ (Tend\text{-}point_n)/n$; where Tend-point is the temperature at which the end-point was reached for individual 1, individual 2, individual n, divided by the total number of individuals (n). The end-point was the appearance of either spasmodic motions (vibrations of the pleopods and/or sudden contraction of the abdomen without any coordinated movement) or loss of equilibrium (LOE, when the shrimp rested on the bottom in either an « upside-down » or a « sideways » position for >2 s). The experiment ended when the shrimp experienced LOE for >30 s. The trial was done for 10 shrimp for both acclimated groups (10 °C and 20 °C). Following the CTmax experiment, the shrimp were quickly returned to their acclimation temperature, and survived for several weeks thereafter. The protocol was similar for larvae and post-larvae except that each individual was placed in an ice-cube tray with a white background, rather than a beaker, to facilitate the observation. The end-point was the appearance of spasms, since the LOE was not clearly identifiable and could easily be confused with immobility. The trial was done for 4 individuals for each sampled instar, with a sampling approximately every day or every 2 days.

2.3. Heat-shock experiments

Heat shocks were conducted as previously described in Ravaux et al. (2012) for *Palaemonetes varians*. Shrimp were transferred from the aquarium in which they had been acclimated to 20-L tanks maintained at the desired shock temperature : 17, 20, 23 °C for the specimens acclimated at 10 °C, and 23, 26, 29 °C for the 20 °C-acclimated group (Fig. 1). After a 1 h-heat exposure, shrimp were transferred back to their previous acclimation temperature (10 °C or 20 °C) in floating cages for 2 h recovery. Shrimp directly sampled in the rearing tanks served as controls for both acclimation groups. The tissues from abdomen muscles were dissected, subsequently frozen and stored in liquid nitrogen until further analysis.

Shrimp individuals of different developmental stages were also submitted to a 1 h-heat shock. Individuals ($n = 4$) were sampled every 7 days during development until 28 days, which corresponds to zoea 3, zoea 5, zoea 5 + and post-larval stages. The heat shock was obtained by transferring the individuals from the beakers at a rearing temperature of 20 °C towards trays immersed in a temperature controlled water bath. The water temperature in the trays, monitored using an electronic thermometer, was 26 °C. After a 2 h-recovery period at 20 °C, the individuals were frozen and stored in liquid nitrogen.

2.4. Identification of hsp70 genes in P. elegans

The total RNA was extracted from grounded tissues using RNeasy Mini kit (Qiagen) and QIAshredder (Qiagen) in accordance with the manufacturer protocols. The RNA (0.5 μg) was treated to remove DNA contamination by using the Turbo-DNAse kit (Ambion), and then reversely transcribed to cDNA with the oligo$(dT)_{18}$ primer and Superscript II reverse transcriptase kit (Invitrogen) according to the manufacturer instructions. The cDNA encoding putative *hsp70* genes in *P. elegans* (*hsp70* form1, form2 and form3) were amplified by PCR amplification, using the degenerated primers HSP1, HSP2, HSP3 and