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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

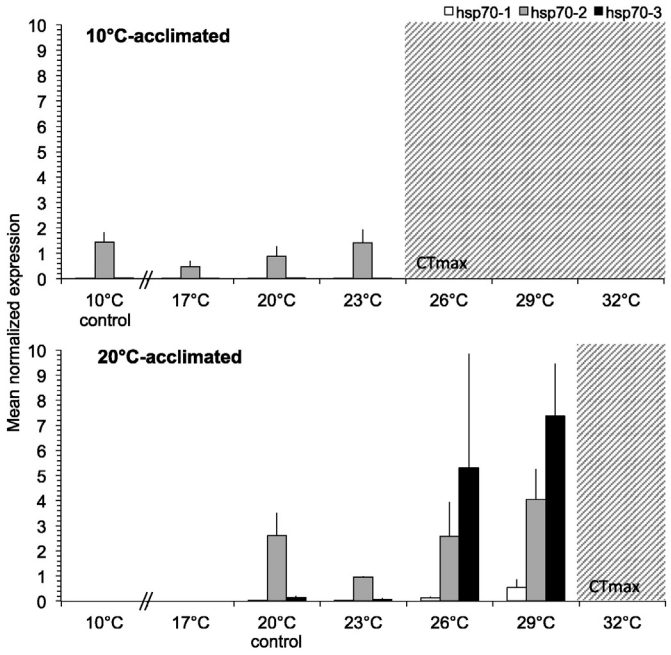

Fig. 1. Capacity for acclimation of both the heat shock response (*hsp70* expression) and upper thermal limit (CTmax) in *Palaemon elegans* adult specimens. Levels of *hsp70* mRNA were measured after a 1 h-heat shock followed by a 2 h-recovery period, and are expressed as means for n individuals (± SEM) of relative *hsp70* amount normalized to *RPL8* abundance (reference gene); $n = 4$–5 for 10 °C-acclimated specimens (except $n = 1$ at 23 °C for *hsp70* form 3), and $n = 2$–4 for 20 °C-acclimated specimens. The hatched areas represent the temperatures above the CTmax (onset of spasms: 25.7 ± 0.8 °C for 10 °C-acclimated shrimp and 31.7 ± 0.8 °C for 20 °C-acclimated shrimp).

HSP4 (see Table S1 in supplementary material). The PCR amplifications were performed following a previously published protocol (Ravaux et al., 2007). Separation of PCR products was performed on a 1.5% agarose gel and DNA purification was carried out using the QIAquick gel extraction kit (Qiagen). The resulting amplicons were cloned using the TOPO TA cloning kit (Invitrogen), and further sent for sequencing to GATC Biotech. The resulting nucleotide sequences were then deposited in the GenBank database under the accession numbers **KT151557** (*hsp70* form1; consensus sequence from 10 clones), **KT151558** (*hsp70* form2; consensus sequence from 25 clones), and **KT151559** (*hsp70* form3; consensus sequence from 46 clones). Specific primers were further designed for each gene for RT-qPCR (Table S1).

2.5. Quantification of hsp70 expression using real-time quantitative RT-PCR

The expression of *hsp70* gene was assessed by qPCR with specific primers (Table S1). Each reaction of qPCR was run in triplicate, and corresponded to 3–5 individuals for each experimental condition. All reactions were performed on the LightCycler® 480 II Real-Time PCR Detection System (Roche, France), using Sybr Green I Master (Roche, France). The PCR program consisted of an initial 13.5 min step at 95 °C, followed by 45 cycles consisting of 30 s of denaturation at 94 °C, 30 s of annealing at the optimal annealing temperature (56 °C) and 30 s at 72 °C. The measurement of fluorescence during the 70 to 95 °C melting curve showed a single and discrete peak for all primers tested. One negative control and one dilution series protocol of pooled cDNA were included in each run. The dilution series were used to construct a relative standard curve to determine the PCR efficiencies and for further quantification analysis. In all experiments, all primers gave amplification efficiencies of 90–100%. Data were analysed with the LightCycler® 480 software and the *hsp70* expression was normalized to the reference gene *RPL8* (60S ribosomal protein L8, previously used to study the *hsp70* expression in another palaemonid species ; Ravaux et al., 2012).

3. Results

3.1. Critical thermal maximum (CTmax) and onset of the heat shock response (HSR) in P. elegans adults acclimated to 10 °C versus 20 °C (Fig. 1)

A first apparent loss of locomotory coordination, which results in the onset of spasmodic movements of the pleopods and/or abdomen with no resulting displacement ('spasms'), was observed at 25.7 ± 0.8 °C for 10 °C-acclimated shrimp, and 31.7 ± 0.8 °C for 20 °C-acclimated shrimp. This disorder of locomotory activity was later observed as the shrimp lost their balance (LOE response) upon reaching 27.5 ± 0.8 °C for 10 °C-acclimated shrimp or 33.2 ± 1.5 °C for 20 °C-acclimated shrimp.

