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Is the deep-sea crab *Chaceon affinis* able to induce a thermal stress response?

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ABSTRACT

Fluctuations in the stress level of an organism are expressed in behavioural and molecular changes that can affect its ecology and survival. Our knowledge of thermal adaptations in deep-sea organisms is very limited, and this study investigates the critical thermal maximum (CTmax) and the heat-shock response (HSR) in the deep-sea crab *Chaceon affinis* commonly found in waters of the North East Atlantic. A mild but significant HSR in *C. affinis* was noted and one of the lowest CTmax known amongst Crustacea was revealed (27.5 °C at 0.1 MPa; 28.5 °C at 10 MPa). The thermal sensitivity of this species appears to be reduced at in situ pressure (10 MPa), given the slightly higher CTmax and the significant 3-fold induction of stress genes *hsp70* form 1 and *hsp70* form 2. Although *C. affinis* deep-sea habitat is characterized by overall low temperature this species appears to have retained its ability to induce a HSR. This capability may be linked with *C. affinis'* occasional exploitation of warmer and thermally instable hydrothermal vent fields, where it has been found foraging for food.

1. Introduction

Environmental temperature, and more specifically the thermal regime of a habitat, strongly impact the geographic distribution, life history, metabolism, and survival of species, explaining the diversity of thermal adaptations among organisms (Angilletta, 2009). The HSR is a modification of heat-shock gene expression that is commonly used to assess levels of thermal stress and thermal tolerance limits (Parsell and Lindquist, 1993). Amongst heat-shock genes, *hsp70* is a biomarker of heat stress and plays a central role in tolerance to high temperatures by allowing cell survival during and after thermal stress (Morris et al., 2013). Since the characteristics of the heat-shock response contribute to setting the acute upper thermal limits of most organisms, defining the set points of the HSR is crucial for understanding a species' thermal biology.

The HSR characteristics, usually examined through laboratory studies, provide information on a species' ability to respond to thermal change in its natural environment. Indeed, there is evidence that marine organisms from thermally distinct habitats (i.e. stable, moderately or highly variable) vary in their ability to express a heat-shock response in a way that suggests that some use the response more frequently than others (see Tomanek, 2010). The extreme case would be that of strongly stenothermal organisms, like some Antarctic species, which have lost their ability to induce heat-shock proteins or lack a HSR altogether (Buckley et al., 2004; Clark and Peck, 2009; Tomanek, 2010). However, others have retained their ability to generate a heat-stress response, like the gastropod *Benedictia limnaeoides ongurensis* from Lake Baikal, found at depths from 5 to 120 m where temperatures range from 3.5 to 6 °C (Axenov-Gribanov et al., 2014).

To date, only a few studies have focused on the heat-stress response in deep-sea organisms. Most of the studies were conducted with species endemic to hydrothermal vents (Ravaux et al., 2003, 2013; Cottin et al., 2008, 2010; Boutet et al., 2009) or Antarctic fishes (Buckley et al., 2004). The present study provides the first insights into the thermal biology of the deep-sea crab *Chaceon affinis* (Milne Edwards and Bouvier, 1894) by investigating its thermal limits and response to acute thermal stress. This species is confined to the North East Atlantic, and is distributed along a wide bathymetric range from 130 to 2047 m (Manning and Holthuis, 1981; Biscoito, 2006), with the highest abundance occurring from 700 to 900 m water depth (Pinho et al., 2001). The annual average temperature of the high-abundance zone in the Azores region is 8 to 10 °C (Pinho et al., 2001). *C. affinis* has also been observed on hydrothermal vent mussel beds feeding on *Bathymodiolus spp.* (Colaço et al., 1998,

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2002; Biscoito, 2006) and is frequently found on the outskirts of vents (Desbruyères et al., 2001). The occurrence of this species in contrasted thermal environments, i.e. cold stable deepsea vs thermally instable vents, questions the assumed cold stenothermy of *C. affinis*.

The aim of this work was to determine *C. affinis* thermal limit by using a common index of thermal tolerance, the critical thermal maximum (CTmax), and to investigate the species' response to heat stress (HSR) through *hsp70* gene expression analyses. As a first step in the HSR analyses, the *hsp70* mRNA sequences of two *hsp70* forms for *C. affinis* were obtained. Both thermal limit and HSR experiments were conducted at in situ (10 MPa) and acclimatization (0.1 MPa) pressure in order to evaluate the possible effect of acclimatization/hydrostatic pressure on thermal sensitivity. The results on *C. affinis* present valuable information on the thermal biology of this deep-sea species, and will ultimately help to understand better how this species is able to temporarily access warmer conditions prevailing at hydrothermal vents.

2. Material and methods

2.1. Animal collection and acclimatization

In August 2010, *C. affinis* specimens were sampled in the south coast of Pico Island, Azores (38°24′N 28°29′W), from irregular rocky/muddy bottom at depths from 820 to 950 m. Crabs were obtained using traps (Fathoms Plus®) baited with sardines. Each set of traps was composed of 5 to 7 traps each connected to one long rope attached to a buoy (for a schematic representation of the fishing gear see Fig. 2 in Pinho et al., 2001). The time between setting and hauling of each set of traps was about 48 h. An autonomous pressure and temperature data logger (SP2T4000, NKE instrumentation) was placed inside the bait cylinder of one of the traps to record time, temperature, and pressure during one of the fishing trials. Collected crabs were wrapped in seawater-humidified cloths, quickly placed inside isothermal chilled boxes, and transported to the laboratory. Although *C. affinis* can reach up to 17 cm in carapace length (CL; Pinho et al., 2001) only immature young adults with a CL of 7.9 ± 0.5 cm and a mean carapace width (CW) of 9.5 ± 0.6 cm were used (see specimens details in Supplementary File 1), given the space restrictions inside the high-pressure chamber IPOCAMP (*Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds*; Shillito et al., 2014).

