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Eco-physiological responses to salinity changes across the freshwater-marine continuum on two euryhaline bivalves: *Corbicula fluminea* and *Scrobicularia plana*

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ABSTRACT

The influence of global climate change will potentially alter the salinity of aquatic ecosystems. This represents a tremendous challenge for societies worldwide. Different sources of salinization (natural or anthropogenic) amplify the introduction of salt in rivers and streams, causing an increase of salt flowing down to estuarine and coastal areas. In this study, *Corbicula fluminea* and *Scrobicularia plana* have been selected because of their large tolerance for salinity variation (euryhaline organisms). They will allow the study of effect on the whole spectrum of salinity from fresh to marine waters respectively. The aim was to study the impact of experimental salinity stress at physiological, biochemical and behavioral levels by exposing both species to a salinity close to their limit range of tolerance, 15 practical salinity unit (psu), and at their field salinity (1.5 psu and 30 psu for *C. fluminea* and *S. plana* respectively) in the presence or absence of food during 2 and 7 days of exposure. Negative impacts of hyper saline condition for *C. fluminea* (15 psu) and hypo saline condition for *S. plana* (15 psu) have been measured at biochemical, physiological and behavioral levels. At sub-individual and individual levels, structural and energetic parameters and behavioral impairments seemed to be suitable biomarkers to assess salinity stress on *C. fluminea* and *S.plana.* After exposure to the limit of salinity tolerance (15psu) for both organisms, fitness modifications could appear, and may participate in endangering populations.

1. Introduction

Global change is predicted to induce major ecological impacts in the next century, on every ecosystem. Aquatic ecosystems are particularly threatened, undergoing acidification, multiple sources of pollution, temperature and salinity changes (Field et al., 2014). Global climate changes present tremendous challenges for the worldwide societies. The alteration of salinity levels in the aquatic environment can have a natural origin (primary salinization such as weathering of rocks, wind and rain depositing salt) and anthropogenic sources (secondary salinization such as salt mining, industrial discharges or road de-icing). All these sources amplify the introduction of salt in rivers and streams, leading to an increase in the amount of salt flowing down to estuarine and coastal areas (Cañedo-Argüelles et al., 2013). On the other hand, in estuary and coastal areas undergoing tidal events, freshwater inputs coming from rivers or extreme precipitations could induce hyposaline stress (Levinton et al., 2011).

Aquatic organisms are able to tolerate only defined ranges of water salinity and so the input of salt in freshwater induced by the anthropogenic sources combined with climate change has been defined as one of the most important stressors for freshwater ecosystems. These changes may have social and economic consequences (safe drinking water, water used for irrigation in agriculture) and affect biodiversity and ecosystem functioning (Reid et al., 2005). Estuarine organisms are continuously subjected to wide variations of salinity depending on the season, the river flow and the tidal rhythm. A growing attention is paid in ecotoxicology to the fresh waters – marine water continuum, with the variations of physico-chemical conditions that affect the fate and ecotoxicity of toxicants (Nõges et al., 2016). Benthic bivalves are defined as good aquatic bioindicators that represent both water column and sedi-

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ment (Carregosa et al., 2014). *Corbicula fluminea* and *Scrobicularia plana* belonging to this group have been widely used as sentinel organisms in biomonitoring programs of freshwater and coastal areas respectively (Mouneyrac et al., 2008; Sousa et al., 2008; Fossi-Tankoua et al., 2011). They both have a key role in ecosystem structure and functioning, and get a wide geographical distribution. Both species are infaunal benthic organisms that can either feed in suspended particles or remove organic matter from the sediment by pedal feeding. Therefore, considering these organisms as ecosystem engineers (bioturbation activity), they have various impacts on habitat structure, biomineralization, oxygenation and benthic and planktonic community structures (Karatayev et al., 2007).

In the present work, we focused on these two euryhaline bivalves, *C. fluminea* and *S. plana*, presenting an overlap in their range of salinity tolerance, and encompassing the whole spectrum of salinity in fresh and estuarine/marine waters. The global aim was to understand biochemical, behavioral and physiological consequences of salinity changes on those two species in the framework of biomonitoring.

To extract the particular assemblage of halite, sulfates, carbonates and clays, several mines were opened 150 years ago in the Lorraine region (Fanlo and Ayora, 1998). Additionally, the artificial increase in salinity caused by human activity is widely documented in Europe, where potassium and sodium industries are highly developed. Consequently, an increase of salinity has been measured in the Moselle River caused by runoff events and the proximity of two tributaries that flow on salt bedrock. This combination of geological factors and anthropogenic activities led to a high salinity (1.5 practical salinity unit (psu)) for a freshwater ecosystem. *C. fluminea* tolerates a range of salinity between 1 and 13 psu (Morton and Tong, 1985) and after acclimatization they may survive at the maximal salinity of 24 psu (Evans et al., 1977). In estuarine and coastal areas, organisms are regularly subjected to huge variations of salinity under the influence of tidal change and run-off from rivers. *S. plana* optimal salinity is 20–30 psu and their lowest tolerance limit is 5 psu (Verdelhos et al., 2015). Despite a general acceptance of osmoregulatory mechanisms set up by freshwater and estuarine bivalves to cope with salinity variations, there is little evidence of salinity effects at the biochemical (Carregosa et al., 2014) and behavioral levels. Several experiments showed in freshwater organisms that hyper salinity variations induced a reduction of survival rate and modification of behavior responses such as valve closure leading to soft tissues isolation and the activation of anaerobic metabolism (Ferreira-Rodríguez and Pardo, 2016; Evans et al., 1977; Gosling, 2003). Decrease of water content and modification of uptake of L- and D-alanine (Matsushima and Yamada, 1992; Koyama et al., 2015) have been observed on freshwater organisms under hypersaline stress. These studies have shown that euryhaline organisms have a high capacity to cope with salinity variation, due to metabolic changes.

