Thermal tolerance of the zoea I stage of four Neotropical crab species (Crustacea: Decapoda)

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ABSTRACT

Although larval stages are often considered particularly vulnerable to stressors, for many marine invertebrates studies of thermal tolerance have focused exclusively on adults. Here we determined the upper thermal limit (LT_{50}) of the zoea I of four Caribbean crab species (*Macrocoeloma trispinosum, Aratus pisonii, Armases ricordi,* and *Minuca rapax*) and compared their thermal tolerance over time and among species. The zoea from the subtidal species *M. trispinosum* and semiterrestrial species *A. pisonii* had a lower thermal tolerance, 35°C and 38.5°C respectively, than did the more terrestrial *A. ricordi* and *M. rapax*. In all four species tested the estimates of thermal tolerance depend on the duration of exposure to elevated temperatures. Longer exposures to thermal stress produce lower estimates of LT_{50}, which decreased by ~1°C from a two- to a six-hour exposure. Crab embryos develop on the abdomen of the mother until the larvae are ready to hatch. Therefore the thermal tolerances of the embryos which need to coincide with the environmental conditions experienced by the adult stage, may carry over into the early zoea stage. Our results suggest that terrestrial species, in which embryos may need to withstand higher temperatures than embryos of subtidal species also produce larvae with higher thermal tolerances. Over the short term, the larvae of these tropical crab species can withstand significantly higher temperatures than those experienced in their marine habitat. Longer term rearing studies are necessary to determine the temperature at which chronic exposure has a negative impact on embryonic and larval survival.

Key words: Larvae, survival, temperature, exposure time, Caribbean

Environmental temperature influences the physiology and ecology of marine organisms across all the stages of their complex life cycles (Storch et al 2009, 2011; Byrne et al. 2010, Hammond & Hofmann 2010; Byrne 2012). It is well-known that temperatures slightly above the optimal can result in negative impacts, including increased ventilation rate and cardiac activity, and can provoke insufficient O_2 supply (Frederich & Pörtner 2000; Harley et al. 2006; Metzger et al 2007). In many cases, this results in reduced performance and survival at temperatures only slightly above those commonly experienced in the field (e.g., Collin & Chan 2016). Available information on tropical marine species suggest that although they are generally more tolerant of heat than are temperate species, adults may live closer to their upper thermal limits, reducing their safety factors and making them especially susceptible to increases environmental temperatures (Somero 2010; Nguyen et al 2011; Madeira et al. 2012). For crustaceans, there is little information on the temperature tolerance and safety factors of tropical species, since most studies have focused on temperate species (e.g. Anger et al. 2003; Storch et al 2009, 2011; Weiss et al. 2009; Fowler et al. 2010; Schmalenbach & Heinz-Dieter 2010; Franke Schiffer et al. 2014; Tepolt & Somero 2014), and few have focused on determining their upper thermal limit (e.g. Ravaux et al. 2016).

For marine invertebrates most studies of temperature tolerance have focused on adult stages (Stillman & Somero 2000; Gilman 2006). However, early life stages may be particularly vulnerable to environmental stressors such as temperature (Anger et al. 2003; Storch et al. 2009; Hammond & Hofmann 2010; Zippay & Hofmann 2010), since oxygen consumption and damage at a molecular level can increase as temperature increase (Carreja et al. 2016; Ravaux et al. 2016). Studies of larval thermal tolerance can provide vital information to promote understanding of the potential impacts of thermal stress on survival, dispersal, and recruitment of marine species (Gilman, 2006; Sanford et al. 2006; Storch et al. 2009).

Considering the limited knowledge on the larval thermal tolerance of tropical crustaceans species, here we determined the upper thermal limit (UTL) of the zoea I stage of four Neotropical crab species, as the first step to determine their vulnerability to environmental warming. Ovigerous females carrying eggs close to hatching as evidenced by embryos with well-developed clearly visible eyes were collected by hand from around the Smithsonian Tropical Research Institute's Bocas del Toro Research Station (09°20'N, 82°14'W), on the Caribbean coast of Panama. Female decorator crabs, *Macrocoeloma trispinosum* (Latreille, 1825), which were

covered with the red-orange sponge *Lissodendoryx colombiensis* (Zea & van Soest, 1986), were collected underwater on *Rhizophora mangle* roots. The sesarmid crab *Aratus pisonii* (H. Milne-Edwards, 1853) was found on branches and roots of *R. mangle*, while female *Armases ricordi* (H. Milne-Edwards, 1853) were found among rocks and leaf litter. Female fiddler crabs *Minuca rapax* (Smith, 1870) were collected between rocks and on sand flats near the mangroves. Ovigerous females (see Table 1) were placed individually in plastic containers with 1 liter of seawater with a salinity of 36 ‰, at an ambient temperature of approximately 28-30 °C. Containers were checked twice per day for hatching larvae. Thermal tolerance assays were conducted on mornings that larvae (zoea I) hatched.

