Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.2014JCLM-2014-0057

Heat shock response of the blue crab *Portunus pelagicus*: thermal stress and acclimation

Suhaila Qari*

Department of Biological Sciences, University of King Abdulaziz, Jeddah City, Saudi Arabia

PEER REVIEW

Peer reviewer

Dr. David Hyde, Durham University, Staff in the School of Biological and Biomedical Sciences, UK.
Tel: +44 (0) 191 37 43337
E-mail: david.hyde@durham.ac.uk
Co-reviewers: Dr. Mirela Cuculescu, Newcastle City, UK. Dr. Madeira, Lisbon, Portugal. Dr. Richard Hopkin, Preston, UK.

Comments

This research study acclimation and heat shock of the tropical crab species. The result conclude that heat shock caused significant rises in CTMax, however this increase was progressively reduced with longer recovery times at the acclimation temperature. This research provides new information that enables evaluation of that issue.
Details on Page 612

ABSTRACT

Objective: To determine the effect of prior heat shock on the CTMax of differently acclimated *Portunus pelagicus* (*P. pelagicus*) as well as the time course of the changes in CTMax post heat shock.

Methods: Crabs *P. pelagicus* were held in laboratory aquaria in tanks, which were supplied with filtered and aerated seawater. Crabs were acclimated at 20 °C, 25 °C, 30 °C and 35 °C for 3 weeks before their CTMax was determined. The CTMax was recorded for each crab as the median temperature during the 5 min period when a crab was not able to right itself, the average CTMax was calculated. The effect of heat shock on subsequent CTMax was measured. Crabs were heat shocked at temperature 1 °C lower than the CTMax for 20 min, followed by either 0.5 h, 1 h or 1.5 h recovery at 20 °C. The same procedure was repeated at other acclimation temperatures (25 °C, 30 °C and 35 °C).

Results: Temperature acclimation of *P. pelargicus* from 20-35 °C progressively increased the CTMax. Acclimation at 35 °C the CTMax was 42.66 °C, whereas acclimation at 20 °C the CTMax was 39.8 °C. In *P. pelagicus* acclimated, at 20 °C the CTMax values after heat shock were significantly higher than crabs in control for 30 min, 1 h and 1.5 h after heat shock. In the 25 °C and 30 °C acclimated crabs, the CTMax values after heat shock were significantly higher than control only in 30 min and 1 h after heat shock. No significant differences in 35 °C acclimated crabs between control and heat shocked crabs were found after recovery for 30 min, 1 h, or 1.5 h.

Conclusions: Heat shock caused significant rises in the CTMax, however, this increase was progressively reduced with longer recovery times at the acclimation temperature. For 20 °C acclimated crabs, the increased CTMax was still evident after 90 min, but for 25 °C and 30 °C crabs, the response was over after 90 min. Heat shock of 35 °C crabs was problematical, the CTMax gave no increased thermotolerance. It must be concluded that the combination of a high heat shock temperature and CTMax determinations were too damaging.

KEYWORDS
CTMax, Temperatre, Acclimation, Heat shock, *Portunus pelagicus*

1. Introduction

Thermal environment is an important leading to genetic divergence of population[1], due to the important role of temperature on a biochemical and physiological process[2]. Temperature stress may eventually reduce the organism's competition and reproduction abilities[3]. Environmental stress that threaten the functional integrity of an organism result in the expression of a set of genes the protein products of which serve to protect the organism from

*Corresponding author: Suhaila Qari, Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah City, Saudi Arabia, P.O. Box 42604.
Tel: +96626926381
E-mail: sqari@kau.edu.sa
Foundation Project: Supported by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah [Grant No. (14/247/1433)].