Three mRNA sequences were isolated and identified as members of the *hsp70* family, and therefore named "*Palaemon elegans hsp70* form 1", "*Palaemon elegans hsp70* form 2", and "*Palaemon elegans hsp70* form 3" (see Supplementary Fig. 1 for alignment and description). The relative abundance of these *hsp70* transcripts was quantified for each acclimation group, following heat shocks of 1 h at temperatures ranging up to about 3 °C below the thermal limit : 23 °C for the 10 °C-acclimated group and 29 °C for the 20 °C-acclimated group (Fig. 1). The expression patterns clearly characterized the *hsp70* form 1 and form 3 as inducible forms, since their expression is very low in control specimens at 10 °C and 20 °C, and can increase at higher temperatures. The *hsp70* form 2 displayed an expression pattern typical of a constitutive form in both acclimation groups, with a higher basal expression level when compared to the other forms, and no significant overexpression following heat shocks (Kruskall-Wallis test, $p = 0.07$ for the 10 °C-acclimated group and $p = 0.26$ for the 20 °C-acclimated group). For the 10 °C-acclimated group, the expression of the *hsp70* form 1 and 3 was not induced in the temperature range tested (Kruskall-Wallis test, $p = 0.65$ for *hsp70* form 1 and $p = 0.23$ for *hsp70* form 3). For the 20 °C-acclimated shrimp, a massive induction of *hsp70* form 1 and 3 expressions was observed at 26 °C, which corresponds to an average 70-fold induction for *hsp70* form 1 and 37-fold for *hsp70* form 3 when normalized to the expression levels of the control shrimp at 20 °C. This over-expression increases to 268-fold induction for *hsp70* form 1, and 51-fold for *hsp70* form 3, at 29 °C.

3.2. Critical thermal maximum (CTmax) and heat shock response (HSR) of P. elegans larvae and post-larvae (Fig. 2)

The mean CTmax values for zoea larvae ranged from 25.5 ± 1.6 °C to 29.1 ± 0.7 °C (Fig. 2A), and were significantly lower than the adult CTmax of 31.7 ± 0.8 °C using the onset of spasms as the end-point (Mann-Whitney two-sided test $p = 0.006$ for all larval stages, except for day 4/5 (zoea 2 or 3) $p = 0.007$ and day 11/12 (zoea 5) $p = 0.04$). The mean CTmax values for post-larvae (PL) of 31.2 ± 1 °C for day 25/26 PL and 31.6 ± 2.2 °C for day 30 PL, were identical to the adult CTmax (Mann-Whitney two-sided test $p = 0.43$ for day 25/26 PL and $p = 1$ for day 30 PL).

Instars of *P. elegans*, sampled every 7 days from hatching until after metamorphosis, were exposed to a 26 °C-shock, in order to explore the heat shock response (Fig. 2B). The *hsp70* transcripts showed relatively low abundances in the zoea larval stages, even for the *hsp70* form 2 that is constitutively expressed in relatively high abundance in adults. The abundances of the inducible *hsp70* form 1 (mean normalized level of 0.004) and *hsp70* form 3 (mean normalized level of 0.13) in zoea 3 after the heat shock were similar to that obtained for the adults maintained at constant 20 °C (0.002 for *hsp70* form 1 and 0.14 for *hsp70* form 3, see 20 °C control data from Fig. 1). A massive HSR was observed only in post-larvae, as witnessed by the increase