In estuarine organisms, studies conducted to define the impacts of hyposaline variation showed an increase of oxygen uptake and a reduction of filtration rate (Hamer et al., 2008; Navarro, 1988; Navarro and Gonzalez, 1998; Akberali, 1978) and negative correlation between acetylcholinesterase activity and valve closures (Freeman and Rigler, 1957; Pfeifer et al., 2005). Hyper salinity induced oxidative stress in the bivalve *Venerupis philippinarum* revealed by the induction of defence biomarkers as catalase, total antioxidant capacity, glutathione-S-transferase. In order to maintain the ionic equilibrium through osmoregulation processes, it can be hypothesized that organisms exposed to a gradient of salinity will rapidly mobilize their energetic reserves such as glycogen and protein contents (Carregosa et al., 2014).

Therefore, the purpose of this study was to investigate ecophysiological impacts of salinity stress by laboratory exposure of the *C. fluminea* and *S. plana* to a salinity of 15 psu, close to their limit range of tolerance. Both species were also exposed to 1.5 or 30 psu, corresponding respectively to their optimum salinity medium. We made the hypothesis that bivalves exhibited biochemical, physiological and behavioral responses to cope with the higher oxidative-stress and energy demand resulting from hyper and hypo saline conditions for *C. fluminea* and *S. plana*, respectively. Biomarkers at the subindividual level involved in oxidative-stress defences (CAT, TAC, glutathione peroxidase: GPx, GST, metallothionein: MT), cellular damage (lipid hydroperoxides: LOOH acid phosphatase: ACP), apoptosis induction (caspase-3 like: CSP) and energy and structural demand (protein: Prot, triglycerides: Trigly, cholesterol: Chol, electron transfer system: ETS, lactate dehydrogenase: LDH) have been quantified in bivalves exposed to different salinity regimes in the presence or absence of food during 2 and 7 days of exposure. At the individual level, behavioral biomarker (burrowing activity) has been measured.

2. Materials and methods

2.1. Chemical compounds used

All chemical reagents were purchased from Sigma Aldrich (Chemical Co, St Louis, MO) and Thermo-Scientific (Waltham, Massachusetts) products. To provide replication of medium for all experiments, tests were conducted using artificial water Tropic Marine® (Tropicarium Buchshlag Dreieich Germany) (Mouneyrac et al., 2002).

2.2. Collection and acclimatization of organisms

C. fluminea and *S. plana* organisms were collected in June 2014 at Argancy in the Moselle River (49° 19′48′58″W, 6° 19′81.11 N, France) and in the intertidal mudflat located on the French Atlantic coast (1° 59′04′80′íW, 47° 01′50.35″ N, Bay of Bourgneuf, France), respectively. Physico-chemical parameters of surface water of the collection sites were measured with the multiparameter system ODEON (Ponsel, Caudan, France) in the Moselle river (pH: 7.5; salinity: 1.5 psu; conductivity: 2.38 mS cm^{-1}) and in the Bay of Bourgneuf (pH: 8.1; salinity: 31.2 psu; conductivity: 30.88 mS cm^{-1}). Only 15–20 mm ranging shell length bivalves were selected, avoiding potential influence of sexual maturity. Animals were then transported to the laboratory in plastic coolers alongside sediment extracted from the site. Bivalves were placed into aerated artificial water (Tropic Marine®) at 1.5 psu or 30 psu for *C. fluminea* or *S. plana* respectively, during three days in a temperate controlled room at 15 °C (temperature measured at the time and site of collection).

2.3. Experimental design

The experimental design is illustrated in Fig. 1. From the field salinity, 1.5 and 30 psu for *C. fluminea* and *S. plana* respectively, bivalves were progressively transferred, by a 3-day step by step acclimatization, placing them into plastic tanks (50 L) with artificial water at salinity of 5, 10 and 15 psu for *C. fluminea* and 20 and 15 psu for *S. plana* under continuous aeration. For each bivalve species, a control group of organisms was kept in artificial water adjusted to the field salinity: 1.5 psu for *C. fluminea* and 30 psu for *S. plana*.

Bivalves (n = 10 for each species and per tested salinity) were introduced into beakers (3L) containing artificial water at both tested salinities (*C. fluminea*: 1.5 and 15 psu and *S. plana*: 15 and 30 psu) and aerated 2 h/day to maintain oxygen levels near saturation. Salinity, pH, conductivity, oxygen rate were measured every day. The species were exposed during 2 and 7 days under two diet conditions (unfed or fed). *S. plana* and *C. fluminea* were fed with