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The dinoflagellate *Prorocentrum cordatum* at the edge of the salinity tolerance: The growth is slower but cells are larger

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ABSTRACT

In this study we examine how the projected climate change driven decrease in the Baltic Sea salinity can impact the growth, cell size and shape of the recently invaded dinoflagellate *Prorocentrum cordatum*. In laboratory treatments we mimicked salinity conditions at the edge of the mesohaline south-eastern Baltic and oligohaline-to-limnic Curonian Lagoon. We used an innovative computer-based method allowing detection of *P. cordatum* cells and quantitative characterization of cell contours in phytoplankton images. This method also made available robust indicators of the morphometric changes, which are not accessible for an expert studying cells under light microscope. We found that the salinity tolerance limit of *P. cordatum* ranges between 1.8 and 3.6, and that the mean cell size of its population is inversely proportional to both salinity and nutrient content. Under ambient south-eastern Baltic salinity (7.2) the nutrients were stimulating the growth of *P. cordatum*; while at the edge of its salinity tolerance the nutrient availability did not have such effect. We suggest that in the future Baltic the decline in salinity and increase in nutrient loads may result in larger cells of *P. cordatum* and extended duration of their presence in plankton, causing longer periods of algal blooms.

1. Introduction

Projected climate change is of growing interest, especially for estuarine and coastal areas, such as the Baltic Sea. Model simulations predict that the climate change in the Baltic Sea will result in a strong increase in air temperature, longer ice-free periods, precipitation and river discharge which, in turn, will lead to decrease in salinity and increase in nutrient load (Neumann, 2010; Neumann et al., 2012; Friedland et al., 2012; Meier et al., 2012; Dahl et al., 2013). The projected changes in salinity, temperature and nutrient concentrations may result in the increased frequency of algal blooms, including harmful algal blooms, and a decrease in the diatoms—dinoflagellates biomass ratio during the blooms (Wasmund et al., 2008). The projected environmental changes may also result in wider spread of invaders from warmer seas with broader physiological tolerance (Meier et al., 2012; Dahl et al., 2013). One of such invaders is the dinoflagellate *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, 1975 (former name: *Prorocentrum minimum* (Pavillard) Schiller 1933), which penetrated into the Baltic Sea, including its inner oligohaline parts during recent three decades (Kimor et al., 1985; Hajdu et al., 2000; Pertola, 2006; Olenina et al., 2010).

P. cordatum, initially described as *Exuviaella minima* Pavillard from the Gulf of Lion, Mediterranean Sea (Pavillard, 1916), occurs in many marine and brackish waters, from temperate to tropical regions: along the west and east coast of the USA, Gulf of Mexico, Sea of Japan, Caspian and Black Seas, and north-west European seas (Smith, 1975; Marasovic et al., 1990; Tyler and Seliger, 1978;

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Stoecker et al., 1997; Hajdu et al., 2000; Pertola, 2006). The species can spread by sea currents (Tyler and Seliger, 1978) and, probably, by human-mediated transport as *P. cordatum* cells were found in a ship's ballast water tank (Olenin et al., 2000). Since the temporary cysts remain viable in the dark for at least 3 months (Manoharan et al., 1999; Grzebyk and Berland, 1996), *P. cordatum* has the potential to tolerate even extended transports in ballast tanks.

P. cordatum is a bloom-forming species, found in vast numbers in coastal waters and especially in estuarine areas (Smith, 1975; Tyler and Seliger, 1978; Smayda and Reynolds, 2001; Heil et al., 2005; Carstensen et al., 2015). Under intensive bloom conditions (abundance exceeding 10^6 cell per litre) the impact of *P. cordatum* on the Baltic Sea plankton community, pelagic habitat and ecosystem functioning has been assessed as "strong-to-massive" (Olenina et al., 2010). *P. cordatum* is known to cause fish and shellfish mortality (Wikfors and Smolowitz, 1995; Tango et al., 2005), seriously affect aquaculture (Alonso-Rodriguez and Páez-Osuna, 2003; Azanza et al., 2005) and human health (Denardou-Queneherve et al., 1999), although according to the recent research a specific chemical compound has not been identified as 'the toxin' (Saba et al., 2011).

P. cordatum cells are small (14–22 μm long to 10–15 μm wide), bivalvate, armoured, laterally flattened. Their shape is variable: cells range from triangular to oval and to heart-shaped. Changes in salinity and nutrient concentration are known to effect cell size and morphology of dinoflagellates (Jensen and Moestrup, 1997; Laabir et al., 2011; Röder et al., 2012) and other phytoplankton groups (Latała, 1991; Aizdaicher and Markina, 2011; Balzano et al., 2011; García et al., 2012; Mukherjee et al., 2013) as well as dinoflagellate cyst community structure, abundance and morphology (Dale et al., 1999; Sildever et al., 2015).

