Comparative Biochemistry and Physiology, Part A 168 (2014) 69–75

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Physiological and metabolic responses to rising temperature in *Gammarus pulex* (Crustacea) populations living under continental or Mediterranean climates

N. Foucreau [a,*], D. Cottin [a,b], C. Piscart [a,1], F. Hervant [a]

[a] UMR 5023 Ecologie des Hydrosystèmes Naturels et Anthropisés, Université de Lyon, Université Lyon 1, ENTPE, CNRS, 6 rue Raphaël Dubois, 69622 Villeurbanne, France
[b] UMR CNRS 7208–IRD 207–UPMC, Biologie des Organismes et Ecosystèmes Aquatiques, Muséum National d'Histoire Naturelle, 7 rue Cuvier, CP 32, 75231 Paris Cedex 05, France

ARTICLE INFO

Article history:
Received 17 July 2013
Received in revised form 5 November 2013
Accepted 13 November 2013
Available online 20 November 2013

Keywords:
Adaptation
Amphipod
Ecophysiology
Freshwater ecosystem
Metabolism
Rising temperatures
Thermal stress

ABSTRACT

Latitudinal thermal gradients offer the possibility of comparing the current performance of populations of a single species living in contrasting thermal conditions. The Rhône River Valley (France) presents a 5 °C thermal gradient corresponding to the increase in temperature predicted by climatic models (IPCC, 2007). We studied the thermal tolerance to rising temperature (from 15 to 30 °C) of five populations of the key species *Gammarus pulex* living either in the North (*i.e.* the cold part) or in the South (*i.e.* the warm part) of the river Valley. Individuals were acclimated at 18, 21, 24, 27 or 30 °C during 10 days. After this period, we here measured experimentally the populations' survival, ventilatory rate, oxygen consumption, and glycogen and triglyceride contents. Southern populations have a higher survival rate and higher oxygen consumption at higher temperatures (27 and 30 °C) in comparison with northern populations. Southern individuals also presented a hyperventilation, and higher energy stores compared to northern individuals whatever the acclimation temperature considered. In a global change context, the rising temperatures during the next decades may differently impair the metabolism and the survival of populations of *G. pulex* from different geographical origins. These differences in ecophysiological responses of organisms must be taken into account to predict the consequences of climate change.

1. Introduction

Temperature is an environmental factor affecting ectothermic organisms and driving their survival and their distribution limits (Chown, 2001). In response to climate change, species replacement patterns can occur (Parmesan and Yohe, 2003), reflecting a latitudinal and altitudinal thermal gradients (Somero, 2010). However, local adaptations of population living under contrasted thermal conditions may strongly influence the consequence of rising temperature on the distribution pattern of organisms (Cottin et al., 2012). To determine the consequences of rising temperatures on species distribution and abundance, it seems crucial to determine the thermal tolerance limits among populations of the same species with different latitudinal distributions. Working on different populations of the same species provides several advantages in comparison with studies dealing with several species (Somero, 2010; Whiteley et al., 2011). By reducing any potential effect of the evolutionary history of species, a comparison at the population level could be more accurate to determine either the consequences or the physiological plasticity of animals in the face of rising temperatures, at a time scale (next decades) compatible with the global warming. However, only few studies on crustaceans have focused on the consequences of climate change on different populations of the same species (diLascio et al., 2011; Logan et al., 2012) even if potential adaptation of populations living at the margin of the distribution area may greatly modify the response at the species level even at the local scale (Hampe and Petit, 2005; Somero, 2010; Cottin et al., 2012). Indeed, environmental conditions can strongly differ among sites of the same region and may therefore influence the organism's response – and therefore population preservation – in the context of temperature rise.