Article history:
Received 28 Apr 2014
Received in revised form 8 May, 2nd revised form 24 May, 3rd revised form 5 Jun 2014
Accepted 10 Jun 2014
Available online 19 Jun 2014

the deleterious effects of the stress[4,5]. These proteins were first reported in *Drosophila melanogaster* during exposures to high temperature and so the term "heat shock proteins" (HSPs) was applied[6]. Subsequently, a range of other environmental stresses have been shown to induce HSPs, and the term "stress proteins" has also been used to describe these proteins[7]. There are many reports that evidence the expression of HSPs in response to environmental stresses[5,8-10]. Under natural and laboratory conditions, some of these studies have shown how HSPs levels vary over hours in response to thermal stress[11,12]. HSPs play an important role in maintaining cellular functions under environmental stress[13-18]. The stimulus for the production of HSPs of several different families, is suggested to be the stress induced appearance of denatured proteins with exposed hydrophobic residues. The HSPs bind to these exposed protein sites affording protection from further damage, but these HSPs also have a role in restoring function when the stress has abated[4,18]. However, in stressed cells, denatured proteins may be refolded and function restored, a function of the molecular chaperones, or they may be broken down by a cellular protease[19].

The CTMax, widely used as an index of organism heat tolerance, is proposed by Lutterschmidt *et al.* to be of ecological relevance[20], because it is the temperature at which an organism is unable to organize locomotor activity effectively, it would not be able to escape harmful conditions. Furthermore, Huey *et al.* found that the CTMax and the optimal temperature for an organism are co-adapted[21]. This implies that the CTMax is genetically correlated with performance and activity at intermediate temperatures.

The criterion used here for thermal tolerance is the temperature at which the righting reflex is lost after the crabs were turned onto their dorsal surface (loss of righting response). Recently, there are several studies on thermotolerance and heat shock in different crab species[22-28].

The climate of the Red Sea is the hottest and driest in the world and the rainfall is minimal, mostly in winter. Mean sea surface values range between 17.5 to 27 °C in February, whereas 32 °C in Augest and September. Temperature extremes occur mostly in inshore waters, high values of 36 to 38 °C have been recorded in the Southern Red Sea[29]. Eurythermal crustaceans survive the environmental temperature by developing several capacities and resistance adaptation to change their behavior, physiology, growth and metabolism[30]. There are many studies on *Portunus pelagicus* (*P. pelagicus*) in different subjects such as growth and survival, biological aspects, reproductive biology and biochemical constituents[31-34]. No previous studies of the heat shock effects on the CTMax of this species occur.

The aims of the study were to determine the effect of prior heat shock on the CTMax of differently acclimated *P. pelagicus* as well as the time course of the changes in the CTMax post heat shock. Comparison will be made with an earlier study on the role of acclimation in determining the CTMax, to assess the importance of the two strategies.

2. Materials and methods

2.1. Crabs

Individual *P. pelagicus* were obtained from Thule Corniche, Red Sea, Saudi Arabia. Experiments were carried out between July and October 2013. Crabs of similar size were chosen of carapace width 7-14 cm. Crabs were held in laboratory aquaria in tanks, which were supplied with filtered and aerated seawater. Crabs were fed twice a week with shrimp and the seawater was changed twice a week at least.

2.2. Determination of CTMax

Crabs were held for at least 3 d at seawater temperature prior to acclimation. Then they were acclimated at 20 °C for 3 weeks before their CTMax was determined. Four groups of three crabs were used. The first group was placed in seawater at 20 °C and was then slowly heated at a rate of 0.2 °C/min using a programmed heater. The righting reflex was tested by turning the crab onto its back by hand, and 5 min was allowed to right itself. The CTMax was recorded for each crab as the median temperature during the 5 min period during which a crab was not able to right itself. Then the crabs were transferred back into seawater at 20 °C to recover. This protocol was repeated with other groups. The average CTMax was calculated using the pooled data for all 12 crabs. The same procedure was used with crabs acclimated at 25 °C, 30 °C and 35 °C.