Crabs were maintained at atmospheric pressure in aerated filtered seawater at 10 °C (±1 °C) inside a controlled temperature room (LabHorta; Colaço et al., 2011), to let them recover from the thermal and pressure shock experienced during collection and transport to the lab. Water was partially changed every two days, and crabs were fed ad libitum every 2 days with chunks of fresh deep-sea fish meat (e.g. *Helicolenus dactylopterus dactylopterus*—blackbelly rosefish, *Phycis phycis*—forkbeard, *Mora moro*—common mora). Crabs were acclimatized in these conditions for at least seven days and at most twelve days in darkness before experimental trials.

2.2. In vivo experiments

All in vivo heat-shock experiments were performed at the Department of Oceanography and Fisheries in Horta, Azores, using the flow-through pressurized aquarium IPOCAMP (Shillito et al., 2014). The maximum flow rate achieved in the 19 L IPOCAMP was 10 L h^{-1} at both 0.1 and 10 MPa working pressures. Temperature was regulated by a refrigerated circulator bath that circulates ethylene glycol around the seawater inlet line and through steel jackets that surround the pressure vessel. The temperature of the flowing seawater (filtered at 0.5 μm) was continuously measured by the inlet and outlet temperature probes (±1 °C). More accurate measurements of both temperature (±0.1 °C) and pressure (±1.2 MPa) were obtained by placing an autonomous pressure and temperature data logger (SP2T4000, NKE instrumentation) inside the pressure chamber in all experimental trials. The current technical standard of the IPOCAMP pressure system does not allow to measure dissolved oxygen inside the pressure chamber.

2.3. Determination of thermal tolerance: CTmax

The thermal tolerance was determined by the dynamic method (for example see Ravaux et al., 2012), and 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). The end-point was the onset of spasms.

In each trial, one crab was placed inside the IPOCAMP at 0.1 MPa for 5 h at 10 ± 1 °C and further exposed to a constant rate of water-temperature increase of 0.2 °C min^{-1} until reaching 35 ± 1 °C (Fig. 1). For the in situ pressure trials pressurization up to 10 MPa was reached in about 10 min, corresponding to the average time of the recovery of crabs from the deep upon capture. The total duration of each trial was 7 h. This procedure was repeated for a total of five specimens at 0.1 ± 1 MPa and for five specimens for in situ pressure (10 ± 1 MPa).

The behaviour was video recorded continuously during each trial, through the vessel sapphire windows by using a light source and an endoscope (Fort, Dourdan, France) coupled to a digital microscopy camera. Although the largest possible field of view inside the IPOCAMP did not allow observations of the whole crab, it was still possible to observe the behaviour of each crab. Four behaviour categories were defined: 'Motionless'—no movement detected for 1 min; 'Movement'—any kind of detectable movement like exploring the pressure chamber, interacting with the inlet; 'Active movement'—large scale locomotor-activity with crabs crawling rapidly around the pressure chambers; when active movement behaviour was detected, this would prevail over the other behavioural categories; and 'Spasms'—the onset of tetany (or paralysis), in which often uncoordinated muscular contractions are evident and the body or appendages are subject to muscular inactivity (see also Oliphant et al., 2011).

The upper thermal limit was calculated using the equation (Madeira et al., 2012): $CTmax = \Sigma(Tend\text{-}point_n)/n$, where Tend-point is the temperature at which the end-point was reached for individual 1, individual 2, individual n, divided by the n individuals that were in the sample.

Videos were analysed for 1 min every 1 h (from 30 min to 4 h after the start of the experiment) followed by 1 min every 10 min (from 5 h after the start until the end of the experiment). Around the CTmax time, videos were re-analysed continuously to determine the exact time/temperature for CTmax.

2.4. Heat-shock response

Two crabs were placed inside the pressure vessel in each trial, by positioning one crab in the lower part of the pressure vessel, and another crab in the higher part, i.e. physically isolated from each other with a flow-through platform in between them. In each heat-shock trial, the crabs were maintained for 3 h at 10 °C followed by heating to 24 °C, with an average heating rate of 0.3 °C min^{-1}. The specimens were kept at this temperature for 1 h, after which temperature was reduced until reaching 10 °C and the crabs were maintained at this temperature for further 2 h (see Fig. 2 for temperature profile). Throughout this experiment there was no video recording and no illumination of the inside of the pressure vessel. The total duration of each trial was 9 h. This procedure was repeated 3 times with 2 crabs per trial ($n = 6$ per treatment), for both atmospheric pressure (0.1 MPa) and in situ pressure (10 MPa; pressurization was reached in about 10 min) experiments. Control crabs were maintained at 10 °C inside the IPOCAMP for 9 h for both atmospheric and in situ pressure experiments. For in situ pressure trials pressurization and depressurization took about 10 min each. At the end of control/experimental trials, muscle tissues were sampled from the legs, rapidly frozen in liquid nitrogen, and further stored at −80 °C.