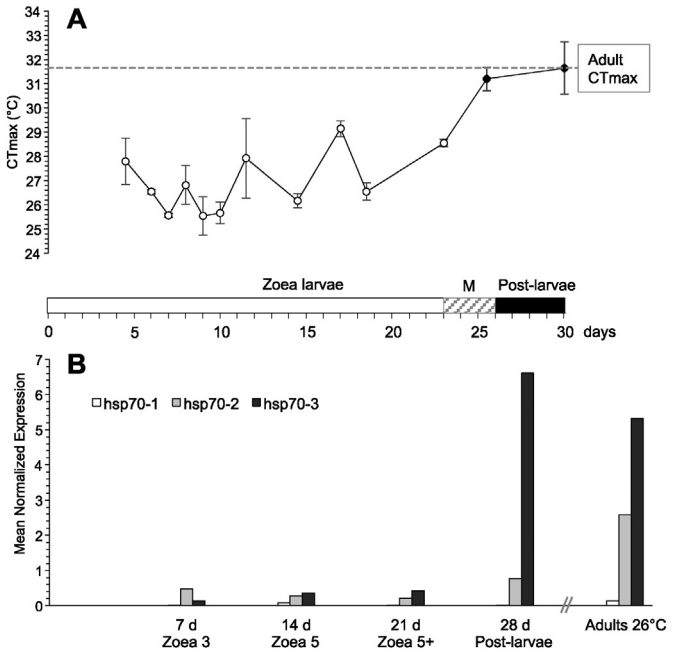

Fig. 2. Upper thermal limit and stress response during ontogeny in *Palaemon elegans*. A. Critical thermal maximum (CTmax) of *P. elegans* larval and post-larval stages. Each dot corresponds to the CTmax (± SEM) for $n = 4$ larvae or post-larvae reared at 20 °C (the white dots correspond to larval stages and the black dots to post-larval stages ; the corresponding days are represented on the horizontal axis ; M, metamorphosis). The end-point for CTmax determination was the first occurrence of spasms. The dotted line represents the CTmax value obtained for 20 °C-acclimated adults, i.e. 31.7 ± 0.8 °C. **B.** Stress gene expression in larval and post-larval stages of *P. elegans* reared at 20 °C and exposed to a 26 °C-heat shock. Each bar corresponds to the quantification of the *hsp70* gene expression (normalized to the *RPL8* reference gene abundance) in a pool of $n = 5$ specimens. The development duration (in days following hatching), and the corresponding instar (according to Fincham, 1977), is indicated below the graph. For comparison, the values obtained for the adults (from Fig. 1 ; 26 °C-heat shock) are plotted on the graph.

of the mean normalized expression of the *hsp70* form 3 to 6.62, a value that is similar to the level of 5.31 obtained for the adults following a 26 °C-heat exposure.

4. Discussion

4.1. Potential for acclimation of the thermal tolerance (CTmax and HSR) in Palaemonid shrimp

In the last decade, the study of thermal tolerance in marine species and their ability to acclimate to changes in environmental temperature has received increased attention. The upper thermal limit and the acclimation capacity, which determine a species vulnerability to temperature changes, were proposed to be related to its thermal habitat (Somero, 2010; Tomanek, 2010; Vinagre et al., 2016). Intertidal organisms were proposed to have a high thermal limit, while nevertheless being very vulnerable to temperature increase because of their limited capacity for acclimation of the upper thermal limit (Somero, 2010) and of the HSR (Tomanek, 2010).

Previous studies on intertidal molluscs and crustacean species showed that the species thermal tolerance limit would be related to the maximal habitat temperature (review in Somero, 2010). The upper thermal limit of cold- and warm-acclimated *Palaemon elegans* was compared to that of the shallow water brackish shrimp *Palaemonetes varians,* originating from the same geographical region (Ravaux et al., 2012; Fig. 3). For both acclimation conditions, *P. elegans* appeared less thermotolerant than *P. varians*, as witnessed by lower CTmax. According to previous assumptions, this difference in the CTmax would imply that these two species encountered different maximal temperatures in their natural environment. The

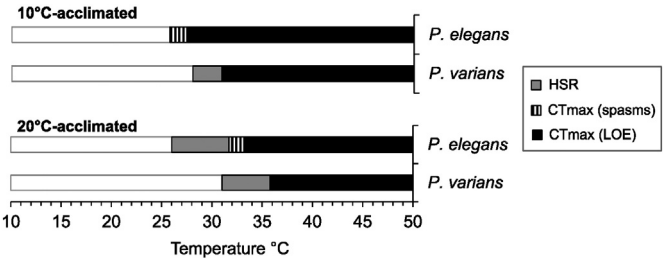

Fig. 3. Comparison of the acclimation potential of two congeneric palaemonid species. The CTmax and HSR (heat-inducible *hsp70* expression threshold) are represented for *Palaemon elegans* and *Palaemonetes varians* acclimated to 'winter' (10 °C) or 'summer' (20 °C) conditions. The colored bars represent the temperature range beyond the HSR threshold (grey) and the CTmax values, with either spasms (vertical stripes) or LOE (dark) as the end-point. The data for *P. varians* are from Ravaux et al. (2012).

distribution of these two species at adult stage is however different, with *P. elegans* occupying the intertidal zone (rockpools) and the shallow subtidal zone, while *P. varians* mainly inhabits the saline marsh pools with an overlapping area of distribution with *P. elegans* in the marine rockpools. A recent comparative approach of the upper thermal limit of *P. elegans* with *Palaemon serratus*, which occupies the lower shore to subtidal habitat, revealed a similar thermal limit for both species in warm-acclimated specimens (Madeira et al., 2015). This suggests that these two species, while inhabiting a different zone of the shore, would encounter the same maximal temperatures in their environment. Adding to the present study, this underlines the need for further studies into the relationship of thermal habitat and the upper thermal limit of Palaemonid shrimp.

