Journal of Thermal Biology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Paralysis and heart failure precede ion balance disruption in heat-stressed European green crabs

Lisa B. Jørgensen *, Johannes Overgaard, Heath A. MacMillan [1]

Zoophysiology, Department of Bioscience, Aarhus University, C.F. Møllers Allé 3, Building 1131, 8000 Aarhus C, Denmark

ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form
9 July 2016
Accepted 1 August 2016

Keywords:
Ion balance
Sodium
Potassium
Heat stress
Crustacean

ABSTRACT

Acute exposure of ectotherms to critically high temperatures causes injury and death, and this mortality has been associated with a number of physiological perturbations including impaired oxygen transport, loss of ion and water homeostasis, and neuronal failure. It is difficult to discern which of these factors, if any, is the proximate cause of heat injury because, for example, loss of ion homeostasis can impair neuromuscular function (including cardiac function), and conversely impaired oxygen transport reduces ATP supply and can thus reduce ion transport capacity. In this study we investigated if heat stress causes a loss of ion homeostasis in marine crabs and examined if such loss is related to heart failure. We held crabs (*Carcinus maenas*) at temperatures just below their critical thermal maximum and measured extracellular (hemolymph) and intracellular (muscle) ion concentrations over time. Analysis of Arrhenius plots for heart rates during heating ramps revealed a breakpoint temperature below which heart rate increased with temperature, and above which heart rate declined until complete cardiac failure. As hypothesised, heat stress reduced the Nernst equilibrium potentials of both K^+ and Na^+, likely causing a depolarization of the membrane potential. To examine whether this loss of ion balance was likely to cause disruption of neuromuscular function, we exposed crabs to the same temperatures, but this time measured ion concentrations at the individual-specific times of complete paralysis (from which the crabs never recovered), and at the time of cardiac failure. Loss of ion balance was observed only after both paralysis and complete heart failure had occurred; indicating that the loss of neuromuscular function is not caused by a loss of ion homeostasis. Instead we suggest that the observed loss of ion balance may be linked to tissue damage related to heat death.

1. Introduction

Ambient temperature has strong effects on the physiology and behavior of ectotherms, whose body temperature approximates that of the environment (Cossins and Bowler, 1987). The geographic distributions of ectotherms, including crustaceans, have repeatedly been associated with the organism's ability to tolerate thermal extremes (Araújo et al., 2013; Stillman and Somero, 2000; Sunday et al., 2012). Temperature fluctuations in marine and terrestrial ecosystems caused by rapid climate changes can thus alter the biogeographical distribution of many ectothermic animal species (Chen et al., 2011; Poloczanska et al., 2013; Walther et al., 2002). Animal thermal performance is an emergent property of temperature effects on biochemical functions integrating through multiple levels of biological organization and across multiple organ systems (Schulte, 2015). The critical thermal maximum and minimum (CT_{max} and CT_{min}) are useful indices of an ectotherm's ability to tolerate temperature extremes, and previous studies have used the endpoints as the outer measures of thermal tolerance since animals often suffer acute mortality outside these boundaries (e.g., Somero, 2002). The critical thermal limits are often measured with different endpoints including the onset of muscle spasms, loss of righting response, paralysis, heart rate inflection point (in an Arrhenius plot), and heart failure (Lutterschmidt and Hutchison, 1997; Tepolt and Somero, 2014), which makes it difficult to compare experimental data (assuming different endpoints). Such differences in methodology have sparked a vivid discussion on the mechanisms determining thermal tolerance (Rezende et al., 2014; Terblanche et al., 2007; Verberk et al., 2016) and in relation to this discussion it is critical to understand what physiological disturbances are responsible for setting each index of thermal tolerance. In this study we examined the physiological changes that occurred in heat stressed moribund crabs

* Corresponding author.
E-mail address: lisa.b.joergensen@gmail.com (L.B. Jørgensen).
[1] Present address: Department of Biology, York University, 4700 Keele St., Toronto, ON, Canada M3J 1P3.

http://dx.doi.org/10.1016/j.jtherbio.2016.08.001
0306-4565/

immediately before and during acute heat mortality (around CT_{max}).

For marine crustaceans, it is not clear what causes the onset of heat coma or paralysis (Verberk et al., 2016). A popular suggestion is that CT_{max} is related to availability of ATP, and it has therefore been proposed that lack of available oxygen ultimately causes a loss of physiological function during heat stress (the oxygen and capacity-limitation on thermal tolerance (OCLTT) hypothesis) (Frederich and Pörtner, 2000; Pörtner, 2001). The consequent reliance on anaerobic metabolism will lead to a toxic rise in anaerobic by-products (e.g. lactate) and insufficient ATP supply to ion motive pumps ultimately causing loss of homeostatic control, dissipation of ion gradients, and eventually organ failure (e.g. heart failure) (Frederich and Pörtner, 2000; Pörtner, 2002; Pörtner et al., 1999). Consequently, heart failure has been proposed as a proximate cause of heat death in some crustacean species, and hence the onset of cardiac failure has been used as a simple measure of CT_{max} (e.g. in the crab *Petrolisthes cinctipes*) (Somero, 2010, 2002; Stillman and Somero, 1996).

