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# Characterization of the salt stress vulnerability of three invasive freshwater plant species using a metabolic profiling approach

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ABSTRACT

The effects of salt stress on freshwater plants has been little studied up to now, despite the fact that they are expected to present different levels of salt sensitivity or salt resistance depending on the species. The aim of this work was to assess the effect of NaCl at two concentrations on three invasive freshwater species, *Elodea canadensis*, *Myriophyllum aquaticum* and *Ludwigia grandiflora*, by examining morphological and physiological parameters and using metabolic profiling. The growth rate (biomass and stem length) was reduced for all species, whatever the salt treatment, but the response to salt differed between the three species, depending on the NaCl concentration. For *E. canadensis*, the physiological traits and metabolic profiles were only slightly modified in response to salt, whereas *M. aquaticum* and *L. grandiflora* showed great changes. In both of these species, root number, photosynthetic pigment content, amino acids and carbohydrate metabolism were affected by the salt treatments.

Moreover, we are the first to report the salt-induced accumulation of compatible solutes in both species. Indeed, in response to NaCl, *L. grandiflora* mainly accumulated sucrose. The response of *M. aquaticum* was more complex, because it accumulated not only sucrose and myo-inositol whatever the level of salt stress, but also amino acids such as proline and GABA, but only at high NaCl concentrations. These responses are the metabolic responses typically found in terrestrial plants.



## Introduction

Human activities can induce salt accumulation in irrigated soils, the discharge of high salinity waters and fluctuating salinities in aquatic ecosystems (Rengasamy, 2006). According to the climatic models, global warming will lead to a sea-level rise and logically will enhance sea water intrusion into coastal marshes and will increase water salinization (IPCC, 2001). Water salinization affects the photosynthesis, productivity and viability of plants (Allakhverdiev et al., 2000; Parida and Das, 2005; McGregor et al., 2007) and consequently the composition and structure of plant communities (Hart et al., 1991; Smith et al., 2009).

High concentrations of salt impose both osmotic and ionic stresses due to reduced water availability and to the accumulation of ions in cells, respectively, which have inhibitory effects on many physiological processes. To overcome such stress, plants have developed strategies which mainly lead to various morphological and physiological adaptations (Greenway and Munns, 1980). Physiological adaptations could be salt exclusion or sequestration of salt ions in vacuoles and accumulation of compatible compounds into the cytoplasm, including amino acids, carbohydrates, and alcohols, to balance the osmotic pressure (Strizhov et al., 1997; Nakamura et al., 2001). Salt stress responses and tolerance vary between plant species (Munns and Tester, 2008). The production of organic solutes in response to salt stress has been investigated in numerous terrestrial plant species. Although freshwater plants could be especially sensitive to water salinization, their ecophysiological responses to salt stress have been little studied, and concern only a few species.

Some studies have shown that chloride may accumulate in the cell vacuoles of freshwater plant leaves (Sculthorpe, 1967) or

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that species such as *Myriophyllum spicatum*, were able to regulate salt intake and maintain cellular concentrations of the ions (Anderson et al., 1966). Other studies focus on the production of compatible solutes in response to salt stress. It was reported that proline and glycinebetaine accumulate in response to salt stress although glycinebetaine does not seem be an ideal osmoprotectant for aquatic plants compared with proline (Tripathi et al., 2007). Synthesis and accumulation of proline was described in several aquatic plants (Rout and Shaw, 1998; Tripathi et al., 2007) such as: *Salivinia natans*, *Hydrilla verticillata*, *Najas indica*, *Najas graminea*, *Spirodela polyrhiza* and *Azolla filiculoides* (Rout and Shaw, 1998; Tripathi et al., 2007; Jampeetong and Brix, 2009; van Kempen et al., 2013). Other compounds like carbohydrates could participate in osmotic adjustment (Parida and Das, 2005), however, there have been few investigations on freshwater plants.

In this study, we examined the changes in morphological and physiological traits and the changes in metabolite profiles in response to salt stress, of three freshwater plants species: *Ludwigia grandiflora*, *Myriophyllum aquaticum* and *Elodea canadensis*. These species have invaded numerous aquatic ecosystems in Europe and are well represented in coastal marshes where they exhibit different tolerances to soil and water salinity levels. *E. canadensis* is primarily a freshwater species, and occasionally grows in brackish water; it tolerates salinities up to 2.5 per mile (Sand-Jensen, 2000). Compared to *L. grandiflora*, *L. peploides* can grow in brackish waters of the Camargue, with salt concentrations of about 10 g $L^{-1}$ (Grillas, 2004; Mesleard and Perennou, 1996). Haller et al. (1974) found that the toxic salt concentration for the growth of *M. aquaticum* was between 10 and 13.3 g $L^{-1}$. In previous work, we showed that increased salt levels induced a decline in growth and photosynthetic activity of *L. grandiflora*, while photosynthetic activity of *M. aquaticum* remained constant up to concentrations of 6 g $L^{-1}$ of salt (Thouvenot et al., 2012). Water salinization of freshwater ecosystems could limit their abundance, distribution and expansion range in the next decades. However, to our knowledge, there are no or few studies about their physiological characteristics or physiological responses to salt stress. The objectives of this study were to determine the short-term physiological responses to salt stress of these three invasive freshwater plants, in order to compare their salt response and sensitivity.