The intertidal shrimp *Palaemon elegans* is able to shift its upper thermal limit when acclimated to cold versus warm temperature, as previously shown for other palaemonid species (Fig. 4). Interspecies comparisons of the CTmax plasticity is achieved by using the acclimation response ratio (ARR ; $\Delta CTmax/\Delta T$; Claussen, 1977), which provides a normalized value of the change of thermal limit as a function of the variation of the acclimation temperature for each species (Table 1). In *P. elegans*, the ARR value of 0.57 is close to the mean ARR value obtained for the seven palaemonid species presented in Fig. 4 (0.53, equivalent to the slope of the function), which represents a genuine acclimatory capacity for this group. A great interspecies variability is however observed,

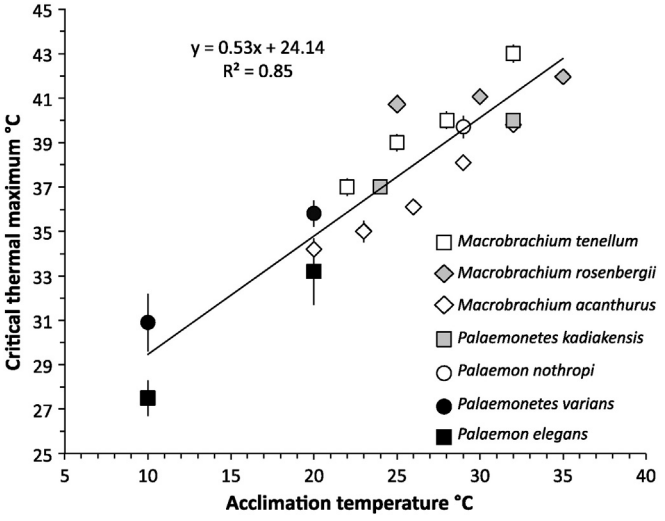

Fig. 4. Critical thermal maxima related to the acclimation temperature in palaemonid shrimp from marine and freshwater habitats. Black squares *Palaemon elegans*; black circles, *Palaemonetes varians*; white circles, *Palaemon nothropi*; grey squares, *Palaemonetes kadiakensis*; white diamonds, *Macrobrachium acanthurus*; grey diamonds, *Macrobrachium rosenbergii*; white squares, *Macrobrachium tenellum*. This graph depicts only values for adults shrimp and from similar protocols for CTmax determination, i.e. LOE and temperature ramp ≤1 °C min^{-1}. See Table 1 for values and references.

Table 1
Critical thermal maxima (CTmax) of adult shrimp acclimated to different temperatures. ARR: Acclimation Response Ratio (ΔCTmax/ΔT; Claussen, 1977). *Values read on the graph. Mean ARR value for each species is indicated in italics.
The CTmax depends on the acclimation temperature and experimental procedures (Rodríguez and Ramirez, 1997; Dìaz et al., 1998; Díaz et al., 2002; Chown et al., 2009). The Loss of Equilibrium (LOE) behavioural response is here chosen for inter-species comparison since it is well established as an indicator of temperature-induced loss of function (e.g. Dìaz et al., 1998; Díaz et al., 2002). This table only reports studies that used similar heating rates, in the 0.5–1.5 °C min^{-1} range, that proved to allow the body temperature to match the environmental temperature and exclude any heat-hardening occurring with lower rates (Lutterschmidt and Hutchinson, 1997a, 1997b). It can however be noted that for some species, the heating ramp would not affect the CTmax value (*Palaemon northropi*, Vinagre et al., 2015). For *P. elegans* similar CTmax values were also obtained in the present study and in Madeira et al. (2015), i.e. 33.4 ± 0.5 °C with a rate of 1 °C h^{-1} (water temperature on the collection site of about 17 °C in both studies, with further acclimation to constant 20 °C at the laboratory).

