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Effect of marine autotrophic dissolved organic matter (DOM) on *Alexandrium catenella* in semi-continuous cultures

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Alexandrium catenella was grown in semi-continuous cultures in L1 growth-medium enriched with concentrated dissolved organic matter (DOM) from a diatom bloom. In medium with full nitrate (880 μM), the average growth rate was 0.32 ± 0.01 day^{-1} (L1, control without added DOM). Adding natural marine dissolved organic nitrogen (DON) at levels of 20–30 μM above background (10 μM) led to a higher growth rate (L1+DOM, 0.40 ± 0.00 day^{-1}). In medium with lower nitrate level (175 μM) and higher DON (L1/5+DOM treatment), both dissolved inorganic nitrogen and DON were used, leading to the highest growth rates (0.43 ± 0.03 day^{-1}). In medium without nitrate [(L1-N)+DOM treatment], the low ammonium concentrations observed throughout the experiment (<1 μM) as well as the uptake kinetics of A. catenella could not have supported the observed growth rates, leading us to conclude that DON was directly used by this organism, rather than using N remineralized by bacteria (from DON). The decrease of bacteria in DOM enriched bioassays could point to a nutrient limitation and competition with A. catenella for organic matter. Viruses likely contributed as an additional factor to keep the bacterial population from becoming dominant.

INTRODUCTION

Dissolved organic matter (DOM) generally constitutes a large portion of the total organic matter in the marine environment (Nagata, 2000). The DOM pool, a mixed composition of dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved organic phosphorous, can be of autochthonous or allochthonous origin, relative to the source in consideration. In marine environments, major sources of autochthonous DOM include phytoplankton exudates, cell lysis (viral and bacterial) and grazing-induced mechanisms, while allochthonous DOM may derive from terrestrial or atmospheric origin (Hansell and Carlson, 2002). According to its nature, DOM is characterized by distinct biochemical and photochemical properties that will ultimately influence its bioavailability (Amon and Benner, 1996; Mopper and Kieber, 2002). Autochthonous DOM is predominantly more labile (turnover times from hours to days) than its allochthonous counterpart (Sondergaard and Middelboe, 1995; del Giorgio and Davis, 2003).

The flux of DOM in the aquatic realm involves diverse trophic levels that influence one another (Nagata, 2000). As an example, heterotrophic bacteria may consume DOM from phytoplankton exudates to grow, but subsequent viral lysis or grazing will either reintroduce this organic matter into the microbial loop or directly into the classical food web if flagellates are in turn grazed by higher trophic levels (Azam *et al.*, 1983; Fuhrman, 1999). Bacterial mineralization of organic matter also reflects its important function in the biogeochemical cycle of DOM (Kirchman, 2000). Bacteria

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and viruses are natural and important components during HABs, influencing their development and decay (Brussaard *et al.*, 2005; Garcés *et al.*, 2007).

Alexandrium catenella (Whedon and Kofoid) Balech is a potentially toxic dinoflagellate that can cause paralytic shellfish poisoning by alkaloid neurotoxins that contaminate bivalves (Hallegraeff *et al.*, 1991), which often leads to the closure of mussel harvesting (Price *et al.*, 1991). This species forms HABs worldwide in a variety of environments, from confined areas impacted by anthropogenic eutrophication (e.g. harbours and lagoons) (Vila *et al.*, 2001; Collos *et al.*, 2004) to upwelling regions with near pristine conditions (Pitcher and Calder, 2000) and offshore waters (Price *et al.*, 1991), reflecting a high physiological plasticity. The ability of *Alexandrium catenella* to use organic elements as nutrients (Carlsson *et al.*, 1998; Legrand and Carlsson, 1998; Collos *et al.*, 2007) contributes to its competitive advantage over strict autotrophs and the wide spread geographical distribution of this species. However, investigations with DOM have only been performed to date with organic matter from terrestrial origin (Carlsson *et al.*, 1998; Doblin *et al.*, 2001). The potential influence of DOM of predominantly autotrophic origin and its possible implications has not yet been addressed.

In general, our work is integrated in a line of previous studies addressing the mixotrophic abilities of potentially harmful algae (e.g. Stolte *et al.*, 2002; Adolf *et al.*, 2006; Burkholder *et al.*, 2008) so as to extend knowledge about the photosynthetic and heterotrophic pathways that may favour their development. In specific, we investigated the effect of predominantly autotrophic DOM in *Alexandrium catenella* growth. The associated bacterial and viral assemblage was also addressed so as to partially mimic the subset of microbial dynamics in natural seawater. We hypothesize that autotrophic DOM has a positive effect on *A. catenella* growth.

METHOD

A strain of *Alexandrium catenella* (VGO565), isolated from Tarragona (Catalonia Coast, Spain), was maintained as a unialgal culture in L1 growth medium (Guillard and Hargraves, 1993) based on natural seawater containing ~8 µM DON and 180 µM DOC. This strain was additionally acclimatized to L1/5 growth medium (one-fifth of the original L1 concentration of nitrate and phosphate), 5 weeks before the experiment, by a total of three transfers to the new media. Although L1/10 cultures (1/10 of the original concentration of nitrate and phosphate) were prepared, after 2 weeks *A. catenella* cells showed signs of apoptosis (Agustí *et al.*, 1998; Veldhuis *et al.*, 2001) and this culture was therefore excluded from this experiment. Both cultures were grown at 31 PSU salinity, 20°C using a 12:12 light:dark cycle. Illumination was provided by fluorescent tubes (Gyrolux, Sylvania, Germany), providing a photon irradiance of 100 µmol photons m^{-2} s^{-1}.

