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An experimental approach to assess *Corbicula fluminea* (Müller, 1774) resistance to osmotic stress in estuarine habitats

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ABSTRACT

Corbicula fluminea arrived in the Miño Estuary in 1989 and, from there, colonized more than 150 km upstream. Our aim was to test the capacity of *C. fluminea* to cope with osmotic stress conditions previously to invade new freshwater habitats through estuaries. Based on previously collected information, the experiment aims to study the response of the species to marine osmotic stress, evaluated by survival and behaviour. Experiments determined the resistance by the species to various levels of osmotic stress, and recovery time after exposure to high salinity levels, representative of the temporal and spatial salinity variation existing in the estuary. Under osmotic stress the semi-maximum response was reached after 19 days exposure. The species tolerance range, measured by individual maintained activity, was at salinity ~20 when exposed to winter temperatures, while when animals were exposed to summer ones its tolerance was reduced to salinity lower than 15. *C. fluminea* show a large physiological flexibility to cope with salinity variations in estuaries. In summer, the temperature increases the metabolic rate thus making the species more vulnerable to osmotic stress exposure. These findings are relevant to preventing new invasions through ship ballast waters ensuring complete mortality if individuals are retained for >26 days.

1. Introduction

The invasive success and subsequent dispersion of non-native species rely on the interplay between biological and behavioural characteristics and abiotic environmental factors (Alpert et al., 2000). These are the principal determinants of the rate of invasion, while the traits characterizing invaders show wide tolerance ranges to environmental factors (Holway, 1998; Sakai et al., 2001). The biotic resistance of each species in the receiving environment appears to play an important role in invasion success or failure and can deter invasions completely, or at least slow down the process (Zenni and Núñez, 2013). The estuarine environment is characterized by highly varying salinity (Elliott and Quintino, 2007). The mixing of waters, strongly fluctuating with tides and seasons, acts as an important abiotic filter for new colonizers (Basset et al., 2013).

The Asian freshwater bivalve *Corbicula fluminea* (Müller, 1774) started its worldwide dispersion at the beginning of the twentieth century as the result of human activities such as global trade, fishing bait and recreational activities (Counts, 1981; Araujo et al., 1993; McMahon, 1999, 2002; Darrigran, 2002). The species colonized lotic and lentic habitats (Vidal et al., 2002) and its distribution in invaded rivers included fresh to brackish waters – with salinity values up to 14 (Pérez-Quintero, 2008; Sousa et al., 2008a; Vohmann et al., 2010).

The distribution and abundance of the species are closely related to its response to fluctuations in environmental factors and these have been widely analyzed (McMahon, 1979; Graney et al., 1984; Byrne et al., 1990; Vidal et al., 2002; Sousa et al., 2006). Where the species is present at downstream sections of rivers in coastal areas, salinity is one of the abiotic factors limiting its occurrence and distribution (Belanger et al., 1985; Morton and Tong, 1985; Sousa et al., 2006, 2008b; Schmidlin and Baur, 2007). To cope with this stress factor, shell valve closure has been identified as a protective response allowing the species to survive under adverse environmental conditions (Doherty et al., 1987; Fournier et al., 2004; Liao et al., 2009). This response is also related to energy metabolism: valve closure behaviour avoids water exchange, important to dissipate metabolic CO_2 and to maintain the low osmolality of body fluids through the interaction between ion transport and acid–base balance (Byrne and Dietz, 1997; Ortmann

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and Grieshaber, 2003). Despite a general acceptance of this mechanism, there is little evidence of the effect and response of the species to highly varying salinity in estuarine environments. The first studies on the *C. fluminea* organismal response to salinity were published in the late 1970s (Gainey, 1978; Evans et al., 1979). Subsequently, with the exception of Ruiz and Souza (2008) and Xiao et al. (2014) who respectively analyzed volume regulation in muscle tissue and metabolic rate in response to salinity, no studies have examined the bivalve response to osmotic stress.

In this study, estuarine salinity conditions were experimentally simulated to test the following hypotheses: firstly, that the species is able to cope with osmotic stress conditions previously to invade new freshwater habitats when passing through brackish water barriers. Secondly, that the mechanism underlying this osmotic resistance is related to animal isolation through valve closure behaviour.