	Species (habitat, Holthuis, 1980)	CTmax	Acclimation temperature	Acclimation duration	ARR	End-point / Temperature ramp for CTmax determination	References
Palaemonidae	*Palaemonetes kadiakensis* (temperate freshwater)	36.7	24	14 days		Complete disorientation / 1 °C per min	Nelson and Hooper, 1982
		37.2	26		0.25		
		38.3	28		0.55		
		39.3	30		0.50		
		40.1	32		0.40		
					0.42		
	Macrobrachium tenellum (tropical freshwater)	37 ± 0.4	22	21 days		Total disorientation / 1 °C per min	Hernandez et al., 1996
		39 ± 0.4	25		0.67		
		40 ± 0.4	28		0.33		
		43 ± 0.4	32		0.75		
					0.60		
	Macrobrachium acanthurus (temperate and tropical fresh and brackish water)	34.2 ± 0.48	20	30 days		LOE / 1 °C per min	Diaz et al., 2002
		35.0 ± 0.5	23		0.27		
		36.1 ± 0.26	26		0.37		
		38.1 ± 0.32	29		0.67		
		39.8 ± 0.28	32		0.57		
					0.47		
	Macrobrachium rosenbergii (tropical fresh and brackish water)	40.73 ± 0.16	25	30 days		LOE / 0.3 °C per min	Manush et al., 2004
		41.06 ± 0.17	30		0.07		
		41.96 ± 0.17	35		0.18		
					0.12		
	Palaemonetes varians (temperate brackish water)	30.9 ± 1.3	10	4 months		LOE / 1 °C per min	Oliphant et al., 2011
		35.8 ± 0.6	20		0.49	LOE / 1 °C per min	Ravaux et al., 2012
	Palaemon nothropi (tropical marine shallow water)	39.7 ± 0.5	29	7 days	–	LOE / 1 °C per min	Vinagre et al., 2015
	Palaemon elegans (temperate marine rockpools and shallow sublittoral zone)	**27.5 ± 0.8**	**10**	**4 to 8 months**		**LOE / 1 °C per** min	**This study**
		33.2 ± 1.5	**20**		**0.57**		
Penaeidae	*Litopenaeus vannamei* (tropical marine)	36	20			LRR / 1 °C per min	Gonzales et al., 2010*
		37.25	23		0.42		Diaz et al., 2013*
		39.6	26		0.78		
		40.6	29		0.33		
		41.75	32		0.38		
					0.48		
Hippolytidae	*Hippolyte obliquimanus* (tropical marine)	34.7 ± 0.8	29		–	LOE / 1 °C per min	Vinagre et al., 2015

with specific mean ARR values ranging from 0.12 for *Macrobrachium rosenbergii*, to 0.6 for *Macrobrachium tenellum*. Based on this observation of interspecies variability of ARR in the palaemonid group, we can suggest that the ability for acclimation of the upper thermal limit is not determined by the phylogenetic affiliation in this group. Further comparison with other shrimp taxa will help to explore this hypothesis, but it is at this time limited to only one species available, the penaeid *Litopenaeus vannamei* (ARR value of 0.48). Comparisons of the acclimation capacity among shrimp from diverse thermal habitats allow to question a potential effect of their native environment, and would not reveal any influence of the thermal habitat on the CTmax plasticity (Table 1). Indeed, the two tropical Macrobrachium species, *M. tenellum* and *M. rosenbergii*, have a very different acclimation capacity. Moreover, *P. elegans* originating from the very variable temperate intertidal zone, and L. *vannamei* originating from the more stable tropical subtidal zone, have a similar acclimation capacity. This would support the previous assumption that the thermal heterogeneity of the environment seems to bear little on the capacity for acclimation of the thermal limit (Angilletta, 2009). On the other hand, this would contradict the recent results of Vinagre et al. (2016), which established a relationship between the acclimation capacity of the CTmax and the native environment, i.e. tropical versus temperate. These authors also obtained ARR values for *P. elegans* of 0.93 (for a change of acclimation temperature from 20 °C to 23 °C), and of 1.06 (for a change of acclimation temperature from 23 °C to 26 °C). Even though comparisons are limited because the CTmax was determined by using different experimental procedures, this emphasizes the existence of a great intraspecies variability of the acclimation capacity depending on the range of acclimation temperature considered (see Table 1 for other examples). Finally, the present study confirmed the capacity of palaemonid shrimp to acclimate their CTmax, but why some species possess a greater plasticity than others, and what determines the plasticity over a species temperature range, remains to be investigated.

The species thermal tolerance is also determined by its ability to respond to thermal stress through activation of the HSR. The cold-acclimated specimens of *P. elegans* did not trigger the HSR after heat-shocks reaching temperatures up to 3 °C below the CTmax, unlike *P. varians* (Fig. 3). Since both the HSR onset and the CTmax are far above the temperatures encountered in this region during the winter season (mean sea surface temperatures of 10–11 °C during a mild winter, Mounier and Gouery, 1992 ; maximum air temperature record registered during winter in Saint-Malo of 23.2 °C in March, http://www.meteofrance.com/previsions-meteo-france/saint-malo/35400), these differences in 'winter' HSR onset and CTmax would therefore be of minor ecological consequences for these species. With a threshold temperature for *hsp70* expression of 26 °C for warm-acclimated specimens, it is highly likely that *P. elegans* triggers *hsp70* synthesis under natural conditions in summer, and during low tide when the temperature can

