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Salinity fluctuation of the brine discharge affects growth and survival of the seagrass *Cymodocea nodosa*

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ABSTRACT

The increase of seawater desalination plants may affect seagrasses as a result of its hypersaline effluents. There are some studies on the salinity tolerance of seagrasses under controlled laboratory conditions, but few have been done in situ. To this end, *Cymodocea nodosa* shoots were placed during one month at four localities: two close to a brine discharge; and the other two not affected by the discharge, and this experiment was repeated four times. The results obtained showed a decrease in growth and an increased mortality at the localities affected by the brine discharge. An increase was detected in the percentage of horizontal shoots in respect to vertical shoots at the impacted localities. It is probably that not only the average salinity, but also the constant salinity fluctuations and slightly higher temperatures associated with the brine that may have caused physiological stress thus reducing *C. nodosa* growth and survival.

1. Introduction

Seagrass meadows are keystone components of coastal ecosystems throughout the world and are important for the health of coastal ecosystems (Durako, 1994; Zieman et al., 1999). Increasing human activities in the shallow water environment often result in impairment, reduction, or decimation of seagrass meadows. These activities, which include trawling fishing, dredging, fish cultures, sewage and waste disposal (Livingston, 1987; Sánchez-Lizaso et al., 1990; Short and Wyllie-Echeverria, 1996; Delgado et al., 1997) as well as the brine discharge from the desalination plants (Férnandez-Torquemada and Sánchez-Lizaso, 2007), constitute perturbations which may adversely affect seagrasses. Brine discharge is the concentrate waste from a desalination plant, which mainly contains a high percentage of salts and additionally some chemicals (such as biocides, antiscalants, coagulants, etc.) depending on the desalination technology employed. In the case of SWRO (sea water reverse osmosis) plants, this reject water is returned back to the sea and spreads according to bathymetry and hydrodynamics (Einav et al., 2002; Férnandez-Torquemada and Sánchez-Lizaso, 2007). The discharged brine may change the salinity and the temperature of the seawater and can cause changes on marine habitats (Lattemann and Höpner, 2003). While salinity may fluctuate in coastal areas or in confined systems due to the relationship between inputs of continental waters and evaporation, in the open sea, where the majority of the brine discharge take place (Fernández-Torquemada et al., 2009), salinity fluctuations are less important.

The increase of desalination plants worldwide has enhanced the necessity to study the effects that salinity changes, associated to brine discharges, may produce in seagrasses. When seagrasses are exposed to salinity changes, they may suffer osmotic stress which is translated into alterations of their growth and survival (Walker, 1985; Vermaat et al., 2000; Fernández-Torquemada and Sánchez-Lizaso, 2005, 2011; Gacia et al., 2007; Ruiz et al., 2009), photosynthesis (Ralph, 1998; Fernández-Torquemada et al., 2005a; Koch et al., 2007), reproduction (Caye and Meinesz, 1986) and synthesis of new solutes (Kirst, 1989; Tyerman, 1989). However, most of those published studies only focus on the tolerance of seagrass species to salinity, but a growing number of recent studies have highlighted the role of salinity interactions with other factors such as pH, temperature, nutrients, light and photoperiod, and sulfide (Pulich, 1986; van Katwijk et al., 1999; Vermaat et al., 2000; Koch and Erskine, 2001; Hackney and Durako, 2004; Fernández-Torquemada et al., 2005a; Fernández-Torquemada and Sánchez-Lizaso, 2011). Most of these works were carried out in mesocosms' stable conditions and some works were done in hypersaline lagoons (Walker, 1985; Walker et al., 1988; Terrados and Ros, 1991) or environments with salinity fluctuations

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(Verhoeven, 1975; Montague and Ley, 1993; Lirman and Cropper, 2003). However, few studies have been done on the tolerance of seagrasses subjected to a real brine discharge (Gacia et al., 2007; Ruiz et al., 2009).

Cymodocea nodosa (Ucria) Aschers is a common species in the Mediterranean and the Eastern Atlantic, from South Portugal to Senegal and around Canary Islands (den Hartog, 1970). It is a relatively small, fast-growing, colonising seagrass with a high turnover and low below-ground biomass (den Hartog, 1970; Pérez and Romero, 1994), that shows a remarkable growth and resource allocation plasticity (Pérez et al., 1994) and can tolerate a wide range of environmental conditions (Phillips and Meñez, 1988). However, the degradation of *C. nodosa* beds has been observed due to several anthropogenic impacts such as a marina construction (Tuya et al., 2002), intensively irrigated agriculture (Lloret et al., 2005), mining wastes (Marín-Guirao et al., 2005), fish cultures (Delgado et al., 1997) and eutrophic conditions (Pérez et al., 1994; Cunha and Duarte, 2005). All these facts make it an ideal species for experimental transplants to study the recovery of an area (Pranovi et al., 2000; Curiel et al., 2005; Zarranz et al., 2010), or the effects of changes in environmental factors (Balestri and Lardicci, 2013).

C. nodosa can inhabit estuarine areas and coastal lagoons with large fluctuations in salinity (Greve and Binzer, 2004). It is common in river mouths or deltas with low salinity (Delta del Ebro), but also in hypersaline lagoons like the Mar Menor (42–47 psu; Terrados and Ros, 1991; Fernández-Torquemada and Sánchez-Lizaso, 2011). However, tolerance of this species to salinity changes may depend on the population (Fernández-Torquemada and Sánchez-Lizaso, 2011) and currently there is no information on the response of Mediterranean populations of this species adapted to open sea environments submitted to a brine discharge. Nevertheless, it has been planned that some SWRO desalination plants discharge its brine on meadows of this species (e.g. SWRO desalination plant of Torrevieja, SE Spain).