The thermal tolerance of actively swimming larvae was tested using a thermal gradient generated by a heated metal block. The heatblock is a custom-made aluminum block with 4 rows divided by 10 columns of evenly spaced holes that snuggly fit 15-mL scintillation vials (Collin and Chan 2016). Each vial was filled with 15 ml of filtered seawater and contained 15 larvae from a single female. Broods from each female were tested separately, as significant differences between females could reflect genetic differences between the half-sib families of larvae or environmental maternal effects, which could be important for understading the potential evolutionary responses to environmental warming.

The temperature gradient ranged from 34 to 44 °C for *A. pisonii*, *A. ricordi*, and *U. rapax* and from 28 to 40 °C for *M. trispinosum*. The temperature inside the vials was recorded with an Omega High Accuracy Digital Thermometer. For each female, one row of ten vials was kept in the heat block for 2h, another row was kept for 4h and another for 6h. After the exposure each larva was scored as alive or dead. Data were analyzed with the statistical software SPSS v.20.0. The effect of temperature on survival was tested using logistic regressions with the binary response as alive/dead after the exposure. The lethal temperature (LT_{50}) was estimated as the temperature at which 50% of the larvae died. To determine if the species differ we used logistic regression to compare the 2h tolerances with species and temperature as factors. Additionally, for each species individually, we determine if there was an interaction between female and temperature for survival at 2 hours., to understand the magnitude of variation among broods. With the exception of *M. trispinosum*, we also used logistic regression to determine if thermal tolerance changed between the 2 and 6h of exposure for each of the species, with temperature and time of exposure as factors.

Thermal tolerance at 2h (Fig. 1a, Table 1) differed significantly among the four species. Logistic regression showed a significant effect of temperature, species, and an interaction between temperature and species on survival of a 2h exposure (Table 2). The two-tailed 95% confidence intervals of the LT_{50} values did not overlap for any of the 4 species. Larvae of *M. trispinosum* had lowest temperature tolerance, with LT_{50} around 35 °C and complete mortality at 37 °C. LT_{50} of the mangrove tree crab *A. pisonii* was around 38.5 °C, with complete mortality at 40 °C. The larvae of *A. ricordi* and *M. rapax* had LT_{50} at 39.9 and 40.7 °C, respectively, and complete mortality by 42°C. Separate analyses for each species, showed a significant interaction between female and temperature in all of the species (Table 2).

Regarding the effect of the duration of exposures on larval survival, in the 3 species for which we have 2h, 4h and 6h exposures, longer exposures generated lower estimates of LT_{50} (Fig 1b-d, Table 1). For each species, logistic regressions showed a significant effect of exposure time, temperature, and an interaction between temperature and duration of exposure (Table 3). The rank order of the LT_{50} among species did not change, and the LT_{50} of a 6h exposure was approximately 1°C lower than a 2h exposure for each species.

Unlike other marine invertebrates in which fertilization and development of early life stages occur in the water column, crabs carry their embryos on their abdomen until the larvae are ready to hatch. This means that embryos must tolerate the environmental conditions of the mother's habitat. Our data show a trend in thermal tolerance with adult habitat, despite the fact that larval habitat is likely similar. Zoea from the subtidal species (*M. trispinosum*) has the lower thermal tolerance, while those from the sand fiddler species, *M. rapax,* have the highest UTL. Our taxon sampling is too sparse to determine to what extent phylogenetic relationships determine thermal tolerances. However, a study on paleomonid shrimps (Ravaux et al 2016), suggest that the ability for acclimation of the upper thermal limit was not determined by the phylogenetic affiliation in that group, but to be related to their thermal habitats.

