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Uptake of dissolved inorganic and organic nitrogen by the benthic toxic dinoflagellate *Ostreopsis* cf. *ovata*

Cécile Jauzein[a,b,*], Douglas Couet[a], Thierry Blasco[a], Rodolphe Lemée[a]

[a] Sorbonne Universités, UPMC Univ Paris 06, INSU-CNRS, Laboratoire d'Océanographie de Villefranche, Villefranche sur mer, France
[b] IFREMER, Centre de Brest, DYNECO PELAGOS, F-29280 Plouzané, France

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ABSTRACT

Environmental factors that shape dynamics of benthic toxic blooms are largely unknown. In particular, for the toxic dinoflagellate *Ostreopsis* cf. *ovata*, the importance of the availability of nutrients and the contribution of the inorganic and organic pools to growth need to be quantified in marine coastal environments. The present study aimed at characterizing N-uptake of dissolved inorganic and organic sources by *O.* cf. *ovata* cells, using the ^{15}N-labelling technique. Experiments were conducted taking into account potential interactions between nutrient uptake systems as well as variations with the diel cycle. Uptake abilities of *O.* cf. *ovata* were parameterized for ammonium (NH_4^+), nitrate (NO_3^-) and N-urea, from the estimation of kinetic and inhibition parameters. In the range of 0 to 10 μmol N L^{-1}, kinetic curves showed a clear preference pattern following the ranking $NH_4^+ > NO_3^- >$ N-urea, where the preferential uptake of NH_4^+ relative to NO_3^- was accentuated by an inhibitory effect of NH_4^+ concentration on NO_3^- uptake capabilities. Conversely, under high nutrient concentrations, the preference for NH_4^+ relative to NO_3^- was largely reduced, probably because of the existence of a low-affinity high capacity inducible NO_3^- uptake system. Ability to take up nutrients in darkness could not be defined as a competitive advantage for *O.* cf. *ovata*. Species competitiveness can also be defined from nutrient uptake kinetic parameters. A strong affinity for NH_4^+ was observed for *O.* cf. *ovata* cells that may partly explain the success of this toxic species during the summer season in the Bay of Villefranche-sur-mer (France).

1. Introduction

Benthic harmful algal blooms represent an increasing threat to human and environmental health worldwide (Parsons et al., 2012; Rhodes, 2011). Toxic dinoflagellates belonging to the genus *Ostreopsis* Schmidt are common components of tropical epibenthic microalgae communities and have also been reported in several temperate waters, including coastal waters of the Mediterranean Sea (Vila et al., 2001), New Zealand (Rhodes et al., 2000) or Japan (Taniyama et al., 2003). Along the Mediterranean coasts, massive *Ostreopsis* cf. *ovata* blooms regularly occurred during the summer season and early fall (e.g. Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2011). Some of them were associated with serious cases of human health disorders (Brescianini et al., 2006; Vila et al., 2016). Symptoms of human illnesses include skin irritations, fever or broncho-constriction, partly due to exposure to toxic marine aerosols (Ciminiello et al., 2014). Blooms of *O.* cf *ovata* can also have deleterious effects on benthic marine invertebrates (Accoroni et al., 2011; Guidi-Guilvard et al., 2012; Pagliara and Caroppo, 2012; Gorbi et al., 2013). The toxicity of *O.* cf *ovata* is associated with the presence of palytoxin-like compounds that include putative palytoxin and ovatoxins-a, b, c, d, e and f (Uchida et al., 2013; Brissard et al., 2014), and mascarenotoxins-a and c (Rossi et al., 2010; Scalco et al., 2012). Palytoxin-like compounds have already been found in Mediterranean fauna (Biré et al., 2015) but no related food poisoning has been reported.

