Repercussions of salinity changes and osmotic stress in marine phytoplankton species

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ABSTRACT

The short-term effect of low salinity was studied using laboratory protocols on some coastal phytoplankton species such as chlorophycea *Tetraselmis suecica*, among diatom the strain *Nitzschia* N1c1 and dinoflagellates *Alexandrium minutum* and *Prorocentrum lima*. All of cultures were exposed to low salinities, and cell growth rate, photosynthetic quantum yield (Φ_{PSII}), and gross photosynthesis (P_g) were analyzed. Growth rate inhibition was similar in all species, and all of them also tolerate short-term exposures to salinities in the range 5–35. There were no significant differences between Φ_{PSII} and P_g endpoints from *Tetraselmis suecica* and *Nitzschia* sp., while *Alexandrium minutum* and *Prorocentrum lima* displayed a higher affectation rate on P_g than on Φ_{PSII} activity. The influence of low salinity was higher on respiration in *T. suecica*, while both dinoflagellates had higher net photosynthesis. *Nitzschia* sp. exhibited similar involvement of the two photosynthetic parameters. Therefore, although the four phytoplankton monocultures studied are able to survive in internal areas of estuaries under low salinity conditions, the photosynthetic activity is more affected than the growth rate in all phytoplankton communities studied except in chlorophycea *T. suecica*, which has increased tolerance for this salinity decrease.

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1. Introduction

Osmotic stress is one of the most significant abiotic cellular stresses and affects every aspect of plant physiology and metabolism. The physiological and biochemical responses are extensively studied to understand how algae respond and adapt to salinity changes (Kirst, 1990). In estuaries, tides usually extend inland the influence of salinity. As a result, the upper reaches of estuaries are freshwater systems characterized by the presence of a tidal regime, the freshwater tidal reaches (Muylaert et al., 2005). While several detailed phytoplankton studies have been carried out in the brackish reaches of some rivers (Soetaert and Van Rijswijk, 1993; Van Spaendonk et al., 1993; Kromkamp and Peene, 1995), there is little information of marine phytoplankton physiological responses in the freshwater tidal reaches, with comparison among growth, photosynthesis and respiration.

In the particular case of estuaries, the continuous river-sea transition and the tidal influence are considered the main determining features of the phytoplankton biomass distribution (Calliari et al., 2005; Domingues et al., 2005; Hagy et al., 2005). However, local physico-chemical and biological factors such as water column depth, light availability, nutrient turnover, grazing pressure and species-specific interactions could eventually mask the effect of the longitudinal hydrological gradient (Lucas et al., 1999; Kocum et al., 2002).

Estuarine circulation is a common phenomenon in all estuaries (Day et al., 1989) and shown to be responsible for the transport of phytoplankton community (Tyler and Seliger, 1978; Malone et al., 1980) from coastal waters into estuaries. Thus, the upper reaches of estuaries are often characterized by massive phytoplankton blooms (Moon and Dunstan, 1990; Cole et al., 1992; Kies, 1997; Muylaert et al., 2005).

The development of models aimed at predicting bloom development and toxicity, which will aid coastal resource managers in their efforts to mitigate the wide ranging effects of harmful algal blooms, is currently a global research emphasis. However, the available field data indicate that bloom toxicity for various harmful algal species can vary considerably depending on the physiological status of the algal cells (Anderson et al., 1990; Poulton et al., 2005) and thereby influence the severity of a bloom event. Multiple

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physico-chemical factors (e.g. temperature, salinity, nutrients) have been reported to change the production of most algal toxins in both laboratory and field populations (Granéli and Flynn, 2006). In order to improve prediction of bloom toxicity, it is essential to understand how and which variables modulate biosynthesis and intracellular accumulation of these toxins.

Blooms of toxic phytoplankton have been always associated with estuarine coastal regions (Larocque and Cembella, 1990; Lim and Ogata, 2005). Giacobbe et al. (1996) showed that the spring blooms of the dinoflagelate *Alexandrium minutum* in Mediterranean Sea coincided with the increase in rainfall and freshwater runoff that increased the stratification of the water column. Similar findings were obtained in studies with diatoms (Doucette et al., 2008) or *Prorocentrum* spp (Morton et al., 1992) blooms under conditions of low salinity. These data seem to establish a relationship between salinity levels in the aquatic environment and the potential risk from toxic phytoplankton blooms and, interestingly, all these species were found in or close to estuarine regions. *Alexandrium minutum* was found in estuarine semi-enclosed lagoon where blooms of this species caused one casualty and six persons were hospitalized (Lim et al., 2002); *Prorocentrum lima* blooms generally occur in zones affected by freshwater inputs (large deltas, estuaries, fjords, lagoons) and/or anthropogenic inputs (Grzebyk and Berland, 1996); and Thessen et al. (2005) reported an abundance of diatoms in coastal areas where salinity varied widely.

As it is well known that the salinity change can result in osmotic stress on cells, uptake or loss of ions and effects on the cellular ionic ratio in phytoplankton, and because the salinity tolerance of phytoplankton differ and based on their tolerance extent they are grouped as euryhaline and stenohaline species, any large-scale change in the phytoplankton community can have a serious ecological impact. For these reasons, the goal of this study was to experimentally test the hypotheses that the physiology of some common phytoplankton species is significantly influenced by tidal salinity changes, and if there are differences in behavior between toxic and non-toxic species. Experiments with these phytoplankton species were conducted to test the inhibitory effects of low salinity on growth rate, photosynthesis and respiration.

