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Short Communication

Anthropogenically driven habitat formation by a tube dwelling diatom on the Northern Patagonian Atlantic coast

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ABSTRACT

The tube dwelling diatom *Berkeleya rutilans* (Trentepohl) Grunow plays a key role as early colonizer and bloom former in coastal zones. Exuding large quantities of extracellular polymeric substances (EPS), it can form dense colonies in mucilaginous macroscopic branches, containing thousands of cells. Due to their pronounced three dimensional growths of its mucilaginous structures, it supports a variety of organisms and traps grains and detritus, which makes it an important habitat former and ecosystem engineer, contributing to sediment stabilization, which is a crucial issue in sedimentary areas. In the present study we investigated the identity and structural morphology of *B. rutilans*, blooming in a tidal channel in Northern Patagonia (S40° 43′ W64° 56′) and experimentally tested its potential physiological responses (e.g. growth rate) to nutrient elevation. The observed morphological plasticity and measured high growth rates under nutrient exposure make *B. rutilans* a likely indicator for eutrophication in sedimentary marine habitats. As to our knowledge the present study provides the first record of *B. rutilans* for Argentinean waters, we discussed the potential reasons for its occurrence and evaluated the ecological impacts of its presence. Due to the observed high colonization capability and rapid response to environmental alterations (e.g. eutrophication, substrate changes) it seems to benefit from human activities, which will consequently favor its further expansion within the disturbed area.

1. Introduction

Forming dense colonies in mucilaginous macroscopic branches, containing thousands of cells, tube dwelling is a common growth form of diatoms in shallow coastal waters (Lobban, 1983; Sommer, 1997). Due to superficial resemblance to filamentous algae of the brown algal order Ectocarpales (Lewin, 1958; Fricke et al., 2008), tube dwelling diatoms (TDDs) were some of the first diatoms noted by naturalists (Round et al., 1990). By exuding large quantities of extracellular polymeric substances (EPS), TDDs form a mucilaginous matrix surrounding the cells and contribute to the biofilm and finally tube formation (Decho, 1990; Hoagland et al., 1993). Movement of the cells is thought to be essential for the tube construction (Houpt, 1987) and cells can be often observed gliding within the tubes (Lobban, 1989). Different pennate genera can form tubes, including *Berkeleya* (Cox, 1975), *Cymbella* (Round et al., 1990), *Gyrosigma* (Round et al., 1990), *Navicula* (Cox, 1988), *Parlibellus* (Camacho and Vidal, 2011), *Mastogloia* (John, 1993), and *Nitzschia* (Fernandes et al., 2002). Diatom tubes show variations in chemistry and morphology, and tubes were long-time used next to frustule characteristics to classify TDDs (Grunow, 1880). Thus the tubes of *Berkeleya rutilans* consist mainly of xylans and mannans (Lewin, 1958), while Cox (1981) speculated that the outer tubes of some species may be partially silicified, since they sometimes survive acid cleaning. The role and advantage of diatom tubes is still unclear. It might serve as grazing protection, by lowering the organic content per volume and hinder digestion, whereas the erect growth form may increase the probability of mechanical removal, as discussed by Sommer (1997). As early settlers they can alter the microstructure of early benthic assemblages, and consequently play a crucial role for the further benthic succession (Davis, 2009; Fricke et al., 2015). Overall their macroscopic growth form makes

Abbreviations: CLSM, confocal laser scanning microscopy; EPS, extracellular polymeric substances; LM, light microscopy; SAO, San Antonio Oeste; TDDs, tube dwelling diatoms.

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TDDs unique, important ecosystem engineers in sedimentary habitats. Trapping and binding grains and detritus in their mucilaginous network, they can strongly contribute to sediment stabilization by altering the laminar flow of sediments, changing bottom structure and chemistry, and consequently shape the entire ecosystem (Neumann et al., 1970; Holland et al., 1974). Sensitive to different environmental parameters, TDDs can show seasonal alterations in abundances in shallow coastal waters (Mizuno 1989). The tube like growth form improves nutrient uptake (Sommer, 1997) and diatom development under eutrophic conditions (Hillebrand and Sommer, 1997). Under these conditions, the tube forming *Berkeleya rutilans* strongly increased under nitrogen (N) and phosphorus (P) enrichment. Anutrient polluted intertidal system at the northern Patagonian Atlantic coast, TDDs were observed, forming dense spring blooms (Fricke pers. obs.). Despite their observed high abundances in our research area and their known ecological importance at other sedimentary habitats (e.g. Lobban, 1989; Hillebrand and Sommer, 2000), so far hardly anything is known on the role of tube dwelling diatoms in Argentinean waters. TDDs were identified as important drivers of the benthic succession at an early stage (Fricke et al., 2015). The present study investigated the identity of the dominant early diatom colonizer to learn more about the ecological role of this nutrient driven habitat former. A combined approach of traditional light microscopy (LM) and modern Confocal Laser Scanning Microscopy (CLSM) was used to study the structure of the TDD colonies and to investigate their relation to their associated biota and environment. A morphological and ecological description of the species is presented, which to our knowledge is the first record for Argentinean coastal waters.