2.3. CTMax after heat shock

The effect of heat shock on the subsequent CTMax was measured. Three groups of three indvidual *P. pelagicus* were chosen that had not previously been heat shocked. They were heat shocked at temperature 1 °C lower than the CTMax value for 20 min, followed by either 0.5 h, 1 h or 1.5 h recovery at 20 °C. The same procedure was repeated at other acclimation temperatures which were 25 °C, 30 °C and 35°C. Crabs acclimated at 35°C were heat shocked at temperature 2 °C lower than the CTMax of control. This heat shock temperature was necessary because exposure 1 °C below the CTMax was damaging for some individuals, so the anomalous results were obtained.

Data were analyzed by using an independent student's

t-test and One-way ANOVA for significant differences. The level of significance used was $P<0.05$.

3. Results

Table 1 shows the CTMax of *P. pelagicus* acclimated at 20 °C, 25 °C, 30 °C or 35°C and of the heat shocked crabs. The CTMax pre-heat shock of 20 °C acclimated crabs was (39.00±0.12) °C, and post-heat shock CTMax was significantly increased to (40.18±0.25) °C after 30 min, (39.90±0.23) °C after 1 h and (39.93±0.05) °C after 1.5 h. In 25 °C acclimated crabs, the CTMax pre heat-shock was (40.07±0.33) °C and after heat shock, the CTMax values were between (42.2±0.21) °C after 30 min and (41.6±0.24) °C after 1 h. There was no significant different between control and post-heat shock after 1.5 h recovery ($P>0.05$). Acclimation at 30 °C pre-heat shock, the CTMax of *P. pelagicus* was (41.25±0.26) °C and after heat shock, the CTMax was (42.23 ±0.34) °C after 30 min. There was no significant difference between control and post-heat shock CTMax after 1 h and 1.5 h recovery. The CTMax pre-heat shock of *P. pelagicus* acclimated to 35 °C was (42.66±0.40) °C. The CTMax of heat shocked crabs was (43.08±0.25) °C after 30 min and (41.5± 0.76) °C after 1 h, but there was no significant difference between control and post-heat shock in both 0.5 h and 1 h at the different time courses of heat shock.

Table 1

Thermal tolerance of *P. pelagicus* acclimated at 20 °C, 25 °C, 30 °C and 35 °C and the effect of heat shock in different time courses.

Acclimation temperature (°C)	CTMax pre-heat shock (°C)	CTMax after 30 min heat shock (°C)	P	CTMax after 60 min heat shock (°C)	P	CTMax after 90 min heat shock (°C)	P
20	39.08±0.12	40.18±0.25	0.0008	39.90±0.23	0.006	39.93±0.05	0.001
25	40.07±0.33	42.20±0.25	0.0005	41.60±0.24	0.004	40.80±0.21	0.070
30	41.25±0.26	42.23±0.34	0.0400	42.03±0.24	0.050	40.75±0.14	0.300
35	42.66±0.40	43.08±0.25	0.2000	41.50±0.76	0.600	–	

Data are expressed as mean±SE. The heat shock degree is 1 °C less than the CTMax pre-heat shock at 20 °C, 25 °C, 30 °C, whereas 2 less than the CTMax pre-heat shock at 35 °C.

4. Discussion

The CTMax values for non-heat shocked crabs were consistent with those presented for *P. portunus* in a previous communication[35]. Increasing acclimation temperatures from 20-35 °C significantly increased the thermal tolerance, which agreed with many previous studies with decapod crustaceans[22,23,27,36,37]. However, the values calculated for the acclimation response ratio. Claussen indicated a moderate acclimation ability in this species[38]. The values obtained increased with rising acclimation from 0.198 (20-25 °C), 0.236 (25-30 °C), 0.282 (30-35 °C) which correspond with the low values obtained in the previous study[35]. It has been reported that the acclimation response ratio between 0.1-0.18 for the intertidal species *Carcinus maenas* (*C. maenas*) was much lower than those for the subtidal species *Cancer pagurus (C. pagurus)* which were between 0.41 and 0.56[22].