2. Methods

2.1. Cultures

The marine microalga *Tetraselmis* spp is a convenient model for physiological and biochemical studies of the mechanisms of the adaptation to salinity (Strizh et al., 2004), and for the maintenance of cellular ion homeostasis in particular when they are exposed to decreasing salinity concentrations. Therefore this species was included in this study together with *Nitzschia* sp. N1c1, *Alexandrium minutum* and *Prorocentrum lima*. Furthermore, *Tetraselmis* spp grows naturally in estuaries, and high tolerance to low salinity is expected. As diatoms are the major group of photoautotrophic organisms inhabiting intertidal fine sediments in estuaries (Underwood et al., 1998), we have included the strain *Nitzschia* sp. N1c1 as a benthic phytoplankton representative of them. Finally, we have included two representative harmful bloom-forming species, *Prorocentrum lima* and *Alexandrium minutum*, involved in diarrheic (DSP) and paralytic (PSP) shellfish poisons, respectively.

Strains of chlorophycea, *Tetraselmis suecica*, (non-toxic) diatom, *Nitzschia* N1c1 (toxic), and dinoflagellates *Alexandrium minutum* and *Prorocentrum lima* (toxic) were obtained from algal culture collection of Genetics, Faculty of Veterinary, Complutense University, Madrid, Spain. Cells were axenically grown in cell-culture flasks with 20 mL of artificial seawater (30) enriched with F-2 medium (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), at 20 °C and a photon irradiance of 60 μmol m^{-2} s^{-1} over the waveband 400–700 nm, in a 16:8 h light-dark photoperiod. Cells were maintained in mid-log exponential growth by serial cell transfers to fresh medium. Prior to the experiments, the culture cells were recloned (by isolating a single cell) to ensure genetic homogeneity in all the cultures. Inoculations were taken from precultures set up three days before the experiment and cultured/grown under the same conditions.

2.2. Toxicity tests

Sea salt (free from nitrate, phosphate and silicate) was purchased from SERA® (Heinsberg, Germany) and was dissolved in distilled water. As SERA® sea salt consists of many different compounds that are present at a wide range of concentrations, the results presented in this work will be expressed as Practical Salinity Scale which is defined in terms of the ratio K_{15} of the electrical conductivity of the seawater sample, at a temperature of 15 °C and a pressure of one standard atmosphere, to that of a potassium chloride (KCl) solution, in which the mass fraction of KCl is 32.4356×10^{-3} at the same temperature and pressure.

Toxicity tests were performed to determine the inhibitory effects of low salinity on algal growth, photosynthetic quantum yield and light-dark balance of oxygen at different salinites. The tests were performed in sterile 5 mL polystyrene culture tubes (Sarstedt Co., Nümbrecht, Germany) filled with F-2 medium. Previous studies determined the suitability for using polystyrene culture tubes in toxicity assays, ensuring that chemicals and cells do not adhere to the wall tubes (Costas et al., 2001; García-Villada et al., 2002). The water used for media preparation was of ultrapure quality, distilled by means of Milli-Q device (Millipore, Bedford, MA, USA) and sea salt was added to the culture medium. Four salinity concentrations (10, 15 20, and 25), as well as four controls (30), were established and tested. In addition, as an internal quality control for testing the cultures, the bioassays were also performed on the reference chemical potassium dichromate (K$_2$Cr$_2$O$_7$), and each assay was repeated eight times (n = 8). Both control and test tubes were inoculated with 10^4 cells mL^{-1} as initial concentration.

All the cultures (control and treatments) were incubated for 72 h at 20 °C in a thermostatically controlled chamber (Velp Scientifica, Usmate, Italy) at 60 μmol m^{-2} s^{-1} to ensure exponential algal growth. Every 24 h the algal density was quantified under the light microscope with a Neubauer's chamber.

The concentration causing 50% growth inhibition of algae was determined by using a light microscope and Neubauer's chamber (Brand, Wertheim, Germany). The quantity causing 50% inhibition of photosynthetic quantum yield (Φ_{PSII}) was obtained by means of the dual-channel PAM chlorophyll fluorometer (ToxY-PAM, Heinz Walz GmbH, Germany). The ToxY-PAM dual-channel yield analyzer obtains highly sensitive measurements of effective quantum yield of the algae pigment system II centers via assessment of the chlorophyll fluorescence yield and the saturation pulse method (Schreiber et al., 2002). The light-dark oxygen balance was analyzed using a Clark-type O$_2$ electrode. Dissolved O$_2$ was measured in a 1 mL reaction chamber from a Chlorolab 2 System (Hansatech, Norfolk, UK). Chlorolab 2. This system allows the study of respiration and photosynthesis processes from liquid samples, under automated illumination from red (660 nm) LED light and darkness. In the toxicity assays, measurements were taken at 20 °C and 975 μmol m^{-2} s^{-1} irradiance. Light-dark oxygen balance, or gross photosynthesis rate (P$_g$) was estimated from the formula:

$$P_g = P_n + R$$

where P$_g$ corresponds to the oxygen production rate in light