The processes that shape dynamics of benthic dinoflagellate populations and facilitate the development of specific toxic species are still poorly understood, mainly because benthic dinoflagellates have received considerably less attention than their planktonic counterparts (Parsons et al., 2012). Among potential controlling factors, temperature may represent a key factor in the seasonality of *O.* cf *ovata* blooms in temperate areas (Mangialajo et al., 2008; Accoroni et al., 2014; Accoroni and Totti, 2016). The control of bloom dynamics by water temperate has still to be clarified,

Abbreviations: EA – IRMS, Elemental Analysis – Isotope Ratio Mass Spectrometry; PC, particulate carbon; PN, particulate nitrogen.

* Corresponding author at: IFREMER, Centre de Brest, DYNECO PELAGOS, F-29280 Plouzané, France.
E-mail address: cecile.jauzein@ifremer.fr (C. Jauzein).

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however, as its appeared to vary with geographical areas (Accoroni and Totti, 2016). Concerning other physical parameters, several studies reported higher abundances of *O.* cf *ovata* in sheltered sites compared to the ones exposed to wave action (e.g. Totti et al., 2010; Selina et al., 2014). This suggests that hydrodynamic conditions can have strong effects on bloom development and maintenance; according to Accoroni and Totti (2016), this influence of hydrodynamics on *O.* cf *ovata* bloom may be particularly pronounced under high levels of abundance (Accoroni and Totti, 2016).

The growth and maintenance of microalgae populations are also directly dependent on nutritive sources that are fueling the blooms. The regulation of *O.* cf. *ovata* bloom dynamics by the nutrient resource is largely unknown. Cells of *Ostreopsis* are expected to be mixotrophic, able to complete their autotrophic growth (based on photosynthesis and uptake of inorganic sources) by the use of organic matter (Burkholder et al., 2008). Among potential organic sources, the phagotrophy of preys by *Ostreopsis* cells was investigated (Faust et al., 1996; Barone, 2007) but is still a matter of debate (Escalera et al., 2014). The potential use of dissolved organic phosphorus by *O.* cf *ovata* cells was tested by Pistocchi et al. (2014), when the uptake of dissolved organic nitrogen sources has not been analyzed yet. Concerning the inorganic sources of nutrients, previous studies reported conflicting results regarding relationships between nutrient availability and occurrence of *Ostreopsis* blooms (Accoroni and Totti, 2016). Several field studies conducted in the NW Mediterranean Sea did not show any relationship between epiphytic *O.* cf. *ovata* abundances and concentrations of dissolved inorganic nutrients (dissolved inorganic nitrogen, DIN, and phosphate) (Vila et al., 2001; Accoroni et al., 2011). Conversely, Parsons and Preskitt (2007) found that *Ostreopsis* sp.1 abundance was positively correlated with nutrient concentrations in the waters surrounding Hawai'i. A positive correlation between phosphate concentration and *O.* cf. *ovata* abundance was also reported by Cohu et al. (2013) in the NW Mediterranean Sea. In the Northern Adriatic Sea, phosphate pulses in the bloom onset period may possibly stimulate *O* cf. *ovata* growth in these coastal waters (Accoroni et al., 2015).

The importance of the availability of nutrient sources and their contribution to *O* cf. *ovata* growth during bloom development and maintenance need to be quantified in marine coastal environments. In the present study, the control of *O* cf. *ovata* growth by several nitrogen (N) sources was investigated under controlled conditions, using cultures. The main goal of the present work was to characterize N-uptake of dissolved inorganic and organic sources, using the ^{15}N-labelling technique and taking into account potential interactions between nutrient uptake systems as well as variations with the diel cycle.