The results of the present study also showed the changes in CTMax at different times after heat shock for crabs acclimated to 20 °C, 25 °C, 30 °C and 35 °C. Heat shock caused significant rises in the CTMax (except for 35 °C acclimated crabs) after 30 min, however this increase was progressively reduced with longer recovery times at the acclimation temperature (60 min and 90 min). For 20 °C acclimated crabs the increased CTMax was still evident after 90 min, but for 25 °C and 30 °C crabs the response was over after 90 min. Heat shock of 35 °C crabs was problematical, a heat shock temperature of low CTMax gave anomalous results, where CTMax was lower than in pre-heat shock crabs, even using heat shock temperature 2 °C below the CTMax gave no increased thermotolerance. It must be concluded that the combination of a high heat shock temperature and the CTMax determinations were too damaging.

A number of recent studies on crabs have emphasized the relationship between habitat temperatures and life styles of different crab species and their thermotolerance. It has been reported that warm acclimation increased greater in subtidal species than in intertidal species[23]. A similar result was found for *C. maenas* and *C. pagurus* by Cuculescu[22]. Stillman *et al*. concluded that upper thermal limits of a species evolved in response to maximal habitat temperatures[23], and so may be close to habitat temperature maxima. Hopkin *et al*. have also reported that *C. maenas* may experience rock pool temperatures in summer close to the CTMax[24]. Madeira *et al*. reported intraspecific differences in thermal tolerance and in HSP expression in *Pachygrapsus marmoratus* that reflected different habitat usage[27].

Kelley *et al*. reported that thermal tolerance of *C. maenas* from southern populations were greater than those from northern habitats[26], that reflected the differences in habitat temperature, but the northern populations gave a greater response to a change in acclimation temperature. They also found that the southern populations expressed a higher level of HSPs than the northern populations.

What emerges is that crab species that are intertidal in distribution have relatively poor acclimation responses. However, they have a high inherent thermal tolerance and a robust heat shock response and are likely to have higher constitutive expression of HSPs than subtidal species. This adaptive strategy has value for crabs that experience daily short term fluctuations in temperature with the tidal cycle, and may also be exposed to near lethal temperatures. As Stillman *et al*. suggest those intertidal species are likely to be adversely affected by global warming that would affect their distribution[23].

Conflict of interest statement

I declare that I have no conflict of interest.

Acknowledgments

I would like to thank Prof. Bowler for his input on the manuscript. This research was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah [Grant No. (14/247/1433)]. The author, therefore, acknowledge with thanks DSR technical and Financial support.

Comments

Background

The climate of the Red Sea region is the hottest and driest in the world and the rainfall is minimal, mostly in winter. Temperature extremes occur in inshore waters, with high values of 36 to 38 °C recorded in the southern Red Sea. Eurythermal crustaceans survive in environmental temperature by developing several capacity and resistance adaptations to change their behavior, physiology, growth and metabolism. The present study is focused on the intertidal zone crabs *P. pelagicus* in the Red Sea that is characterized by marked diel temperature fluctuations.

Research frontiers

The aim of present research work was to determine the effect of prior heat shock on the CTMax of differently acclimated *P. pelagicus* as well as the time course of the changes in CTMax post heat shock.

Related reports

CTMax is widely used as an index of organism heat tolerance. Environmental stresses that threaten the functional integrity of an organism result in the expression of a set of genes the protein products (hsps) of which serve to protect the organism from the deleterious effects of the stress.

Temperature acclimation of Portunus pelargicus from 20 to 35 °C progressively increased CTMax. In *P. pelagicus* acclimated, at 20 °C the CTMax values after heat shock were significantly higher than in control crabs 30 min, 1h and 1.5 h, after heat shock. In 25 and 30 °C acclimated crabs, the CTMax values after heat shock were significantly higher than controls only 30 min. and 1h after heat shock. No significant differences in 35 °C acclimated crabs between control and heat shocked crabs were found after recovery for 30 min, 1 h, or 1.5 h.

Innovations and breakthroughs

No previous information exists on the thermal tolerance of a tropical crab species. The research also explored the relative importance of acclimation and heat shock as strategies to survive in a fluctuating high temperature environment.