reach or even exceed 30 °C (Madeira et al., 2015 ; Vinagre et al., 2016 ; maximum air temperature record registered during summer in Saint-Malo of 39.4 °C in August, http://www.meteofrance.com/previsions-meteo-france/saint-malo/35400). On the contrary, *P. varians* was previously shown to have a threshold temperature for *hsp70* expression well above the temperature range that they currently experience in the field, and would only rarely activate the HSR in their thermal niche (Fig. 3; late summer temperature profile in Fig. S2 ; Ravaux et al., 2012). According to Tomanek (2010), the organisms inhabiting highly variable environments (variations up to >20 °C) would induce the HSR frequently to cope with recurrent thermal extremes, while organisms inhabiting moderatly variable environments (variations <10 °C) would not. Since both *P. elegans* and *P. varians* occupy thermally variable environments, a more detailed characterization of their thermal habitat would be necessary to determine if some differences in temperature ranges, temperature extremes or pattern of variations could account for the difference of thermal tolerance in these two species. This should be done by keeping in mind that *in situ* temperature probing in fluctuating environments is challenging, since the thermal profile of marsh pools and intertidal rockpools can significantly differ depending on their depth, the seawater influx, and the level of solar exposure.

The shrimp *P. elegans* proved here to have a limited capacity for HSR acclimation, if any. Indeed, the onset for heat-inducible *hsp70* expression did not acclimate together with the CTmax, unlike observed in the congeneric species *P. varians* (Fig. 3). The extent of acclimation of the HSR, estimated as the change in induction temperature per unit change in rearing temperature, is of about 0.3 in *P. varians* and would then be <0.3 in *P. elegans*, if not zero (Fig. 3) (0 to 0.3 for marine invertebrates, Barua and Heckathorn, 2004 ; 0.4 for *Macrobrachium malcolmsonii*, Selvakumar and Geraldine, 2005). Tomanek (2010) established a relationship between the HSR acclimatory capacity and the variability of the thermal habitat. This would mean that the difference in acclimation capacity of these two palaemonids would be explained by a difference in the thermal variability of their habitats. Here again, a detailed description of their thermal habitat will help to further elucidate its influence on the HSR plasticity. Finally, the lower potential for acclimation of the HSR, as well as the lower onset of the HSR in 'summer' conditions, of *P. elegans* implies that this species is more sensitive to temperature increase than its congener *P. varians* to temperature increase and is more likely to be affected by climate change.

4.2. Acquisition of thermal tolerance during development in P. elegans

We provide here some insights into the thermal tolerance of *P. elegans* during ontogeny. This palaemonid shrimp occurs at adult state in rockpools and other situations where the temperature may show large fluctuations, while larvae usually develop in the open ocean under more constant conditions (Rochanaburanon and Williamson, 1976). This environmental change during development may be accompanied by a change in thermal tolerance. Our study supports this view, by showing a higher CTmax of *P. elegans* adults when compared to larvae, with CTmax ranging from 25 to 29 °C for larvae and being about 32 °C for adults (onset of spasms for 20 °C acclimated shrimp, Fig. 2). The shift of the upper thermal limit to higher temperatures occurred upon metamorphosis of larvae to post-larvae, thus identifying this stage as a physiological milestone in the process of thermal tolerance acquisition in *P. elegans*. Previous studies in another palaemonid shrimp, the giant river prawn *Macrobrachium rosenbergii*, compared the upper thermal limit at two other stages of physiological readjustment, i.e. the development of post-larvae to juveniles and of juveniles to adults, and found similar CTmax at all these developmental stages (Diaz et al., 1998; Manush et al., 2004). It could be interesting to address the thermal tolerance limit of larvae *versus* post-larvae to determine if a shift also occurs upon metamorphosis in this palaemonid species, especially when this also corresponds to a change of habitat. Indeed, larvae of *M. rosenbergii* complete their development in estuarine or coastal lagoon environments and metamorphose in post-larvae that migrate upstream into freshwater conditions (Holthuis, 2000). Further studies on other palaemonid species, and possibly other shrimp species, will help to determine if the acquisition of the upper thermal limit always occurs during the metamorphosis.

The instars of *P. elegans* showed a stage-dependent heat shock response (HSR), and probably gained the ability to trigger a strong HSR during metamorphosis of zoeal larvae to post-larvae. A HSR has previously been demonstrated in crustacean larvae (*Artemia* nauplii, McLennan and Miller, 1990; spider crab *Hyas araneus* zoeal and megalope larvae, Schiffer et al., 2014) and showed a variable pattern of stage-specific activation. In the spider crab *Hyas araneus*, a 20 °C-heat shock caused the upregulation of *hsp70* (1–4) with fold ranging from 1.4 to 5.5 for all developmental stages. The early larval stages seemed to be less responsive to heat stress that the later stages, which was proposed to reflect a narrowing of thermal tolerance or improved resilience with progressive development (Schiffer et al., 2014). In *P. elegans*, the thermal tolerance limit increased in the post-larval stages, and the strong upregulation of the heat stress gene expression at this stage could therefore rather be interpreted as an improved resilience to thermal damages.

