The Effect of Season and Acclimation on the Heat and Cold Tolerance of the Red Sea Crab, *Portunus pelagicus.*

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Abstract: Without further acclimation the CTMax of summer caught crabs was 42.7°C significantly higher than 41.5°C for winter crabs. Acclimation from 20 to 30°C increased CTMax significantly from 40.09 to 42.32°C. Acclimation from 30° to 20°C progressively and significantly lowered CTMin from 15.3 to 11.57°C. Acclimation response ratios for 20-25, and 25-30°C for CTMax were 0.136 and 0.31 respectively, whereas for CTMin the values were 0.49 and 0.256. This suggests a greater capacity for low temperature acclimation in this species.
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1. Introduction

Temperature is an important environment parameter which plays a role in limiting the distribution of ectotherms and is an important determinant of their activity. This is evidenced by their ability to make several types of response to predicted or actual temperature changes, they can adjust their physiology and biochemistry in an adaptive fashion **(Somero, 1997)** responses that are suggested to contribute to increased fitness **(Bowler, 2005)** but they can also behaviorally avoid potentially harmful temperatures **(Dillon et al., 2012).** These responses contribute to the suite of changes that will be necessary for ectotherms to develop the capacity to deal with climate change **(Stillman, 2003).**

Blue crabs *Portunus pelagicus* are a tropical species and are widely distributed from East Africa to Tahiti, Japan, Australia, Philippines, and the Mediterranean **(Stephenson, 1972).** They are an important commercial species throughout the entire Red Sea region and have a high commercial value for human consumption **(Johnson et al., 2011).** It is known that the intertidal zone of the Red Sea is characterized by marked diel temperature fluctuations **(Morley, 1975; Eshky, 1985);** in the north Red Sea summer temperatures are 20-26°C whereas in the south they can be 25-31°C.

Determinations of CTMax as an index of organism heat tolerance were developed many years ago **(Cowles & Bogert, 1944; Hutchison, 1961; Brattstrom, 1968)** and CTMax was also argued to be of ecological relevance. **Lutterschmidt & Hutchison, (1997)** in a review of the field reasoned that as CTMax is the temperature at which an organism is unable effectively to organize locomotor activity, it would not be able to escape harmful conditions.

In the present work the criterion used for the thermal tolerance (CTMax) is the temperature at which the righting reflex is lost after the crabs were turned onto their dorsal surface (loss of righting response or LRR). In this study we also extend this criterion to cold tolerance in the determination of CTMin. There are many studies of the heat tolerance of crabs and other decapod crustaceans from early studies on crayfish **(Bowler, 1963; Bowler et al., 1973; White, 1983; Layne *et al*., 1987)** and lobster **(McLeese, 1956),** however, more recent studies primarily concern crabs **(Taylor & Wheatly, 1979; Cuculescu et al., 1998; Stillman & Somero, 2000; Hopkin et al., 2006; Metzger et al., 2007; Kelley, et al., 2011)** although many have been concerned with studies on heat shock responses, that are not a subject of this work, In contrast, there are few studies that deal with determination of CTMin in crabs. Most studies of cold tolerance in crabs have focused on the physiological mechanisms that are limiting for crabs in the cold (**Frederich, et al., 2000; Metzger, et al., 2007; Wittmann et al., 2012).** However, juvenile and larval stages of *P. pelagicus* are reported to be vulnerable at temperatures below about 17°C (**Bryars & Havenhand, 2006; Yan, et al., 2008; Johnson et al., 2011)** which has consequences for the management of crab fisheries.

The aims of the study are to determine the effect of acclimation on the CTMax and CTMin of adult *P.peliagcus* from the Red Sea and so the acclimation temperatures chosen reflect the prevailing sea temperatures. Further to report the relationship between season and CTMax and CTMin. It is emphasized that no earlier studies of the thermal tolerance of this tropical species occur.