2. Material and methods

2.1. Study area

San Antonio Bay (northern Patagonia, Argentina, S40° 43′ W64° 56′, Fig. 1**a,b**) is a macrotidal system (up to 9 m) of 80 km^2 (Aliotta et al., 2000), which shows contrasting trophic conditions within two inner tidal channels (SAO and CONTROL channel) (Martinetto et al., 2010; Teichberg et al., 2010). Providing comparable environmental conditions exposed to similar variations in temperature and salinity, this bivalent channel system offers an unique opportunity to examine the effect of anthropogenic N inputs on receiving waters by comparing the biogeochemical characteristics as done in different studies (Martinetto et al., 2010, 2011; Fricke et al., 2015). Further information on the vegetation and benthic composition can be found in Martinetto et al. (2010) and Fricke et al. (2015), who reported a high abundance of bloom forming green algal genera in the eutrophic SAO channel.

2.2. Nutrient regime

To investigate differences in nutrient concentrations between the two channels during the study period, samples were taken for nutrient analyses at three days (October 21 and 27, November 1 2012). At each sampling day three replicates were collected from each channel at ~10 cm below low tide level, using a sterile (60 ml) syringe, extended with a plastic tube (1.5 m long). Water samples were filtrated (Whatmann GF/F) right after taken and kept cooled (below 4 °C) in 50 ml PE bottles for later standard colorimetric measurements (Kattner, 1999). Concentrations for phosphate, nitrite and silicate were measured for all three days. In addition concentrations for nitrate were determined for the SAO channel at the last two and for the CONTROL channel at the last day.

2.3. Sampling and preservation of field material

A benthic microalgal bloom was observed during October 2012 (spring season, Fig. 1**c, d**). Tubes of bloom forming algae were randomly sampled from different hard substrates (e.g. stones, rubber of old tires) present in the channel (at approximately 30–50 cm water depth). To study benthic colonization processes, a succession study was initiated, using settlement substrates (PolyEthylene Terephthalate = PET, 1.5 × 2 cm) that were exposed in the eutrophic (SAO) and pristine (CONTROL) channels at 40 cm water depth (below low tide) at sites showing comparable hydrodynamic environments (Fricke et al., 2015). Substrates were sampled (n = 4) after 4 (October 25, 2012), 8 (October 29, 2012), 11 (November 1, 2012) and 40 days (November 29, 2012). More details on sampling procedures can be found in Fricke et al. (2015).

2.4. Mounting and staining

In the laboratory, the substrates and algal material were mounted in semi-permanent slides (SLIDEs) using a corn syrup (Karo^{TM} light) solution, diluted with distilled water (1:1) and few drops of 4% formaldehyde to avoid bacterial and fungal growth. Material selected to CLSM analyses was remounted and stained with Congo Red following the protocol of Fricke et al. (2017).

2.5. Microscopical investigation of field material

To investigate the abundances, structure and identity of TDDs and other related benthic taxa, SLIDES were investigated in a three-step microscopic approach: i) *TDD growth rates,* each SLIDE was investigated under LM. To avoid underestimation of patchy distributed colony forming taxa 4 day old assemblages were scanned twice for 15 visual fields (VFs) at 40× (2.55 mm^2) and for 10 VFs at 20× (6.8 mm^2), whereas 8 and 11 days old assemblages were scanned for 15 VFs at 20× (5.1 mm^2) and 40 days old for 15 VFs at 11.5X (0.36 mm^2). To investigate the percentage contribution of TDDs to the developing assemblages, measured TDD cover (T) was related to the total taxa cover, calculated as sum from the individual taxa cover (C) as: T*100/C. For the following studies, the material was split into two parts. For ii) *TDD colony structures*, one part of the samples (partly stained, see above) were investigated at 20 times magnification, by LM (0.7 numerical aperture; Nikon Eclipse 80i) and CLSM, using a Leica TCS SP5 equipped with a DM5000B upright microscope (Leica, Wetzlar, Germany) and 2 visible light lasers: DPSS 10 mW 561 nm (at 68% power) and HeNe 10 mW 633 nm (at 45% power), combined with the software LAS AF 2.2.1. Leica Application Suite Advanced Fluorescence. CLSM was set following the protocol of Fricke et al. (2017), with excitation range of 620–680 nm and 540–560 nm, emission range of 575–609 nm (ch1) and 665–712 nm (ch 2), gain and offset of ch 1:667 and ch 2:487, pinhole aperture of 59.9 μm, and the amount of light that reached the detectors of ch1:-4.2 and ch2:-0.9. The CLSM data were presented as maximum intensity projections, a sharply focused image using the brightest value along the z-axis for each pixel. Finally for iii) *Species identity*, based on valve characteristics, the other part of the material was removed from the object slides, transferred to a glass vial and prepared according the protocol of Al-Handal et al. (2016). Each sample was mounted on a stub, sputter coated with gold-palladium (SCD 050 Bal-Tec), and investigated using a Tescan VEGA3 microscope (Elekronen-Optik-Service GmbH, Dortmund, Germany) at 15 kV with an SE detector.

3. Results

We observed a pronounced microbenthic spring bloom at the beginning of our study in October 2012 within the highly eutrophic