2. Materials and methods

2.1. Salinity and temperature background

Salinity and temperature are considered the variables with the highest potential to influence the species distribution and survival in estuarine environments. Our experimental design was based on previous field studies developed in a temperate estuary from the biogeographic Atlantic region (River Miño Estuary; Ferreira-Rodríguez and Pardo, 2014). In general, estuarine bivalves are alternately exposed to fresh and brackish water with tidal succession. The experiment was designed to test the survival and recovery capacity of *C. fluminea* when confronted by increasing periods of salinity exposure causing osmotic stress, as a proxy for its spatial presence in the estuary, and comprising the annual range of temperature variation. Boundary conditions used in the experiment were established from abovementioned previous research. The distribution range of the species ranged from freshwater, upstream sites without tidal influence, to brackish water with maximum salinity values of 15 during high tide in the population distribution limit, at downstream sites. Between both salinity conditions, aquatic community and specifically *C. fluminea* populations inhabiting estuaries are exposed to osmotic stress (salinity >15) for short time periods ($\leq$1 h) at upstream sites, and for long periods ($\geq$5 h) at downstream sites. The mean seasonal temperature in ranged from 11.6 °C in late winter (in March, minimum 8.2 °C) to 19 °C in late summer (in September, maximum 23.6 °C).

2.2. Experimental material

Corbicula fluminea individuals were collected from the upper River Miño Estuary (10 km from the river mouth) in March 2013 with a kick net (1 mm mesh size). A total of 140 adult individuals of shell 20 ± 1 mm (Aldridge and McMahon, 1978) and 0.071–0.091 g ash free dry mass (AFDM) were collected. The use of a single age class in the laboratory experiments despite temperatures representative of winter and summer conditions was justified because of the continuous recruitment pattern of the species in some temperate Atlantic estuaries (Franco et al., 2012; Ferreira-Rodríguez and Pardo, 2014).

Animals collected in the field were conditioned in the laboratory in 5 l freshwater aquaria. The aquaria were maintained with constant aeration under subsequent experimental conditions of temperature and photoperiod for a seven day period, sufficient time for a significant physiological adaptation in experiments performed on bivalves (Sarà et al., 2008).

2.3. Resistance to osmotic stress

The experiment tested the *C. fluminea* resistance to osmotic stress using a one factor design with six salinity levels (tolerance range: salinity values of 0.07, 5, 7 and 15; over-tolerance range: salinity values of 20 and 24). The different salinities (Practical Salinity Scale) were obtained by mixing freshwater from the upper estuary (salinity of 0.07) and marine water from the river mouth (salinity of 32.3) until the desired concentrations were reached and measured using a Thermo Orion 115A salinometer. Experiments were performed in microcosms with four individuals per microcosm in an incubation chamber at a constant temperature of 11 °C, under 12:12 h photoperiod. Each microcosm container of 1000 ml capacity was fitted with a 3 cm bottom layer of the same sediment composition (organic matter content: 5.48%). On the same day that the bivalves were collected, freshwater (salinity of 0.07) and river sediments from the same location (ED50 UTM 29N, coordinates 517.987, 4.641.291) were brought to the laboratory and used as the experimental media.

The experiment was continued for 40 days, with daily monitoring and recording of mortality as the dependent variable. A total of 20 experimental microcosms, 5 controls of at salinity of 0.07 and 3 of each other salinity level were set up. Dead individuals were removed when detected. Daily salinity and temperature values were measured, evaporation losses being replaced with deionized water.

2.4. Activity recovery under tidal fluctuations

Experiments were performed in 150 ml plastic container microcosms with one *C. fluminea* individual in each chamber at constant temperature under a 12:12 h photoperiod. The experiment consisted of a three factor design:

i) two temperature levels representing winter and summer temperature conditions (11 and 20 ± 0.1 °C respectively);
ii) three tidal cycles, the first and third cycles used to evaluate the recovery time of *C. fluminea* at different exposure times, and between them, in the second cycle all individuals were exposed to same osmotic stress conditions (salinity of 17) for 6 h (representative of a spring tide) as a restart period and to ensure the independence of both measures;
iii) exposure time to brackish water (salinity of 17) for 5 time periods (from 1 to 5 h) in the first and second tide representing the range of osmotic stress exposure from the up- to the down-estuary.

A total of 30 microcosms were set up at 11 °C and 30 more at 20 °C (n = 60: 2 temperatures * 3 replicates of salinity and 3 replicates of freshwater controls * 5 exposure times). The experiment extended over 36 h (three complete tidal cycles; Fig. 1). After osmotic stress exposure, bivalves were monitored visually during a period of 2 h to determine when the valves opened (recovery time) once re-immersed in freshwater. To reduce the manipulation influence and maintain salinity in the control (salinity from 0.07 to 0.07) and experimental microcosm (salinity from 17 to 0.07), water exchanges were made by siphoning. In the analysis, records from individuals that did not react to re-immersion in freshwater within the 2 h of monitoring period were discarded. The same individuals exposed to brackish water per each time period during the first tidal cycle, were also used for the same exposure time during the third tidal cycle.

2.5. Data analysis

The survival analysis of *C. fluminea* individuals exposed at six