Taken together, these results suggest that *P. elegans* larvae have adapted to the low variability of the open ocean thermal environment, with a thermal limit lower than the adults and a limited capacity of stress response mechanisms. The more advanced stages that occur in the highly variable thermal conditions of the intertidal zone would enhance their capacity for thermal tolerance, by increasing their thermal limit and activating mechanisms of cellular damage repair. The physiological adjustment occurring during the metamorphosis from the larval to the post-larval instars would thus be a major genetically-programmed physiological milestone for the acquisition of the adult thermal tolerance. Further studies on thermal tolerance during ontogeny of *P. elegans* will aim at assessing the capacity for acclimation of the thermal limit in larvae, and also the potentially irreversible modifications induced by rearing temperature that would impact the thermal tolerance of adults.

5. Conclusion

This study showed an acclimation capacity of the thermal tolerance in *P. elegans* through plasticity of its thermal limit. The question remains whether the shift of the thermal tolerance limit to higher temperatures is sufficient to face temperature increase, since the CTmax of warm-acclimated specimens is not far above maximal temperatures encountered in summer. Moreover, the HSR threshold is within the thermal range that could be experienced during low tide in summer, and has a limited capacity for acclimation, if any. This species could therefore potentially be more sensitive to global warming than its congener *Palaemonetes varians*. Further studies should help to determine the acclimation capacity of larval stages in both species, and to possibly characterize the developmental acclimation. This latter comprises the irreversible adjustments occuring during the early ontogeny in response to the environmental temperature, which may determine the thermal tolerance of the adult.

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References

Angilletta, M.J., 2009. Thermal adaptation. Oxford University Press Inc., New York.

Barua, D., Heckathorn, S.A., 2004. Acclimation of the temperature set-points of the heat-shock response. J. Therm. Biol. 29, 185–193.

Chown, S.L., Jumbam, K.R., Sørensen, J.G., Terblanche, J.S., 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Funct. Ecol. 23, 133–140.

Claussen, D.L., 1977. Thermal acclimation in ambystomatid salamanders. Comp. Biochem. Physiol. A 58, 333–340.

Dalley, R., 1980. Effects of non-circadian light/dark cycles on the growth and moulting of *Palaemon elegans* reared in the laboratory. Mar. Biol. 56, 71–78.

Dìaz, H., Uribe, E.S., Ramirez, L.F.B., Mora, A.G., 1998. Critical thermal maxima and minima of *Macrobrachium rosenbergii* (Decapoda: Palaemonidae). J. Therm. Biol. 23 (6), 381–385.

Díaz, H.F., Sierra, E., Re, A.D., Rodríguez, L., 2002. Behavioural thermoregulation and critical thermal limits of *Macrobrachium acanthurus* (Wiegman). J. Therm. Biol. 27, 423–428.

Diaz, F., Re, A.D., Sanchez, A., Cruz, H., Gonzalez, R.A., Sanchez, L.N., Licea, A., Ponce-Rivas, E., Munoz-Marquez, M.E., Giffard, I., Rosas, C., 2013. The effects of exposure to critical thermal maxima on the physiological, metabolic, and immunological responses in adult white shrimp *Litopenaeus vannamei* (Boone). Mar. Freshw. Behav. Physiol. 45 (6), 365–374.

Fincham, A.A., 1977. Larval development of British prawns and shrimps (Crustacea : Decapoda : Natantia). 1. Laboratory methods and a review of Palaemon (Paleander) elegans Rathke 1837. Bull. Br. Mus. Nat. Hist. (Zool.) 32 (1), 1–28.

Gabrowski, M., 2006. Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. Aquat. Invasions 1 (3), 116–123.

Gonzales, R.A., Diaz, F., Licea, A., Re, A.D., Sanchez, L.N., Garcia-Esquivel, Z., 2010. Thermal preference, tolerance and oxygen consumption of adult white shrimp *Litopenaeus vannamei* (Boone) exposed to differant acclimation temperatures. J. Therm. Biol. 35, 218–224.

Hernandez, R.M., Bückle, R.L.F., Diaz, H.F., 1996. Critical thermal maximum of *Macrobrachium tenellum*. J. Therm. Biol. 21 (2), 139–143.