Local thermal gradients can be caused by fine-scale variation in conditions such as altitude or solar exposure (Stillman and Somero 2000). Adults of *M. trispinosum* are found on shallow waters to 60 m (Lemaitre 1981; Keith 1985) at habitat temperatures are controlled primarily by water temperature. *M. rapax* is a highly active, semiterrestrial species found frequently on sand flats near mangroves. It is likely, therefore to experience higher solar radiation levels, and therefore, higher temperatures than the other species. Although this species can thermoregulate

by retreating into a burrow when body temperature approaches lethal temperatures (Smith and Miller 1973; Thurman 1998). Also, the adults of *M. rapax* have a high resistance to water loss, preventing dissecation (Smith and Miller 1973; Thurman 1998). Compared to the other species, the larvae of *A. pisonii,* a semiterrestrial crab, showed intermediate UTL values. Adults of *A. pisonii* are constantly in air, however, this species is exposed to lower solar radiation and cooler microhabitat due to the shade from the mangrove canopy. It may also face less desiccation stress due to frequent trips to the water surface to rehydrate (Wolcott and Wolcott 2001; personal observations). Therefore, it is likey that these species encounter different maximal temperatures in their natural environment.

Environmental monitoring in Bocas del Toro has provided data on both water temperature and air temperature near the site of our study (Kaufmann and Thompson 2005; Collin et al. 2009; Collin and Chan 2016). Data measured less than 200 meters from our study site (http://biogeodb.stri.si.edu/physical_monitoring/research/bocas) shows air temperatures from May 2002 until June 2016 well below the thermal tolerance of the zoea. Mean air temperature over this period was 26.3°C, 95% of the 15 minute temperature averages did not exceed 29.6°C and the maximum temperature recorded during this period was 34.4 °C. In contrast the mean water temperature during the same period was 28.8°C, 95% of the observations did not exceed 30.4°C and the maximum during this period was 31.7°C.

This shows that water temperature in the shallow-water habitats relevant to the early developmental stages of these species is generally warmer than the air temperature. However, extreme air temperatures are higher in terrestrial habitats, and terrestrial microhabitats receiving direct solar radiation may significantly exceed reported air temperatures. The 2h assays suggest that due to the 3-7°C difference between the 2h UTLs and the maximum recorded water temperatures, the early zoea stages of these species are unlikely to ever encounter lethal temperatures. However, we also demonstrated that the duration of exposure impacts the estimates of UTL, with the LT_{50} decreasing similarly in the 3 species tested, indicating that long-term thermal stress may have negative impacts at lower temperatures.

Parental thermal history of marine organism can also influence the temperature tolerance of the offspring (Fujisawa 1995; Bingham et al. 1997; Zippay & Hofmann 2010). Maternal effects can be considered as a shared phenotype that influence simultaneously both maternal and offspring fitness (Marshall & Uller 2007). Therefore, the significant effect of mother on the

thermal thresholds exhibited by the zoea I of these species could result from either genetic differences or plastic responses to the microhabitat conditions of the mother or both. Either way, such variation between individuals is fundamentally important for a species ability to respond to environmental change.