In the present study, short-term (1 month) transplanting experiments were carried out to estimate the effect of a real brine discharge from a SWRO desalination plant on the seagrass *C. nodosa*. We hypothesized that salinity increment associated to a desalination brine discharge may have a negative effect in the vitality of *C. nodosa* at lower salinities that those observed in mesocosm studies (Fernández-Torquemada and Sánchez-Lizaso, 2011); so we expected to find a lower leaf growth and an increment of mortality of this seagrass when it is exposed to a brine effluent.

2. Material and methods

2.1. Description of the desalination plant and its brine discharge

The study was carried out in Alicante Bay (Northwestern Mediterranean Sea), close to a brine discharge from two SWRO desalination plants. One of the desalination plants began operations in September 2003, with an initial production of 50,000 $m^3\ d^{-1}$ of freshwater and a discharge of 66,000 $m^3\ d^{-1}$ of brine with a salinity of 68 psu. In January 2006, the desalination plant was enlarged to produce up to 68,000 $m^3\ d^{-1}$ and a second desalination plant with the same capacity started to operate in summer 2008, when the discharge was doubled. Following this increase in capacity, the option of diluting the brine with seawater prior to discharge was adopted. Although the dilution of the brine is not constant, and has been oscillating between 1.5 and 5 parts of seawater to each part of brine according to the results of the monitoring program (Fernández-Torquemada et al., 2009, 2013). In this monitoring program (2004 to present) it has also been found that the discharge is mainly characterized by an increase in salinity (slightly in temperature), and that it is not accompanied by other environmental problematic compounds that sometimes have been associated with this type of activity, such as orthophosphates or sodium bisulfite (Lattemann and Höpner, 2003).

Brine discharge is located directly on the shoreline south of Alicante city, since this was an area degraded by previous impacts caused by different local disturbances, such as the influences of the Alicante city and its port, bottom trawling, boat anchoring and sewage and industrial outfalls. *Posidonia oceanica* meadow in this area is in decline; currently its upper limit is at 17 m, whilst between 17 and 5 m depth, dead *Posidonia* matte, covered by muddy sediments and opportunistic macroalgae, dominate the benthos. During the summer period (June–September), it is detected a seasonal thermocline (12–13 m depth) in the area that avoids highest salinities affecting at this *Posidonia oceanica* meadow, so brine dilution is usually reduced and it is noticed an increase in salinity at shallower depths (Fernández-Torquemada et al., 2009, 2013).

2.2. Plant sampling and experimental design

Plant fragments of *C. nodosa* with intact attached rhizome-connected shoots and roots were collected by SCUBA diving from well-preserved meadows at 6 m depth located at a single undisturbed site at 15 km south from the desalination brine discharge from June to September 2009. The rhizomes were extracted by digging under the sediment by hand; this technique allowed us to minimise disruption of the root-rhizome layer. Plants were collected at the edge of a patch where it was easier to remove and may be better suited for transplanting (Thom, 1990). The plants were brought to the University of Alicante (Spain) in coolers from the sampling site and were attached to a rigid plastic net (40 × 40 cm). In each net, five randomly intact rhizome fragments with bare roots and containing 10–15 connected shoots were attached, as a similar method based on apical rhizome fragments has been employed by Balestri and Lardicci (2012, 2013) with a relatively high success. Shoots were marked at the beginning of the experiment using the modified Zieman method (1974). Following the day of collection, three nets were placed at the sea bottom, at 1 m intervals, at four localities at the same depth (10 m) in Alicante Bay (Fig. 1). Two localities were selected under the influence of the brine discharge of both SWRO desalination plants, impact localities (I1 and I2), and the other two, of similar physical and biological conditions (seabottom with dead *Posidonia* matte covered by muddy sediments and opportunistic macroalgae) and remote of the brine discharge, considering them as control localities (C1 and C2).

A continuous record of salinity, temperature and irradiance every 10 min for each locality and experiment was obtained. Salinity and temperature were measured by placement of a CT-Compact model (Alec Electronics, resolution 0.001 °C and 0.001 mS/cm) at 10 cm from the bottom, which was the average height of *C. nodosa* leaf canopy above the seafloor. All data collected by the CTs were processed eliminating those erroneous data caused by fouling.

The continuous irradiance measurements were taken using an underwater photometer (Ultra-Miniature Light Intensity Recorder (MDS-Mk V/L), resolution 1 $\mu m/m^2\ s$) and, in order to avoid the possible effects of shading caused by fouling, the data taken during the first ten days of each experimental period was used (González-Correa et al., 2008). Percentage of surface irradiance (% Is) and the daily integrated PPFD (400–700 nm Photosynthetic Photon Flux Density, mol quanta $m^{-2}\ d^{-1}$) were calculated for the recorded period in order to estimate the effect of silt–clay fraction on the water column turbidity (González-Correa et al., 2008). To calculate percentages, the underwater radiation was compared with the air global radiation (10 $kJ\ m^{-2}\ s^{-1}$) from the weather bureau of