Due to climate change, the increase in atmospheric temperatures will range between +1 °C [0.3–1.7 °C] and +3.7 °C [2.6–4.8 °C] (IPCC, 2007, 2013) over the next decades, affecting freshwater ecosystems too (Daufresne et al., 2004; Winder and Schindler, 2004; Rosset and Oertli, 2011). This increase may induce thermal stress, which would imply changes in metabolism of organisms (Pörtner, 2002). Therefore, energy stores can be altered in case of thermal stress. The change of glycogen content, an essential energy storage molecule, has often been studied during numerous environmental stresses (Hervant et al., 1995, 1999; Maazouzi et al., 2011). Triglycerides are the main energy storage molecules in crustaceans (Hervant et al., 1999; Koop et al., 2008; Becker et al., 2013), and this body store can be used by an

---

[*] Corresponding author.
E-mail address: natacha.foucreau@univ-lyon1.fr (N. Foucreau).

[1] Present adress: UMR CNRS 6553 Ecosystèmes, Biodiversité, Evolution, Université Rennes 1, 263 avenue du Général Leclerc, CS 74205, 35042 Rennes Cedex, France.

1095-6433/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2013.11.006

organism to fuel to stress resistance mechanisms (Anguilletta et al., 2002), when it is exposed to stressful conditions (such as temperature increase: Wijnhoven et al., 2003; Issartel et al., 2005).

Here, we studied in total five different populations of the freshwater amphipod *Gammarus pulex* (Crustacea) widely distributed throughout Europe and Asia (Pinkster, 1972). There is increasing interest in its use as a model in environmental change studies (Maazouzi et al., 2011; Kupisch et al., 2012), as *G. pulex* is considered a key species in aquatic ecosystem functioning (Piscart et al., 2011) and as an eurythermal species, individuals can be found with a wide range of temperatures (10–20 °C) with a possibility of adjustment to “extreme temperatures” (5–27 °C) depending on the populations considered (Roux and Roux, 1967; Pinkster, 1972; Sutcliffe et al., 1981; Maazouzi et al., 2011). These populations are distributed along a 5 °C thermal gradient of the Rhône River Valley (France) in the northern hemisphere, where the southern area is warmer than the northern one. This range corresponds to the increase in temperature predicted by climatic models (IPCC, 2007). Notably, southern *G. pulex* populations that live at the southern limit of the species distribution may have to develop mechanisms of thermal tolerance in comparison with populations living in the northern part of the Valley (Cottin et al., 2012). We hypothesized that there is an effect of geographical origin of *G. pulex* populations on their thermal tolerance (heat tolerance), and consequently southern gammarid should present a higher thermal tolerance. To answer this question, we experimentally studied the thermal tolerance of males and females from each population through the survival rate, and a more specific approach through the study of metabolic and physiological parameters.

2. Material and methods

2.1. Sampling sites of animals and rearing conditions

Animals from three populations of *G. pulex* were sampled in the north of the Rhône River Valley (N1, N2 and N3) and two populations living in the south of the Valley (S1 and S2) were studied (see Cottin et al., 2012 for a presentation of the thermal gradient of the valley). These populations live a 500-km long latitudinal gradient corresponding to an increase of 5 °C of the mean annual temperatures.

Two southern populations were sampled (S1, sampled near Aix-en-Provence: 05°23′57″E, 43°34′42″N; S2, sampled near Aubagne: 05°36′13″E, 43°18′15″N). Three northern populations were sampled: N1, sampled near Balan (05°06′23″E, 45°49′35″N), N2, sampled near Dijon (04°53′0.9″E, 47°24′13″N), and N3, sampled near St Maurice-de-Gourdans (05°13′51″E, 45°50′06″N). All five populations were sampled in June 2012 with a hand net: 150 couples were collected, and males and females were separated directly in the field.

In the laboratory, individuals were settled with water from their own site during four days at 15 °C (12:12 Ligh:Dark) in a rearing chamber in order to standardize their physiological and metabolic parameters as described in Foucreau et al. (2013). Animals were feed (*ad libitum*) with alimentary chips (Novo Crabs®, JBL GmbH & Co, Germany) until the start of the experiments.

