Aquatic Botany 122 (2015) 20–26

Contents lists available at ScienceDirect

Aquatic Botany

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

Foreseeable global warming will differentially affect *Chara vulgaris* populations from different altitudes

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ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 5 December 2014
Accepted 8 December 2014
Available online 7 January 2015

Keywords:
Climate change
Common garden
Local adaptation
Phenotypic plasticity
Acclimatization
Morphological architecture
Respiration rate

ABSTRACT

A 2–4 °C increase in temperature is foreseen for the Mediterranean region by the end of the 21st century due to climate change. This warming is expected to occur as heat waves, and will be particularly important for shallow water bodies which are the main freshwater ecosystems in the Mediterranean. We subjected four populations of *Chara vulgaris* to a common garden experiment. Each population came from locations at different altitudes, hence living at different mean temperatures. Individual charophyte cultures were initiated with specimens from four origins and acclimatized at 20 °C in a controlled environment for 3 weeks. After this, all the populations were incubated under 3 water temperature treatments: 20 °C (control), 22 °C and 24 °C. A two-way ANOVA (temperature × population origin) showed that the temperature treatment significantly, and positively, affected the growth calculated on normalized dry weight, the length of the main axis, the number of branches per node, the horizontal biomass distribution and the respiration rate. Moreover, a significant effect of the temperature × population-origin interaction was detected for biomass production, total length and branch production. These results suggest that lower-altitude populations have a greater tolerance to temperature increase, and they would be favored by climate change. These different intraspecific responses to warming could impact the ecotype distribution and diversity.

1. Introduction

Climate change is imposing new selection pressures which appear to have already changed the distribution and abundance of widespread species (Lovejoy and Hannah, 2005). Species and populations with high climatic tolerances are likely to survive and adapt to these changes, whereas those with more specific needs will be threatened, leading to the extinction of some parts of the species' ranges (Lovejoy and Hannah, 2005). Species and populations living in highly variable environments could well be more tolerant to climate change variations in temperature. Most of them can alter the expression of some of their phenotypic features, phenotypic plasticity (Walters, 2005), in response to environmental variability (Piersma and Drent, 2003).

By the end of 21st century, a 2–4 °C temperature increase is expected in the Mediterranean region in spring (Stocker et al., 2013). Freshwater ecosystems in this climatic region are often shallow water bodies or small lakes, hence they are particularly sensitive to climate change (Álvarez-Cobelas et al., 2005; Parcerisas et al., 2012), including foreseen increases in the frequency of extreme events such as heat waves (Barrera-Escoda et al., 2014), and a severe reduction in the water level (Mariotti et al., 2008; Lelieveld et al., 2012). These rapid changes in the environment may well have serious implications for the biodiversity of Mediterranean freshwater ecosystems and how they function (Beklioglu et al., 2007; Rodrigo et al., 2007; Rojo et al., 2008, 2012; Martínez et al., 2014).

Submerged macrophytes are a key group in freshwater ecosystems (Jeppesen et al., 1997; Burks et al., 2006; Beklioglu et al., 2007; Rojo et al., 2008). Among them, charophytes have a recognized influence on structuring the planktonic food web (Hilt and Gross, 2008; Pakdel et al., 2013; Rojo et al., 2012, 2013) and are highly involved in biogeochemical processes through nutrient transfer (Rodrigo et al., 2007; Rojo et al., 2008). Therefore, any change that might alter the populations in a species-specific way may well imply a shift in the submerged macrophyte community, thus modifying the trophic structure and the ecosystem function. There is an extensive bibliography on how changes in temperature affect microalgae and phanerogams. Generally, an increase in growth and metabolic rates is reported directly proportional to the temperature increase (Barko and Smart, 1986; O'Neal and Lembi, 1995; Graham et al., 1996; Berry and Lembi, 2000). Recent publications have

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http://dx.doi.org/10.1016/j.aquabot.2014.12.002

pointed out the need to find the thermal thresholds of macroalgae (Martínez et al., 2014) and charophytes among other drivers (Auderset Joye and Rey-Boissezon, 2015). However, no experimental studies have been carried out specifically on temperature effects in charophytes in order to discover, for example: how common charophyte species will actually respond to the expected rise in temperature over the next century.

The goal of this study is to discover how different populations of *Chara vulgaris* Linnaeus will respond to a foreseen gradient of temperature increase. *C. vulgaris* is a widely distributed charophyte (Rey-Boissezon and Auderset Joye, 2015) which is ubiquitous in the Mediterranean (Cirujano, 2008). For this study, different populations were sampled in freshwater ecosystems at different altitudes, thus inhabiting water bodies featuring different spring temperatures. In detail, we will test whether, for different levels of temperature increase, four *C. vulgaris* populations show enough phenotypic plasticity to acclimatize and survive under rapid temperature variations and, if all the studied populations respond in a different way under the same temperature change. The acclimatization capacity of each population would determine if it will either benefit from, or be adversely affected by variation in temperature. This will depend on how close each population currently lives to its upper thermal tolerance limits (Somero, 2010). We performed an experiment with a common-garden design, similar to studies carried out with other macrophytes and trees (Santamaría et al., 2003; Vitasse et al., 2009). The main idea of the common garden approach is to culture, under the same homogeneous conditions, populations from different localities (therefore subjected to different environmental conditions). Environmental variability is nullified and all the variations observed between populations depend on genetic factors. Common garden approaches have usually been used in genetic studies (Santamaría et al., 2003; Hofstra and Clayton, 2012), but they are increasingly being used to foresee the effects of climate change (Vitasse et al., 2009). Populations living at lower altitudes are subjected to higher and more variable temperatures during spring, particularly from March to May the most important part of the growth period of *C. vulgaris* in the Mediterranean region (Rodrigo et al., 2013). We thus hypothesize that they will have adapted to show a wide phenotypic response to variations. On the other hand, populations inhabiting high altitudes are likely to have adapted to slighter temperature changes in a lower and less variable temperature regime. The effect of these climatic conditions has already been observed in trees and macrophytes inhabiting different altitudes and latitudes (Santamaría et al., 2003; Vitasse et al., 2009; Quiroga et al., 2012; Hyldgaard and Brix, 2012).