2. Material and methods

2.1. Culture conditions

Two strains of *Ostreopsis* cf. *ovata*, MCCV 054 and MCCV 055, were obtained from the Mediterranean Culture Collection of Villefranche (MCCV). They were both isolated in 2014 from Villefranche Bay, South of France (43°41′34.83″ N and 7°18′31.66″ E), during the same bloom event. Non-axenic stock cultures were grown in modified K/10 medium (originally defined by Keller et al. (1987)), where addition of silicate and Tris base was omitted, phosphorus was added as KH_2PO_4 (final concentration of 4 μM) and ZnSO4 was added at a final concentration of 0.08 nM. Culture medium was prepared using autoclaved old seawater filtered on 0.2 μm (FSW) at salinity 38. Cultures were maintained at 23 °C, under 250 μmol photons m^{-2} s^{-1}, with a 16:8 h light:dark cycle. Stock cultures were grown in batch mode without bubbling, in 15 mL of culture medium. Culture flasks were maintained in flat culturing conditions in order to optimize the surface area for gas exchange and growth of benthic cells. Before each experiment, one stock culture in exponential phase of growth was successively diluted in order to scale up the culture volume from 15 mL (in flask of 25 cm^2 surface area) to 350 mL (in flask of 300 cm^2 surface area). The final large volume culture was used to inoculate three or four replicated cultures of 350 mL. Experiments were run using a set of replicated cultures in exponential phase and characterized by a cell density higher than 1500 cell ml^{-1}.

2.2. Micro-algal cell resuspension in low N medium

Experiments were conducted under controlled conditions of nitrogen (N) availability in order to help for a precise characterization of N-uptake capabilities of *O.* cf. *ovata* cells. Each experiment started with the resuspension of micro-algal cells in culture medium where no NH_4^+ or NO_3^- addition was performed (–N medium). Concentrations of NH_4^+ and NO_3^- were determined for the —N medium used for running the experiments. Full resuspension of *O.* cf. *ovata* cells was completed in about 1 h. Cells were collected on an 8 or 10 μm mesh size net by gravity filtration, then rinsed with —N medium before being resuspended in —N medium. To ensure that the net was not clogged due to mucus accumulation, these collection, rinsing and resuspension steps were performed on successive aliquots of 35 or 40 mL of culture and a new piece of net was used every four aliquots. A gentle agitation of the net in —N medium did not allow for passive resuspension of *O.* cf. *ovata* cells. Thus, for each aliquot of culture, micro-algal cells concentrated on the net were collected by pipetting repeatedly and carefully ~1 mL of —N medium above the net, then this volume was finally poured in a culture flask (75 cm^2 surface area) filled with 40 mL of —N medium. The resuspension and rinsing steps allowed for the removal of most of the bacteria present in the growth medium and limited their contribution in the resuspended cultures (Rausch de Traubenberg and Soyer-Gobillard, 1990).

The resuspended culture flasks were kept aside in the culture chamber, under initial culture conditions, during 1–2 h before starting the incubations. This lag reduced the potential impact of stress associated with the resuspension step on uptake rates and also contributed to start incubations under really low N concentrations.

2.3. Kinetic experiments

Uptake kinetics of three potential N-sources, nitrate (NO_3^-), ammonium (NH_4^+) and urea, were characterized for the two *O.* cf. *ovata* strains, MCCV 054 and MCCV 055. For each strain, *O.* cf. *ovata* cells were resuspended from three replicated cultures of 350 mL in exponential phase. Each mother culture allowed for the creation of one series of eight 40 mL samples and was used to characterize the uptake kinetics of one N-source. Incubations started with the addition of ^{15}N ($^{15}NO_3^-$, $^{15}NH_4^+$ or ^{15}N-urea) at eight graded concentrations (0.1, 0.2, 0.5, 1, 2, 3, 5, and 10 μmol N L^{-1}). Samples were incubated for 1 h under initial culture conditions. At the end of the incubation, samples were filtered through precombusted (4 h at 450 °C) A/E filters (Gelman Sciences) and rinsed with 20 mL of FSW. Filters were dried at 60 °C overnight and analyzed by EA-IRMS (Elemental Analysis – Isotope Ratio Mass Spectrometry) for measurements of particulate carbon (PC), particulate nitrogen (PN) and $^{15}N/^{14}N$ isotopic ratios.

An additional experiment was conducted in order to characterize N-urea uptake capabilities of *O.* cf. *ovata* cells taking into account the potential role of preconditioning effects. Cells of *O.* cf.