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Progressive acclimation alters interaction between salinity and temperature in experimental *Daphnia* populations

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HIGHLIGHTS

- The halotolerance of *D. galeata* was negatively temperature-dependent.
- Salinity × temperature interaction changed after multigenerational acclimation.
- Temperature-dependence was nullified when animals were acclimated to both stressors.
- Risk assessment practices must take into consideration potential complex interactions.

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ABSTRACT

Environmental stressors rarely act in isolation, giving rise to interacting environmental change scenarios. However, the impacts of such interactions on natural populations must consider the ability of organisms to adapt to environmental changes. The phenotypic adaptability of a *Daphnia galeata* clone to temperature rise and salinisation was investigated in this study, by evaluating its halotolerance at two different temperatures, along a short multigenerational acclimation scenario. Daphniids were acclimated to different temperatures (20 °C and 25 °C) and salinities (0 g L^{-1} and 1 g L^{-1}, using NaCl as a proxy) in a fully crossed design. The objective was to understand whether acclimation to environmental stress (combinations of temperature and salinity) influenced the response to the latter exposure to these stressors. We hypothesize that acclimation to different temperature × salinity regimes should elicit an acclimation response of daphniids to saline stress or its interaction with temperature. Acute (survival time) and chronic (juvenile growth) halotolerance measures were obtained at discrete timings along the acclimation period (generations F1, F3 and F9). Overall, exposure temperature was the main determinant of the acute and chronic toxicity of NaCl: daphniid sensitivity (measured as the decrease of survival time or juvenile growth) was consistently higher at the highest temperature, irrespective of background conditions. However, this temperature-dependent effect was nullified after nine generations, but only when animals had been acclimated to both stressors (high salinity and high temperature). Such complex interaction scenarios should be taken in consideration in risk assessment practices.

1. Introduction

Fluctuations in environmental conditions are responsible for environmental stress, modulating structure and abundance dynamics of natural populations and, consequently, leading to significant impacts on ecological processes (Bijlsma and Loeschcke, 2005). In fact, environmental stress is considered a driver in the adaptation and evolution of populations to changing environments (Bijlsma and Loeschcke, 2005; Van Doorslaer et al., 2009). Therefore, the success of natural populations is dependent on their ability to cope with new environmental conditions (Bijlsma and Loeschcke, 2005; Van Doorslaer et al., 2009), by changing their behaviour, life history and physiological responses (Boersma et al., 1998; Bijlsma and Loeschcke, 2005; Castro et al., 2007; Van Doorslaer et al., 2009). On the short-term, organisms adapt via acclimation to new environmental conditions – phenotypic

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plasticity (Bijlsma and Loeschcke, 2005; Castro et al., 2007). On the longer term, natural selection acts on genotypes by selecting the fittest ones under the new environmental conditions – local adaptation (De Meester, 1996a,b; Van Doorslaer et al., 2007, 2009). Thus, the impacts of environmental change on natural populations must be investigated taking into account the organisms' ability to adapt to such change.

Among sources of environmental change, temperature has received considerable attention given the foreseen (direct and indirect) impacts of global warming on freshwater systems (Noyes et al., 2009; IPCC, 2014). Temperature is a key driver in biological systems, modulating seasonal patterns and interacting with biotic processes (Willmer et al., 2005). Changes in temperature alone can modify growth, metabolic rate and life history of organisms (Chopelet et al., 2008; Chen and Stillman, 2012), and, in a broader scale, affect community structure (Van Doorslaer et al., 2009; Kaya et al., 2010; Benincà et al., 2011) and ecosystem processes (Brown et al., 2004; Dang et al., 2009). Concomitantly with changes in temperature, ecosystems face the threat of chemical contamination, and interactive scenarios between both stressors bring uncertainty onto environmental risk predictions (Noyes et al., 2009). In freshwater ecosystems, a relevant source of disturbance comes from natural or anthropogenic salinisation (James et al., 2003; Cañedo-Argüelles et al., 2013), as most freshwater taxa are stenohaline. Salinity rise has been shown to have implications in the structure and dynamic of populations (Schallenberg et al., 2003; Sarma et al., 2006; Nielsen et al., 2008; Loureiro et al., 2012) and species richness (Hall and Burns, 2002; Nielsen et al., 2008; Brucet et al., 2010; Duchet et al., 2010). Furthermore, salinisation of freshwaters is likely to be enhanced by climate change via increased evaporation, extended droughts and reduced rainfall (Chopelet et al., 2008; Nielsen and Brock, 2009; IPCC, 2014). Temperature increase and salinisation will therefore co-occur as stressors in vulnerable freshwater ecosystems.