2. Materials & Methods

2.1 Crabs

Individual *Portunus pelagicus* (L.) were obtained from Thule Corniche, Red Sea, Saudi Arabia. For acclimatization, experiments were carried out in winter and summer seasons between December 2010 - January 2011 and June – July 2011. The crabs for 20°C, 25°C and 30°C acclimation were caught in April to July 2011. The photoperiods in winter and summer were approximately 11-h light/13-h dark and 13-h light/11-h dark, respectively. The sea temperatures were between 25-28°C and 30-37°C in winter and summer respectively. Crabs of similar size were chosen of carapace width 9-15 cm. Crabs were held in laboratory aquaria in tanks, which were supplied with filtered and aerated seawater. Crabs were fed between twice or three times a week on squid or shrimp and the seawater was changed twice a week at least.

2.2 Acclimation and tolerance determination.

On arrival crabs were held for at least three days at sea water temperature prior to acclimation. Crabs were then acclimated at 20°C, 25°C or 30°C for 3 weeks before CTMax and CTMin measurements were made. To determine CTMax the crabs were placed into aerated sea water at their acclimation temperature and the water was then heated at a constant rate of 0.2°C min^{-1}. CTMax was the temperature at which a crab was unable to right within 5mins, after being turned onto its back. In some cases CTMax was also determined immediately after capture to establish its dependence on season. This procedure was repeated by cooling the sea water at a constant rate of 0.2°C min^{-1} to determine CTMin, again the temperature at which crabs could not right themselves after being turned onto their backs within a 5 min. period.

Data were analysed, using an independent Student t-test and one way ANOVA for significant differences. The level of significance used was $P<0.05$.

3. Results.

This study reports the first data for heat and cold tolerance of adult tropical crab *Portunus pelagicus* from the Red Sea. Table 1 shows the CTMax and CTMin of summer and winter caught crabs without further acclimation. Summer crabs had a significantly higher CTMax of 42.7±0.09°C as compared with that of winter crabs, 41.4±0.01°C ($p<0.001$). Also summer crabs had a significantly higher CTMin of 17.7±0.13°C as compared with that of winter crabs, 11.8±0.23°C ($p<0.001$).

Table 2 shows the CTMax and CTMin of crabs after laboratory acclimation. Increasing acclimation temperature caused an increase in CTMax from 40.09±0.13°C at 20°C to 42.32±0.3°C at 30°C ($p<0.01$) however there was no significant increase in CTMax between 20 and 25°C acclimation, but both were significantly lower than CTMax at 30°C. Table 2 also shows the effect of acclimation temperature on CTMin of Portunus. Here falling acclimation temperature caused a significant lowering in CTMin from 15.3±0.26°C at 30°C to only 11.57±0.26°C at 20°C ($p<0.001$). Acclimation at 25°C also significantly increased CTMin (to 14.02±0.4°C) over that at 20°C ($p<0.05$), but it was significantly lower than the value determined after acclimation at 30°C.

Claussen, (1977) introduced the concept of the acclimation response ratio (ARR =ΔCTMax/Δacclimation temperature), to provide an index of the effectiveness of acclimation on CTMax. Table 3 shows the ARR values for CTMax of the crabs, it also extends to values of ARR for CTMin. Acclimation at 20° or 25°C, resulted in a low ARR value for CTMax that indicates, the ability for warm acclimation is limited at these temperatures, however, warm acclimation at 30°C is relatively strong. In contrast, the ARR value of 0.49 for CTMin between 20-25°C suggests a strong effect of cold acclimation, which lessens with acclimation at 30°C. If it is assumed that in winter the crabs experienced a temperature of about 20°C, and in summer a temperature of close to 30°C then the AZRR (Acclimatization Response Ratio) can be estimated to show the seasonal effect (see Table 1). From these data the AZRR derived is 0.24 which is in close agreement with the value of 0.223 from the acclimation data in Table 3.

Table 1. The seasonal dependence of CTMax and CTMin of the crab *Portunus pelagicus* from the Red Sea. (*p*<0.001)

Season	n	CTMax°C ±SEM	n	CTMin°C ±SEM
winter	14	41.4±0.01	8	11.8±0.23
summer	13	42.7±0.09	8	17.7±0.13

Table 2. The effect of acclimation at 20°, 25° and 30°C on CTMax and CTmin of the crab *Portunus pelagicus* from the Red Sea. (Means with the same letter are not significantly different. Means with different letters are significantly different, see text).