## Materials and methods

### *Biological material*

*Myriophyllum aquaticum* (Haloragaceae) is an aquatic (or semi-terrestrial) perennial plant native from tropical and subtropical South America (Aiken, 1981), which was introduced into France in about 1880 (Sheppard et al., 2006) and has invaded many areas in Europe (Les and Mehrhoff, 1999). *M. aquaticum* is often found in eutrophic water bodies and could be used in the phytoremediation of polluted water (Souza et al., 2013).

*Ludwigia grandiflora* (Onagraceae) is an aquatic (or semi-terrestrial) perennial plant, native to South America which was introduced in 1820 in south-eastern France (Dandelot et al., 2008) and has also colonized many countries in Europe (Dutartre et al., 2007; Hussner, 2009). The growth of *L. grandiflora* is enhanced by increasing nutrient availability but the plant is able to develop in oligotrophic waters (Hussner, 2010).

*Elodea canadensis*, (Hydrocharitaceae) is an aquatic species, coming from North America which was introduced in Europe in the early 19th century and became widely distributed in Europe (Cook and Urmi-Konig, 1985). It is well adapted to a broad range of environmental conditions (Cook and Urmi-Konig, 1985) and is used for pollution monitoring (Samecka-Cymerman and Kempers, 2003) due to its abilities to accumulate pollutants (Kähkönen et al., 1997; Thiébaut et al., 2010).

### *Experimental design*

The plants were collected in natural environmental conditions from a pond called Apigné, in Brittany, France (48°05′31.3″N; 01°44′41.3″W). At the beginning of the experiment, individuals of the plants corresponded to apices of 10 cm, without roots, buds, and lateral stems. Plants were cleaned gently by hand to remove epiphytic algae. The plant sample consisted of a pool of at least three individuals, and the biomass of the plant sample was measured. Samples were randomly placed in each container with 500 mL of saline solution. The three salt concentrations tested corresponded to concentrations found in coastal marshes. Saline solutions were created by using a Hoagland's solution (Hoagland and Arnon, 1950) in combination or not (control) with two salt concentrations: 3 g $L^{-1}$ (≈50 mM) and 6 g $L^{-1}$ (≈100 mM). Three replicates were used for each saline solution and species. The containers were placed randomly in growth chambers at 20 °C day/16 °C night and in a 14 h day/10 h night cycle. The light intensity in the growth chambers was 420 μmol $m^{-2}$ $s^{-1}$ of PAR. Morphological traits and plant metabolism were studied after 7 days of exposure to saline solution.

### *Morphological traits analysis and determination of the water content*

At the end of experiment, the length of each individual and the number of roots were measured. Each plant sample was divided into two parts. The submerged and emerged parts were weighed separately (fresh weight; FW), then freeze-dried in liquid nitrogen and lyophilized for 48 h. After lyophilization, each part of the sample was weighed (dry weight; DW). Then, dry plant material was ground, and stored at −80 °C before subsequent metabolite analysis. Metabolism analysis and measurement of photosynthetic pigments were realized on the emerged parts of *L. grandiflora* and *M. aquaticum* and on the submerged part of *E. canadensis*.

The growth of macrophytes was evaluated by the Relative Growth Rates (RGR) which was adapted from Hunt (1990): $RGR = (\ln FW2 - \ln FW1)/(T1 - T2)$, where FW1 and FW2 refer to total Fresh Weight of sample (or length of each fragment) at times 1 and 2. The Water Content (WC) was then calculated as $[(FW - DW)/FW] \times 100$.

### *Determination of photosynthetic pigments*

Tissues were freeze-dried in liquid nitrogen, lyophilized and homogenized with a 4 mm steel ball for 1 min at 30 $s^{-1}$ frequency using a mixer mill (Mixer Mill MM 400, Retsch). Photosynthetic pigments were extracted with 80% acetone (150 μL $mg^{-1}$ DW) for 1 h under 1000 rpm agitation. After centrifugation at 13,000 × *g* for 1 min, supernatants were recovered. Pellets were re-suspended in 80% acetone (100 μL $mg^{-1}$ DW), then after centrifugation at 13,000 × *g* for 1 min, supernatants were recovered and pooled with those obtained from the first extraction. Absorbance of the extracts was measured at 663 nm, 647 nm and 470 nm. The chlorophyll and carotenoid contents were then calculated according to Lichtenthaler's (1987) equations.

### *Metabolite analysis*

Metabolic profiling was performed on the 27 samples, using gas chromatography (GC) for carbohydrates and ultra performance