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Influence of salinity, nutrients and light on the germination and growth of *Enteromorpha* sp. spores

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Abstract

In many shallow coastal areas worldwide, several species of opportunistic macroalgae (mainly Chlorophyta) have an excessive growth, as a consequence of eutrophication processes. Therefore, bloom-forming macroalgae become the dominant primary producers within these coastal systems. However, frequently the ecology and the ecophysiology of adult macroalgae have been insufficient to explain their seasonal abundances. Thus, it is essential to understand the factors that regulate the germination and growth of spores of opportunistic green macroalgae. In the present work, we assessed the effects of nutrients (N and P), salinity and light on the germination and growth of *Enteromorpha* spores. Overall, the results highlight the fact that, such as for adult macroalgae, spore germination and growth are adversely affected by low salinities. Growth of the spores is significantly decreased at 5 psu, while salinities of 20 and, especially of 35 psu, clearly promote the spore growth. Additionally, *Enteromorpha* spores seem to be particularly sensitive to PO_4–P limitation and to NH_4–N toxicity, which suggests a higher sensitivity to the variation of external nutrient concentrations than adult macroalgae. The present results contribute to increase the understanding about the factors that control macroalgal growth at its early phases of development. In particular, the results suggest that the growth of spores from opportunistic green macroalgae is strongly salinity-dependent. Consequently, in highly hydrodynamic systems such as most shallow estuaries, salinity variations may play a determinant role in the yearly abundances of green macroalgae, since it controls macroalgal growth from the spores to the adults.

Keywords: *Enteromorpha*; Light; Macroalgal blooms; Nutrients; Salinity; Spores

1. Introduction

Eutrophication of estuaries and shallow coastal areas is a worldwide worrying phenomenon (e.g. Flindt et al., 1997; Raffaelli et al., 1998; Martins et al., 2001; Naldi and Viaroli, 2002; Huang et al., 2003; Kamer et al., 2004). Massive proliferations of fast-growing green macroalgae frequently occur as a response to the increased

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concentration of growth limiting nutrients (Duarte, 1995; Valiela et al., 1997; Schramm, 1999; Flindt et al., 1999; Cardoso et al., 2004). These bloom-forming species are characterized by rapid nutrient uptake and high growth rates (Fujita et al., 1988; Hein et al., 1995; Taylor et al., 1998), becoming the dominant primary producers in these eutrophic systems (Valiela et al., 1997; Flindt et al., 1997; Hauxwell et al., 2001; Cardoso et al., 2004). Following the macroalgae biomass bloom, decomposition processes become more frequent and consequently oxygen depletion in the water column and sediment surface may occur, possibly conducting to anoxic situations during night periods (Flindt et al., 1999), which will be harmful for the estuary communities at different trophic levels (Valiela et al., 1997; Raffaelli et al., 1998; Bolam et al., 2000; Pardal et al., 2004). Due to its effects at the system level, it becomes fundamental to study the biotic and abiotic conditions that originate from the enhanced proliferations of opportunistic green algae.

Enteromorpha intestinalis (*Ulva intestinalis*, according to the recent revision of Hayden et al., 2003) is the most common bloom-forming macroalgae species occurring in intertidal flats of the Mondego estuary (Western coast of Portugal) (e.g. Martins et al., 2001; Pardal et al., 2004; Cardoso et al., 2004; Lillebø et al., 2005). As a fast-growing and opportunistic macroalgae, this species can proliferate in a wide range of abiotic (temperature, light, nutrients, salinity) and biotic (herbivory, competition) conditions (e.g. Poole and Raven, 1997; Martins et al., 1999; Schories et al., 2000; Lotze and Worm, 2002).

Several studies have shown that early-life stages and its conditioning factors assume an essential role on determining the development patterns of macroalgal blooms (Lotze et al., 1999, 2000) and that "banks of microscopic forms" (*sensu* Chapman, 1986) from marine macroalgae, which consist of settled propagules, micro-recruits and microscopic life stages, constitute an important overwintering mechanism in temperate ecosystems, allowing algal recruitment in the following growth season (Schories, 1995; Lotze et al., 1999, 2000; Schories et al., 2000). Additionally, since spores of bloom-forming macroalgae have a greater sensitivity to different abiotic and biotic factors than adult stages (Lotze et al., 1999, 2000; Cordi et al., 2001), they are crucial to the knowledge of all life history stages.

Previous works had already accounted for the influence of abiotic and biotic factors on *E. intestinalis* recruitment (e.g. Lotze and Worm, 2002), suggesting that typical spring and summer high temperatures and light have a positive influence on the germination and growth of *E. intestinalis* spores (Lotze et al., 1999; Lotze and Worm, 2002). Experimental evidence also suggests that *Enteromorpha* spores can survive more than 10 months in darkness (Schories, 1995) and germinate (*E. intestinalis* and *Enteromorpha compressa*) in total darkness (Santelices et al., 2002). Germlings growth rate (after germination) has been reported to vary depending on light intensity among algal species, even within green macroalgae (Santelices et al., 2002). In spite of these works, the general knowledge of the factors that control the growth of green macroalgae at its early stages is still very scarce.

In the Mondego estuary (Western Portugal), previous works have shown that the abundance of adults from *Enteromorpha* sp. depends on hydrodynamics and on river flow management (Martins et al., 2001). In turn, these factors control the local variation of salinity, current velocities, light extinction coefficients and N:P ratios (Martins et al., 2001). However, except for the evidence that spores can grow attached to shells of *Hydrobia ulvae* (Schories et al., 2000), little is known about the local abundance of *Enteromorpha* spores and the factors that control it. Thus, since blooms of macroalgae constitute a worldwide problem nowadays, the aim of this work was to assess the combined effects of nutrient enrichment (nitrogen and phosphorus), salinity and light on the germination and growth of the early-life stages of *Enteromorpha* sp. Experimental procedure was based on four fully crossed experiments performed in the laboratory, with a two-factor experimental design. The results from this work will contribute to increase the knowledge of the factors that control eutrophication symptoms, in general.

2. Methods

2.1. Sampling strategy and culture conditions

During ebb tide, for each experiment, fifty Plexiglas circular plates (∅ 7 cm) were placed in an intertidal mudflat located in the Mondego estuary. To ensure the attachment of macroalgal spores to the surface of the Plexiglas plates, because the surface properties greatly affect settlement success (Lobban and Harrison, 1994), the plates had a rough surface, which favoured the adhesion of the spores. The replicates were hung from a rope that became submerged during high tide. They were placed 10 cm apart from each other and 20–30 cm above the sediment surface, in order to prevent sediment particles deposition. Two days later, the plates were collected and transported to the laboratory in a cooler within 1 h.

In the lab, 5 plates were immediately treated for quantification of spore biomass (see Experimental procedure 2.3). Depending on the observed germlings growth, each experiment lasted for 10–14 days wherein