Holthuis, L.B., 1980. Shrimps and prawns of the world. FAO species catalogue. An annotated catalogue of species of interest to fisheries. FAO Fish. Synop. (125) (Vol.l).

Holthuis, L.B., 2000. Nomenclature and taxonomy. In: New, M.B., Valenti, W.C. (Eds.), Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, England, pp. 12–17.

Kassahn, K.S., Crozier, R.H., Pörtner, H.O., Caley, M.J., 2009. Animal performance and stress: responses and tolerance limits at different levels of biological organization. Biol. Rev. 84, 277–292.

Kültz, D., 2005. Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. 67, 225–257.

Lutterschmidt, W.I., Hutchinson, V.H., 1997a. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can. J. Zool. 75, 1553–1560.

Lutterschmidt, W.I., Hutchinson, V.H., 1997b. The critical thermal maximum: history and critique. Can. J. Zool. 75, 1561–1574.

Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., 2012. Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. J. Sea Res. 70, 32–41.

Madeira, D., Mendonça, V., Dias, M., Roma, J., Costa, P.M., Larguinho, M., Vinagre, C., Diniz, M.S., 2015. Physiological, cellular and biochemical thermal stress response of intertidal shrimps with different vertical distributions : *Palaemon elegans* and *Palaemon serratus*. Comp. Biochem. Physiol. Part A 183, 107–115.

Manush, S.M., Pal, A.K., Chatterjee, N., Das, T., Mukherjee, S.C., 2004. Thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures. J. Therm. Biol. 29, 15–19.

McLennan, A.G., Miller, D., 1990. A biological role for the heat shock response in crustaceans. J. Therm. Biol. 15 (1), 61–66.

Morris, J., Thatje, S., Hauton, C., 2013. The use of stress-70 proteins in physiology: a re-appraisal. Mol. Ecol. 22, 1494–1502.

Mounier, J., Gouery, P., Costel Team, 1992. Température de la mer autour du Massif armoricain selon les données du satellite NOAA/AVHRR. Norois 155, 305–315 (http://www.persee.fr/web/revues/home/prescript/article/noroi_0029-182X_1992_num_155_1_6436).

Nelson, D.H., Hooper, D.K., 1982. Thermal tolerance and preference of the freshwater shrimp *Palaemonetes kadiakensis*. J. Therm. Biol. 7 (3), 183–187.

Oliphant, A., Thatje, S., Brown, A., Morini, M., Ravaux, J., Shillito, B., 2011. Pressure tolerance of the shallow-water caridean shrimp, *Palaemonetes varians*, across its thermal tolerance window. J. Exp. Biol. 214, 1109–1117.

Ravaux, J., Toullec, J.-Y., Léger, N., Lopez, P., Gaill, F., Shillito, B., 2007. First hsp70 from two hydrothermal vent shrimps, *Mirocaris fortunata* and *Rimicaris exoculata*: Characterization and sequence analysis. Gene 386 (1–2), 162–172.

Ravaux, J., Léger, N., Rabet, N., Morini, M., Zbinden, M., Thatje, S., Shillito, B., 2012. Adaptation to thermally variable environments: capacity for acclimation of thermal limit and heat shock response in the shrimp *Palaemonetes varians*. J. Comp. Physiol. B. 182 (7), 899–907.

Rochanaburanon, T., Williamson, D.I., 1976. Laboratory survival of larvae of *Palaemon elegans* Rathke and other caridean shrimps in relation to their distribution and ecology. Estuar. Coast. Mar. Sci. 4, 83–91.

Rodríguez, M.H., Ramirez, F.B., 1997. Thermal preference area for *Macrobrachium tenellum* in the context of global climate change. J. Therm. Biol. 22, 309–313.

Schiffer, M., Harms, L., Lucassen, M., Mark, F.C., Pörtner, H.-S., Storch, D., 2014. Temperature tolerance of different larval stages of the spider crab *Hyas araneus* exposed to elevated seawter PCO_2. Front Zool. 11, 87–109.

Selvakumar, S., Geraldine, P., 2005. Heat shock protein induction in the freshwater prawn *Macrobrachium malcolmsonii*: acclimation-influenced variations in the induction temperatures for Hsp70. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 140 (2), 209–215.

Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. 213, 912–920.

Tomanek, L., 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. J. Exp. Biol. 213, 971–979.

Vinagre, C., Leal, I., Mendonça, V., Flores, A.A.V., 2015. Effect of warming rate on the critical thermal maxima of crabs, shrimp and fish. J. Therm. Biol. 47, 19–25.

Vinagre, C., Leal, I., Mendonça, V., Madeira, D., Narciso, L., Diniz, M.S., Flores, A.A.V., 2016. Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms. Ecol. Indic. 62, 317–327.