The interactive effects between salinity and temperature in freshwater organisms have received the attention of several authors, particularly for zooplankton (Hall and Burns, 2002; Brucet et al., 2009; Ismail et al., 2011; Kaya et al., 2010; Chen and Stillman, 2012). Zooplankters play a key role in the maintenance of the ecosystem dynamics, as grazers and nutrient cyclers (Lampert, 2006; Jeppesen et al., 2007; Van Doorslaer et al., 2009). Therefore, it is important to understand their ability to survive and reproduce under simultaneous stressors (see reviews by Noyes et al., 2009; Fischer et al., 2013). In fact, several authors reported that interactive effects of stressors compromise trophic interactions, thus compromising ecosystem functioning (Hall and Burns, 2002; Winder and Schindler, 2004; Brucet et al., 2010; Chen and Stillman, 2012). However, this is dependent on the degree of environmental variability and the long-term effects of acclimation (Chen and Stillman, 2012; Stoks et al., 2014). There are still insufficient studies that consider pre-adaptation or acclimation in the sensitivity or resilience of organisms and populations to the interactive effects of simultaneous stressors (Ismail et al., 2011; Jansen et al., 2011; Chen and Stillman, 2012; Fischer et al., 2013).

Bearing this in mind, the present study intended to investigate the phenotypic component of adaptability to salinity and temperature in an experimental population of *Daphnia galeata*. Our objective is to assess whether acclimation of organisms to different temperature (20 °C and 25 °C) and salinity (0 g L^{-1} and 1 g L^{-1}, using NaCl as a proxy) regimes influences their sensitivity to salinity at two different exposure temperatures (20 °C and 25 °C), following a progressive (multigenerational) acclimation scenario. The null hypothesis is that culture conditions (temperature × salinity acclimation regimes) bear no influence on the halotolerance of the laboratorial population. The alternative hypothesis states that acclimation to culture conditions elicits an adaptive response of daphniids to saline stress or its interaction with temperature. Because previous data (Loureiro et al., 2012, 2013a) suggested that acute and chronic responses to NaCl were not coupled, we examined survival time and juvenile growth as measures of halotolerance.

2. Material and methods

2.1. Cultures and test organisms

For this study, four cultures of *D. galeata* were established from an existing culture (clone B, collected in a freshwater reservoir; Loureiro et al., 2013b), which was maintained under a temperature of 20 ± 2 °C and a $16h^{L}$:$8h^{D}$ photoperiod. Daphniids were reared in moderately hard reconstituted water (123 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 96 mg L^{-1} of $NaHCO_3$, 60 mg L^{-1} $CaSO_4{\cdot}2H_2O$, and 4 mg L^{-1} KCl), supplemented with 4 mL L^{-1} of a standard organic additive (algal extract) and vitamins. Culture medium was renewed and organisms were fed – with a *Pseudokirchneriella subcapitata* ration of 1.5×10^5 cells mL^{-1} – three times a week (Monday, Wednesday, Friday). For further details on daphniid culture medium and procedures, as well as algal cultures, see Castro et al. (2007), Gonçalves et al. (2007), and Loureiro et al. (2012). *D. galeata* belongs to a ubiquitous stenohaline species complex that inhabits European lakes and reservoirs (Castro et al., 2007; Castro and Gonçalves, 2007), and this particular genotype is known to be a sensitive ecoreceptor to salinity increase (Loureiro et al., 2013b).

Each new culture, derived from the initial culture (see above), was submitted to one of four different combinations of temperature and salinity: 0 g L^{-1} of NaCl at 20 °C; 1 g L^{-1} of NaCl at 20 °C; 0 g L^{-1} of NaCl at 25 °C; and 1 g L^{-1} of NaCl at 25 °C. Sodium chloride was used as a protective surrogate of seawater (Leitão et al., 2013; Loureiro et al., 2013a,b), because its toxicity to freshwater organisms is comparable or only slightly higher than equivalent seawater dilutions (ongoing results of project Saltfree). Neonates from the initial culture (see above) were used as propagules (F0 generation) to inoculate each new culture. All cultures were reared under the same conditions of the initial culture, except in terms of temperature and NaCl concentration (as described above). These conditions were maintained for nine generations, promoting the gradual acclimation of organisms to the new temperature × salinity conditions. Neonates from F1, F3 and F9 generations (born from F0, F2 and F8 mothers, respectively) were used for halotolerance tests (see below) at two exposure temperatures (20 °C and 25 °C). The time frame required for the acclimation period of the group cultures was approximately three weeks for the F1 generation, two months for F3, and six months for F9. Tests were always conducted with neonates from the four background conditions, i.e. acclimation regimes of temperature × salinity. To assure homogeneous quality and standardisation, culture renewal and tests were always performed with neonates (less than 24 h old) born between the 3rd and 5th broods of each F_x mothers.

2.2. Survival time trials (acute halotolerance)

Survival time (ST) trials were adapted from Ribeiro et al. (2000) and Lopes et al. (2005) and followed the same procedures described by Loureiro et al. (2012). ST was used as an acute measure of halotolerance, since it is more sensitive than the standard acute EC_{50} (Loureiro et al., 2012). It was measured as the time to immobilisation of a batch of neonates individually exposed to 6.0 g L^{-1} NaCl (prepared in moderately hard reconstituted water). These trials were performed at 20 °C and 25 °C, using 9–12 individuals per temperature from the same batch of neonates. Neonates were individually placed in 2 mL of test solution in 24-well culture