2.2. Experimental design

For all experimentations, five acclimation temperatures were tested: 18, 21, 24, 27 and 30 °C. Final acclimation temperatures were reached progressively (3 °C per day) starting from 15 °C, then gammarids of each population were maintained during 10 days at the final acclimation temperature (18, 21, 24, 27 or 30 °C). Seventy individuals per population, per sex and per acclimation temperature were put in 5-L plastic tank with food *ad libitum* (Novo Crabs®, JBL GmbH & Co, Germany) in a 100 L water bath (used as a double boiler) maintained at the acclimation temperature by a thermostatic water pump (Aquarium refrigeration unit, TECO®, Italy). The water was aerated through air pumps continuously to avoid any depletion of the dissolved oxygen, especially at higher temperatures. Two days before the end of the acclimation, individuals were starved to avoid any effect of the digestive metabolism on the physiological parameters measured (Hervant et al., 1999).

2.3. Survival, respiratory metabolism, energy storage

2.3.1. Survival experiment

For all acclimation temperatures, survival of males and females of each population sampled was checked daily for 10 days directly in the acclimation box. Dead animals were removed. Surviving individuals were used to measure physiological and metabolic parameters. For all parameters, 20 individuals (10 males and 10 females) were used. Not enough living individuals remained at 30 °C, so this temperature was not retained for graphical or statistical analyses.

2.3.2. Ventilatory rate and oxygen consumption rate

At the end of the acclimation period (T0 + 10 days), the ventilatory rate was measured in 20 individuals (10 males and 10 females) from each gammarid populations and for each acclimation temperature. The frequency of pleopod beats (ventilatory appendages of malacostracan crustaceans) was measured in 48-h-starved individuals as described in Hervant et al. (1997). All measures were done in the same period of the day, to avoid any potential interference of the circadian rhythm.

Concerning the oxygen consumption, individuals were placed, having been starved for 48 h, into specific respirometer at the corresponding acclimation temperatures for each modality, as described in Maazouzi et al. (2011). The oxygen consumption was recorded during 24 h to avoid any effect of the circadian cycle interfering with the measurement of the basal respiratory metabolism (Rosas et al., 1992). For each temperature, three control respirometers without animal allowed us to estimate the bacterial oxygen consumption. This value was subtracted from the global consumption to determine the “real” gammarid oxygen consumption.

2.3.3. Energy stores

Triglycerides and, to a lesser extent, glycogen are the two major energy stores in crustaceans (Becker et al., 2013). Glycogen is a macromolecule rapidly synthesized and consumed, whereas triglyceride molecules need more time to be synthesized and catabolized by organisms, but provide more energy (Hervant et al., 1999; Koop et al., 2008; Becker et al., 2013). Individuals used for energy store measurements were beforehand lyophilized then weighted. Dry individuals were stored at −25 °C until analyses. Measurements were performed individually. Extraction and assays of glycogen and triglycerides were processed as described by Hervant et al. (1995, 1996). GPO Trinder and Glucose HK specific kits for triglycerides and glycogen, respectively, were purchased from Sigma-Aldrich® (France). All assays were performed using an Aquamate spectrophotometer (Thermo) at 25 °C.

The ecophysiological index (EPI) was calculated following the method described by Marmonier et al. (2013): EPI = percentage of surviving individuals × (glycogen content / (glycogen content + triglycerides content)). This index is high when organisms showed a high survival rate and are able to maintain their body stores despite stress exposure, but the EPI value decreases with a reduction in individual survival rate or in glycogen level. It theory, EPI varies between 0 (when all animals die during the study) and nearly 100 (when all animals survive and glycogen content is maintained at a high level even if it is not possible to achieve a value of 100 because these species naturally store some of their body stores as triglycerides (Marmonier et al., 2013).

2.4. Statistical analysis

For the different parameters, general linear models (GLMs) were used to test the differences between geographical origin, populations sampled, gender and acclimation temperatures with the population