Applications

P. pelagicus is a commercially important species in Saudi Arabia as well as in other tropical countries. The proposed climate change could present additional thermal stress to this and other tropical species, this research provides new information that enables evaluation of that issue.

Peer review

This research study acclimation and heat shock of the tropical crab species. The result conclude that heat shock caused significant rises in CTMax, however this increase was progressively reduced with longer recovery times at the acclimation temperature. This research provides new information that enables evaluation of that issue.

References

[1] Angilletta MJ Jr. *Thermal adaptation: a theoretical and empirical synthesis*. Oxford: Oxford University Press; 2009.

[2] Clarke A. Costs and consequences of evolutionary temperature adaptation. *Trends Ecol Evol* 2003; **18**(11): 573-581.

[3] Tomanek L, Zuzow MJ. The proteomic response of the mussel congeners *Mytilus galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits and metabolic costs of thermal stress. *J Exp Biol* 2010; **213**: 3559-3574.

[4] Parsell DL, Lindquist S. The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu Rev Genet* 1993; **27**: 437-496.

[5] Feder ME, Hoffman GE. Heat-shock proteins, molecular chaperones and the stress response. *Annu Rev of Physiol* 1999; **61**: 243-282.

[6] Ritossa F. A new puffing patterns induced by temperature shock and DNP in *Drosophila*. *Experienta* 1962; **18**(12): 571-573.

[7] Lewis S, Handy RD, Cordi B, Billinghurst Z, Depledge MH. Stress proteins (HSP′s): methods of detection and their use as an environmental biomarker. *Ecotoxicology* 1999; **8**: 351-368.

[8] Qari S. Adaptive responses to temperature and photoperiod in neuromuscular transmission in *Carcinus maenas* (L.) [dissertation]. Durham: University of Durham; 2004, p. 72.

[9] Lockwood BL, Sanders JG, Somero GN. Transcriptomic

responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. *J Exp Biol* 2010; **213**: 3548-3558.

[10] Schoville SD, Barreto FS, Moy GW, Wolff A, Burton RS. Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcription of the copepod *Tigriopus californicas*. *BMC Evol Biol* 2012; doi: 10.1186/1471-2148-12-170.

[11] Malev O, Srut M, Maguire I, Stambuk A, Ferrero EA, Lorenzon S, et al. Genotoxic, physiological and immunological effects caused by temperature increase, air exposure or low deprivation in freshwater crayfish *Astacus leptodactylus*. *Comp Biochem Physiol C Toxicol Pharmacol* 2010; **152**(4): 433-443.

[12] Tomanek L, Somero GN. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (Genus tegula) from different tidal heights. *Physiol Biochem Zool* 2002; **73**(2): 249-256.

[13] Hofmann G, Somero G. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussels *Mytilus trossulus*. *J Exp Biol* 1995; **198**: 1509-1518.

[14] Hofmann GE, Somero GN. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat-shock response and protein ubiquitination. *Mar Biol* 1996; **126**: 65-75.

[15] Sagarin RD, Somero GN. Complex patterns of expression of heat-shock protein 70 across the southern biogeographical ranges of the intertidal mussel *Mytilus californianus* and snail *Nucella ostrina*. *J Biogeogr* 2006; **33**: 622-630.

[16] Dutton JM, Hofmann GE. Biogeographic variation in *Mytilus galloprovincialis* heat shock gene expression across the eastern Pacific range. *J Exp Mar Biol Ecol* 2009; **376**(1): 37-42.

[17] Hammond LM, Hofmann GE. Thermal tolerance of *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced gene expression and biogeographic patterns. *Mar Biol* 2010; **157**: 2677-2687.

[18] Willmer P, Stone G, Johnston I. *Environmental physiology of animals*. London: Wiley-Blackwell; 2004.

[19] Wickner S, Mauizi MR, Gottesman S. Post translational quality control: folding, refolding and degrading proteins. *Science* 1999; **286**: 1888-1893.