In this study, we examine how the projected decrease in the Baltic Sea salinity can impact the growth, cell size and shape of the recently invaded dinoflagellate *P. cordatum* at its salinity tolerance limit and under different nutrient supply. In laboratory treatments we mimicked salinity conditions at the edge of the mesohaline south-eastern Baltic and oligohaline-to-limnic Curonian Lagoon. Besides traditional microscopic measurements, we applied an innovative computer-based method (Verikas et al., 2012, 2014; Gelzinis et al., 2013, 2015) allowing detection of *P. cordatum* cells and quantitative characterization of cell contours in phytoplankton images. This method also made available robust indicators of the morphometric changes, which are not accessible for an expert studying cells under light microscope.

2. Materials and methods

2.1. P. cordatum culture and growth conditions

The non-axenic *P. cordatum* strain KAC 72 was obtained from the Kalmar Algae Collection of the Linnaeus University (Kalmar, Sweden) and grown in modified f/2 medium by following the protocol provided with the culture. The medium was prepared using sterile (ultra-filtered through 100 000 kDa) PES (polyethersulphone) filters (Sartorius) and autoclaved (121 °C for 15 min) ambient south-eastern Baltic Sea water with a salinity of 7.2. KAC 72 was cultured at 16 °C temperature under a light–dark cycle of 16:8 h with 100 μmol photons m^{-2} s^{-1} irradiance. To maintain the culture in exponential growth phase, serial transfers of KAC 72 inoculum to fresh medium were done every 14 days.

2.2. Experimental approach

We used a factorial experiment design in order to measure the effect of salinity, nutrients and combination of both factors on phenotypic (cell size and shape) and growth response of *P. cordatum* strain KAC 72 (Fig. 1). The experiment was conducted using five different salinity and three nutrient levels (Fig. 1).

Each treatment was created using modified f/2 (N – 580 μM L^{-1}, P – 36.3 μM L^{-1}), f/4 (nutrient concentration is 1/2 of f/2) or f/8 (nutrient concentration is 1/4 of f/2) medium prepared from ambient south-eastern Baltic Sea water: undiluted (salinity of 7.2) and diluted in proportion 1:1 (salinity of 3.6), 1:2 (salinity of 1.8), and 1:4 (salinity of 0.9). Treatment (growth medium) corresponding to 'freshwater' (Curonian Lagoon) conditions (salinity of 0.0) was prepared using sterile Curonian Lagoon water. All treatments were prepared in three replicates each of 50 ml volume. Treatments were inoculated with exponential phase cells at the concentration of ~10^3 cells mL^{-1}. *P. cordatum* cells were acclimated to experimental conditions by growing them in lower salinity (3.6) medium over 7 generations (17 days). Acclimation to experimental conditions was verified by the observations of stabilized growth rate of the culture. During experiment cell morphology and abundance was monitored every three days over a 28-days period. Treatments were maintained at culture conditions (temperature, irradiance and photoperiod) as described above over the course of the experiment.

2.3. Cell counts and growth rate calculations

The abundance of *P. cordatum* cell was determined under light microscope (1000× magnification) in three or more 5 μl subsamples in order to count at least 200 cells per sample. The specific growth rate (μ, d^{-1}) was calculated from the slope of the linear regression of natural-log transformed cell abundance *versus* incubation time according to the equation $\mu = (\ln N_2 - \ln N_1)/(t_2 - t_1)$, where N_2 and N_1 were the cell densities at respective time, t_2 and t_1.

2.4. Microscope and computer-based measurements of cell size and morphological alterations

Morphological alterations of *P. cordatum* cells in all treatments were controlled under light microscope (400× magnification) by measuring the maximum dimension (length, μm) of 80–120 cells of *P. cordatum* every third day. In total 42 samples were processed and 4766 cells measured. Five photographs were taken from each sample for further computer-based analysis. The photographs were processed using a computer-based method aimed at identification and counting of the *P. cordatum* cells in phytoplankton images by cell contour detection (Verikas et al., 2012, 2014; Gelzinis et al., 2013, 2015).

In total, the precisely extracted contours for 2576 cells from 140 microscopy images (each with dimensions of 1280 × 960 pixels) were obtained. After the cells were detected and their contours

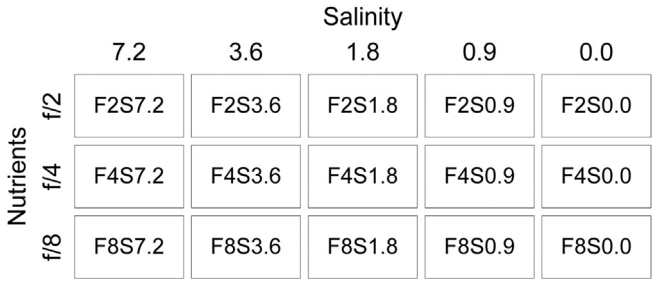

Fig. 1. The principal scheme of experiment design: the salinity and nutrient gradients are in accordance with ambient conditions of the south-eastern part of the Baltic Sea including the Baltic Sea – Curonian Lagoon transitional zone. The abbreviation for the each treatment used in the text below is given in the rectangles.