As an alternative to oxygen limitation it is possible that the upper thermal limit is related to membrane fluidity, protein denaturation, and/or loss of neuronal function. Chronic heat stress has been shown to rapidly and markedly increase extracellular $[K^+]$ in freshwater crayfish *Austropotamobius pallipes* (Bowler, 1963), and neuronal failure at high temperatures could be a consequence of perturbed ion balance, which would depolarize and silence neuromuscular signal transmission (Taylor, 1982). However, insufficient ATP production, protein denaturation, or breakdown of cell and/or epithelial permeability could also cause cell damage and release of $[K^+]$ into the extracellular space (Bowler et al., 1973). Since the result of heat stress ultimately is the same, namely death, it is difficult to discern which (if any) of these factors (hyperkalemia, impaired oxygen transport capacity from cardiac dysfunction, or neuronal failure) is the proximate cause of failure at high temperatures, and which are secondary effects of these, or other heat-induced perturbations. In the present study, we examine the possibility that ion balance disruption is a physiological mechanism that limits the survival of the green crab *Carcinus maenas* following extreme heat stress.

To address this question we first exposed wild-caught individual green crabs to an upward temperature ramp until complete paralysis occurred, and used this state of rigor as a proxy for CT_{max} as the crabs showed no ability to recover after this state had been entered. We then examined the effects of prolonged exposure to two high temperatures (both slightly below the CT_{max}) on extracellular (hemolymph), and intracellular (muscle) ion homeostasis to examine if heat stress causes ion balance disruption in this species. Given that such loss of ion balance may proceed, or follow failure of heart function, we also assessed whether heart failure follows as a consequence of lost ion homeostasis and we furthermore investigated if cardiac failure was the direct cause of heat stupor (i.e. if cardiac failure preceded complete paralysis).

2. Materials and methods

2.1. Experimental animals

Male, adult European green crabs (*Carcinus maenas*) (30–76 g) were obtained from local fishermen operating in the Bay of Aarhus (Denmark) in the autumn of 2014 (mean sea surface temperature 15 °C (September) and 12 °C (October)), and in the spring of 2015 (mean sea surface temperature 3 °C (March) and 10 °C (May)). Crabs were maintained in constant darkness at 12 °C in fresh sea water collected from the Bay of Aarhus (water was changed twice a week and osmolality ranged from 670 and 540 mOsm (September 2014–March 2015)). Crabs were kept for 2–3 weeks without access to food before experimentation such that all animals were post-absorptive and acclimated to the lab conditions before experiments. This species has been found capable of surviving starvation for 3 months at 10 °C (Wallace, 1973), and since all of the crabs were treated in the same manner and randomized into treatment, we are confident that temperature effects are larger than effects of prolonged starvation. Only crabs with a hardened carapace were used for experiments to avoid effects of molting.

2.2. Thermal tolerance during gradual heating

To measure the CT_{max} during gradual heating crabs were placed in a 10 L glass aquarium with sea water. They were then allowed ten minutes to adjust to the new container before the temperature was gradually increased from 12 °C at a rate of 0.25 °C min^{-1} until onset of heat coma. Two to three crabs were tested in a given experimental run and the water was continuously aerated with ambient air (oxygen saturation was never observed to fall below 90%). Water from the aquarium was circuited through a stainless steel coil submerged in a water bath regulated by a thermostat, and returned to the aquarium, to heat the water. Water temperature in the aquarium was continuously monitored using a type-K thermocouple connected to a TC-08 data logger and a PC running PicoLog software (Pico Technologies, Cambridgeshire, UK). The crabs' behavior was continuously observed during heating and the temperatures for two distinct behaviors were recorded: (1) The temperature at which the crabs began uncontrolled muscle spasms and (2) the temperature at which the crabs were paralyzed (no movement could be observed). When a crab had completely ceased to move, we gently poked it to provoke it, and lifted it to the surface of the water to check for movements of the exopodites protruding from the scaphognathites (cessation of this beating has been used as a measure of failure in crustaceans (Ern et al., 2014; Gladwell et al., 1975)). If the crab did not react, and no signs of exopodite movement were observed, it was immediately transferred to a second 10 L tank with fresh 12 °C seawater. Survival was assessed 24 h later.

2.3. Thermal tolerance during chronic and constant heat stress

Thermal tolerance of crabs was also assessed during exposure to two constant heat stress exposures (34 °C and 36 °C, respectively). For these experiments, the aquarium temperature was preset to the high temperature and two to three crabs were placed directly from their holding temperature (12 °C) into the heated aquarium. The time for onset of heat paralysis was registered using a similar protocol as described above and after the onset of heat paralysis the crabs were quickly transferred to 12 °C to allow for assessment of survival 24 h later.

2.4. Measurement of muscle and hemolymph ion and water content

Based on the data from the thermal tolerance experiments we designed experiments to quantify the effects of prolonged high temperature exposure on hemolymph and muscle ion balance in *C. maenas* before, during, and after onset of paralysis. Specifically we took samples at 0, 15, 45, 90, 135, and 180 min for crabs exposed to 34 °C (mean time to paralysis: 172 min) and at 36 °C we took samples at 0, 10, 20, 30, 40, and 50 min (mean time to paralysis: 45 min).

For these experiments we used a large tank (80 L) containing four aquarium air bubblers to maintain high oxygen saturation while crabs were in the tank. For each experimental temperature we randomly assigned 48 crabs in six groups representing the

Please cite this article as: Jørgensen, L.B., et al., Paralysis and heart failure precede ion balance disruption in heat-stressed European green crabs. J. Thermal Biol. (2016), http://dx.doi.org/10.1016/j.jtherbio.2016.08.001