2. Materials and methods

2.1. *C. vulgaris* population origin and culture

Four different populations of *C. vulgaris* were used during the experiment. Each population came from different water bodies at different altitudes, namely: Tancat de la Pipa marsh (Albufera de València Natural Park, 3 m a.s.l., 39°21′51″N 0°20′47″W), Algar pond (Protected Area, 100 m a.s.l. 38°38′52″N 0°05′45″W), Mas dels Arbres pond (Serra Mariola Natural Park, 1000 m a.s.l. 38°45″30′N 0°31″45″W) and Somolinos lake (Sierra de Ayllón Protected Area, 1270 m a.s.l. 41°15′04″N 3°03′54″W). Populations from the first two sites were considered to be the low altitude populations (LAP1 and LAP2) and the other two high altitude populations (HAP1 and HAP2). The charophyte samples were taken in March. Water temperature in the meadow in March–May ranged between 17.2 and 21.8 °C in LAP1, 17 and 22.6 °C in LAP2, 11.3 and 13.0 °C in HAP1 and 9.5 and 12.0 °C in HAP2. The collected charophytes were transported from the field to the laboratory at the University of Valencia. Stock cultures were initiated with the specimens and kept in an indoor culture room at a constant temperature (20 °C) under artificial illumination provided by Sylvania Gro-Lux F58W tubes (100 μmol photons m^{-2} s^{-1}; light:darkness 13:11 h). This irradiance is non-limiting for *C. vulgaris* and other charophytes (Rubio et al., 2015). The stocks were prepared by planting shoot tips of charophytes in small pots (65 mL) using as the substrate (anchoring material) a mixture of commercial sand and sediment in a proportion of volume 2:1. The sediment was taken from Tancat de la Pipa wetland, homogenized, sterilized by autoclave and kept in the laboratory until its use. The same sediment was used for all the studied populations. The cultures were placed in plastic containers previously filled with dechlorinated tap water. After a few days, the charophytes developed a rhizoidal system and began to grow. This culturing and maintenance method has proven effective for growth of charophytes and experimentation in the laboratory (Rodrigo et al., 2013).

2.2. Experimental design and previous statistical analyses

A common garden approach was used for the experiment. The four populations were subjected to the same temperature treatments: water temperatures of 22 °C and 24 °C in addition to a control treatment at a water temperature of 20 °C. These treatments were established taking into account the expected temperature increase in the Mediterranean by the end of this century (Stocker et al., 2013). A two-way ANOVA design was carried out: population-origin treatment (4 levels) × temperature treatment (3 levels). Before the experiment started, several individuals from each population were cultured at the three treatment temperatures for three weeks (pre-experimental cultures), time enough to let the individuals grow and acclimatize (i.e., physiological adaptations that occur through experimentally induced stressors) to the new environment (Rubio et al., 2015). This acclimation was necessary in order to avoid a thermal shock, which could lead to false results. Irradiance conditions were the same as described above.

Once the acclimatization ended, the dry weight (DW)-24 h at 72 °C- of 4 shoot tips randomly selected from each group (population × temperature treatments) was determined in order to obtain an estimation of the initial biomass. Then, the necessary shoot tips (replicates) from each group were randomly selected from the pre-experimental cultures for the experiment. All the shoot tips were the top 4 nodes, and ramifications, if any, were removed in order to obtain the most similar initial biomass and morphology. These shoot tips were individually planted in small pots using the same mixture of sand and sediment used in the stock and pre-experimental cultures.

Once planted, each individual was labeled, and the length above the sediment was measured. Each pot was placed in a tall plastic beaker filled with 1.6 L of dechlorinated tap water. The beakers were then placed in plastic containers (buckets) filled with ∼40 L of dechlorinated tap water. The beakers were used in order to avoid the individuals becoming pseudo-replicates due to a "bucket effect" (Hurlbert, 1984). The water in each bucket was kept at a constant temperature. The water temperature of each individual beaker was measured, and its position in the bucket was changed once a day in order to avoid a site effect (as seen in Niu et al., 2012). The temperature in the buckets for the 22 °C and 24 °C treatments was raised and kept constant by means of an aquarium heater (Eheim Jäger 125 W/150 W for 100 L). The control bucket was kept at the environmental temperature of the culture room, 20 °C. Both the increased temperature treatments and the control were subjected to the same irradiance conditions: those of the culture room.

The lack of a "bucket effect" or "position effect" was tested before the experiment started by calculating an estimation of variability of selected variables (i.e., length, DW, etc.) (Hurlbert, 1984; Faul et al.,