In conclusion, we found that the larvae of these 4 species experience abrupt reduction in survival around the UTL and that zoea from the subtidal species had lower UTLs than did those from the semiterrestrial species. All of these UTLs were significantly higher than both air and ocean temperatures experienced in Bocas del Toro. UTLs differ significantly among females suggesting that acclimation capacity or genetic variation may allow long-term responses to thermal tolerance. Since there is a decrease in the LT_{50} as exposure time increases, longer term rearing studies are necessary to determine the temperature at which chronic exposure to thermal stress has a negative impact on larval growth and survival.
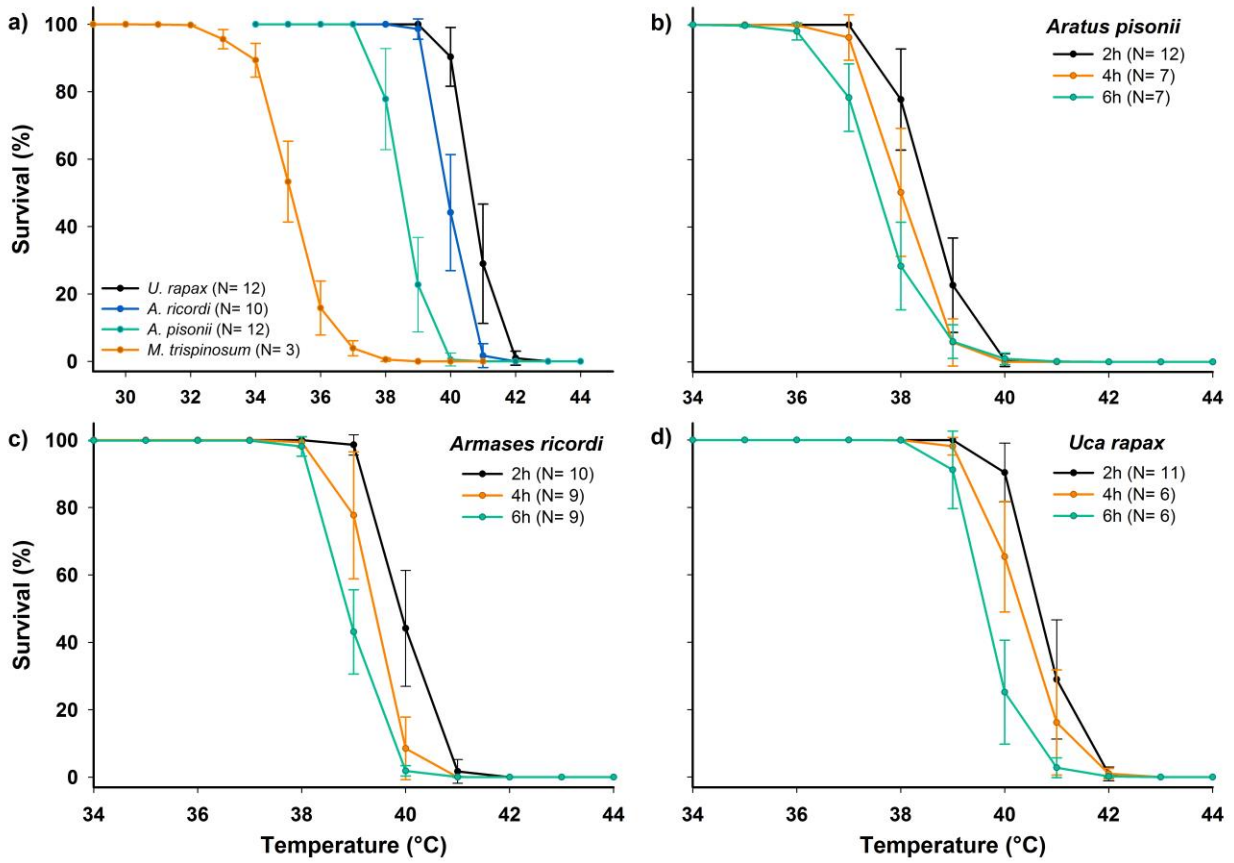
Figure 1. Zoea I thermal tolerance of *Macrocoeloma trispinosum, Aratus pisonii, Armases ricordi,* and *Minuca rapax*: a) Comparison of larval survival after 2h, b-d) Comparison of larval survival within species at 2, 4 and 6h.

Table 1. The overall LT_{50} temperature at which 50% of the zoea I died for the four crab species studied. Mean ± Standard deviation; N= number of females.

Species	LT$_{50}$: Temperature (°C)					
	2 h	N	4 h	N	6 h	N
Macrocoeloma trispinosum	35.3 ± 0.1 (35.2 - 35.3)	3	-	-	-	-
Aratus pisonii	38.5 ± 0.3 (38.2 - 38.9)	12	38.0 ± 0.3 (37.6 - 38.4)	7	37.6 ± 0.3 (37.1 - 37.8)	7
Armases ricordi	39.9 ± 0.3 (39.5 - 40.3)	10	39.4 ± 0.3 (38.9 - 39.8)	9	38.9 ± 0.1 (38.7 - 39.1)	9
Minuca rapax	40.7 ± 0.2 (40.3 - 41.0)	11	40.3 ± 0.5 (39.7 - 40.8)	6	39.7 ± 0.2 (39.2 - 39.9)	6

Table 2. Logistic regression of 1) the larval survival after a 2h exposure for the four crab species, and 2) the interaction between female and temperature for each species individually.

Source	DF	Chi Square	P
1) Species	3	135.59	<0.001
Temperature	1	2835.92	<0.001
Species x Temperature	3	278.43	<0.001
2) Female x Temperature			
Macrocoeloma trispinosum	2	10.21	0.006
Aratus pisonii	11	131.45	<0.001
Armases ricordi	9	64.96	<0.001
Minuca rapax	10	55.76	<0.001

Table 3. Logistic regression of larval survival over time (between 2 and 6h) for each species.

Species	Source	DF	Chi Square	P
Aratus pisonii	Exposure time	1	18.91	<0.001
	Temperature	1	1457.82	<0.001
	Exposure time x Temperature	1	104.73	<0.001
Armases ricordi	Exposure time	1	41.10	<0.001
	Temperature	1	1837.86	<0.001
	Exposure time x Temperature	1	140.69	<0.001
Minuca rapax	Exposure time	1	22.30	<0.001
	Temperature	1	1837.35	<0.001
	Exposure time x Temperature	1	158.56	<0.001

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