[20] Lutterschmidt WI, Huchison VH. The critical thermal maximum: history and critique. *Can J Zool* 1997; **75**(10): 1561-1574.

[21] Huey RB, Kingsolver JG. Evolution to resistance to high temperatures in ectotherms. *Am Nat* 1993; **142**(Suppl): S21-S46.

[22] Cuculescu M, Hyde D, Bowler K. Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *J Therm Biol* 1998; **23**(2): 107-110.

[23] Stillman JH, Somero GN. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influence of latitude, vertical zonation, acclimation and phylogeny. *Physiol Biochem Zool* 2000; **73**(2): 200-208.

[24] Hopkin RS, Qari S, Bowler K, Hyde D, Cuculescu M. Seasonal thermal tolerance in marine crustaceans. *J Exp Mar Biol Ecol* 2006; **331**(1): 74-81.

[25] Metzger R, Sartoris FJ, Langenbuch M, Portner HO. Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. *J Therm Biol* 2007; **32**(3): 144-151.

[26] Kelley AL, de Rivera CE, Buckley BA. Intraspecific variation in thermotolerance and morphology of the invasive European crab *Carcinus maenas* on the west coast of North America. *J Exp Mar Biol Ecol* 2011; **409**: 70-78.

[27] Madeira D, Narcisco L, Cabral HN, Diniz MS, Vinagre C. Thermal tolerance of the crab *Pachygrapsus marmoratus* intraspecific differences at a physiological (CTMax) and molecular level (Hsp70). *Cell Stress Chaperones* 2012; **17**(6): 707-716.

[28] Hyde D, Qari S, Hopkin RS, Bowler K. Thermal acclimation, heat shock and photoperiod: do these factors interplay in the adaptive responses of crab neuromuscular systems to temperature? *J Therm Biol* 2012; **37**(3): 164-170.

[29] McClanahan TR, Sheppard CR, Obura DO. *Coral reefs of the Indian Ocean: their ecology and consevation*. Oxford: Oxford University Press; 2000.

[30] Tomanek L. 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* 2010; **213**: 971-979.

[31] Ikhwanuddin Mhd, Azra MN, Sung YY, Bolong AA, Long SM. Growth and survival of blue swimming crab (*Portunus pelagicus*) reared on frozen and artificial foods. *Agric Sci* 2013; **4**(6A): 76-82.

[32] Abdul-Sahib IM. Some biological aspects of the swimming crabs *Portunus pelagicus* (Linnaeus, 1766) (Decapoda: *Portunidae*) in NW Arabian Gulf. *Mesopot J Mar Sci* 2012; **27**(2): 78-87.

[33] Kamrani E, Sabili AN, Yahyavi M. Stock assessment and reproductive biology of the blue swimming crab, *Portunus pelagicus* in Bandar Abbas coastal waters, Northern Persian Gulf. *J Persian Gulf* 2010; **1**(2): 11-22.

[34] Sugumar V, Vasu P. Effect of temperature on the biochemical constituents of the blue swimmer crab *Portunus pelagicus*. *World Appl Sci J* 2013; **28**(3): 382-391.

[35] Qari S, Aljarari R. The effect of season and acclimation on the heat and cold tolerance of the Red Sea crab, *Portunus pelagricus*. *Life Sci J* 2014; **11**(4): 145-148.

[36] Kumlu M, Turkmen, Kumlu M. Thermal tolerance of *Litopenaeus vannamei* (Crustacean: Penaeidae) acclimated to four temperatures. *J Therm Biol* 2010; **35**(6): 305-308.

[37] Paschke K, Cumillaf ME, Diaz E, Fiebauer P, Rosas C. Relationship between age and thermoregulatory behavior of *Lithodes santolla* (Molina, 1782) (Decapoda, Lithododae) juveniles. *J Exp Mar Biol Ecol* 2013; **448**: 141-145.

[38] Claussen DL. Thermal acclimation in ambystomatic salamanders. *Comp Biochem Physiol* 1977; **58**(4): 333-340.