Acclimation Temperature °C	n	CTMax°C ±SEM	n	CTMin°C ±SEM
20	9	40.09±0.13[a]	9	11.57±0.26[a]
25	9	40.77±0.36[a]	9	14.02±0.4[b]
30	9	42.32±0.3[b]	9	15.3±0.26[c]

Table 3. The Acclimation Response Ratio (ARR) for the values of CTMax and CTMin derived from Table 2.

ΔAT	CTMax ARR	CTMin ARR
20-25°C	0.136	0.49
25-30°C	0.31	0.256
20-30°C	0.223	0.373

4. Discussion.

Portunus pelagicus largely inhabits intertidal zones but no previous data on its thermal tolerance are available. In the Red Sea the intertidal zone is subject to both seasonal and diel temperature variation. Table 1 shows the seasonal in variation CTMax it being higher in summer caught crabs. It is likely that this difference simply reflects the seasonal changes in sea temperature because of the correspondence with the acclimation data in Table 2. It is unlikely that changes in photoperiod would interact with temperature as the summer/winter photoperiods were little different.

Hopkin et al., (2006) found that the seasonal CTMax of eight species of adult marine crustaceans from temperate latitudes were higher in summer captured animals than in winter captured animals. **Cuculescu et al., (1998)** reported similar results for *Cancer pagurus* and *Carcinus maenas*, where the CTMax of winter caught crabs was significantly lower than that of summer or autumn caught animals.

Determinations of CTMax are considered to be ecologically relevant estimations of heat tolerance **(Huey & Kingsolver, 1993). Korhonen & Lagerspetz (1996)** have suggested that CTMax reflects disruption to that region of the CNS the controls the righting reflex. However, the loss of the righting reflex is reversible provided the animals are not maintained for a long period above CTMax. It is significant that, **Stillman & Somero (2000)** and **Somero (2005)** report that LT_{50} of porcelain crabs correlated with maximal habitat temperatures. The CTMax values obtained for *P. pelagicus* were in excess of 40°C and were about 10°C above summer sea temperatures, but intertidal pool temperatures may rise close to CTMax.

The effect of warm acclimation on CTMax has been the focus of many studies on marine crustaceans. Generally, warm acclimation increased CTMax values over those for cold acclimated animals. (e.g. *Cancer pagurus* & *Carcinus maenas*, **Cuculescu et al., 1998**; *Macrobrachium acanthurus*, **Diaz et al., 2002;** *Hemigrapsus nudus*, **McGaw, 2003;** *Litopenaeus vannamei*, **Kumlu et al., 2010). Kelley et al., (2011)** report that invasive *C. maenas* at the southern end of their distribution on the west coast of America had higher CTMax (34.7°C) than did more northerly populations (31.7°C) which corresponded with differences in sea temperature (11-23°C southern crabs and 6-14°C northern crabs), values that are similar to those for *C. maenas* from the North Sea **(Cuclescu et al., 1998). Kelley et al., (2011)** also determined CTMax for both populations after acclimation at 6 or 23°C. The ARR values that can be derived are 0.22 for the northern crabs but only 0.088 for the southern population, values close to those reported by Cuculescu et al., (1998). The ARR values obtained for *P. pelagicus* CTMax were between 0.136 and 0.31 and were similar to those reported for intertidal temperate crab *C. maenas* **(Cuculescu et al., 1998).** The estimated value for AZRR of 0.24 also points to relatively limited acclimation ability for heat resistance in *P. pelagicus*, which in common with *C. maenas* has a value for CTMax comfortably in excess of summer sea temperatures. This asks the question, do these species trade-off warm acclimation and their eurythermy?

It would be reasonable to suggest that CTMin is also an ecologically relevant estimation of cold tolerance. However there is a paucity of information on the cold tolerance of crabs, with most recent studies focusing on the underlying causes of cold mortality **(Frederich et al., 2000; Rome et al., 2005; Wittmann et al., 2012; Ronges et al., 2012)**. CTMin for *P. pelagicus* was also dependent on acclimation temperature and ranged between 11.57±0.26°C for 20°C acclimated crabs to 15.3±0.26°C for 30°C acclimated crabs. No similar data for other crab species exists so no comparisons can be made. The estimated ARR values are given in Table 3 and vary between 0.256 and 0.49, values higher than those for CTMax. This suggests a greater capacity for cold acclimation to extend cold resistance than for warm acclimation to extend heat resistance.

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