Sampling area and DOM concentration

Seawater was collected from the temperate oligotrophic Blanes coastal station, NW Mediterranean Sea (41°40′19″N, 2°47′11″E), 60 km north of Barcelona (NE Spain). The circulation patterns in the Blanes canyon induce a high dilution rate in this area by exchange with offshore waters (Masó and Tintoré, 1991). The water column is generally mixed all year round. Although terrestrial inputs may arise from the nearby Tordera River and from urban point-source runoff, there is a high proportion of autochthonous DOM due to phytoplankton lysis that contributes to bacterial production during blooms and summer conditions (Agustí and Duarte, 2000). The sampling was performed on 5 March 2007 during the characteristic late-winter diatom bloom (Duarte *et al.*, 1999) (phytoplankton database of Microbial Observatory of Blanes Bay, MOBB), so as to maximize DOM autochthonous conditions. Allochthonous DOM was also minimized by sampling during winter when urban discharges are at their seasonal minimum (the population increases during summer owing to the influx of tourists). Winter runoff was also at its minimum due to the very dry winter conditions (monthly mean rain <1.5 mm; monthly mean number of days of rain >1 mm=1.3; meteorological data from the area, Meteocat).

The water (105 L) was filtered through a 0.2 µm Pall Corporation cartridge and subsequently concentrated to 50× its original volume by tangential-flow filtration (Prep/Scale-TFF cartridge, Millipore) to obtain HMW DOM >1000 kDa. The concentrated DOM was then used for the experiment.

Experimental design and analyses

L1 and L1/5 cultures were diluted to $\approx 5 \times 10^4$ cell L^{-1} and distributed to 2.0 L polycarbonate containers (Nalgene) to a final volume of 1.8 L. Three treatments and two controls were run in duplicate: L1+DOM and L1/5+DOM (nutrient-sufficient treatments with DOM addition), (L1-N)+DOM (N-deficient treatment with DOM addition), L1 (L1 without added DOM) and BV+DOM, a bacteria and virus control obtained by removing *Alexandrium catenella* from the L1/5 culture (10 µm filtration). The bioassays were enriched with

200 mL of HMW DOM corresponding to 25 ± 7 μM DON and 73 ± 3 μM DOC. To promote exponential growth throughout the experiment, the cultures were grown in semi-continuous mode replacing 13% of the volume every 2 days (dilution rate=0.07 day^{-1}). The outflow was used for the estimation of chemical and biological variables. Aliquots for inorganic nutrients (nitrate, nitrite, ammonium and phosphate) were stored frozen (−20°C) and measured with an auto-analyzer (Alliance Evolution II) by means of standard colorimetric techniques (Grasshoff *et al.*, 1983). Samples for DOM quantification were filtered through pre-combusted GFF (450°C for 6 h) filters. DOC and total dissolved nitrogen (TDN) samples were fixed with H_3PO_4 (pH≤2) and kept at 4°C in 10-mL flame-sealed glass ampoules (pre-combustion: 450°C, 24 h). Nutrient utilization was calculated according to the equation: $U=(C_{T0}+C_{T0} * D * T)-C_{TF}$, where C_{T0} is the concentration at T_0; D the dilution rate (day^{-1}); T the time of incubation; and C_{TF} the final concentration. Analyses were performed by high-temperature catalytic oxidation using a Shimadzu TOC-V interfaced with a TNM-1 (total nitrogen) detector following the method described by Álvarez-Salgado and Miller (1998). DON was obtained by subtracting dissolved inorganic nitrogen (DIN=nitrate+nitrite+ammonium) from TDN. Chlorophyll *a* (Chl *a*) samples were filter-extracted in acetone for 48 h and analysed with a Turner 10 AU fluorometer according to Yentsh and Menzel (Yentsh and Menzel, 1963). Cells were fixed with Lugol-iodine solution, sedimented (24 h) and quantified with a Leica-Leitz DM-IL inverted microscope (Andersen and Throndsen, 2003). Two subsamples from each duplicate bioassay were collected to increase the accuracy of *A. catenella* quantification. As described by Guillard (Guillard, 1973), species-specific net growth rates (μ, day^{-1}) from each duplicate were calculated from the slopes of the regression lines of ln (N) versus time, with N as the mean cell abundance from the subsamples collected from each duplicate. Considering that semi-continuous mode promotes the exponential growth of cells, all data were taken into account for the calculation of growth rates, with exception of day 0 that was excluded to avoid a possible bias of data from the initial variability observed between treatments. The dilution rate (day^{-1}) was added to the final growth rates. Bacteria samples were fixed with 1% paraformaldehyde, frozen in liquid-N_2, kept at −80° and quantified with a Becton-Dickinson FACScalibur flow cytometer following the recommendations of Gasol and del Giorgio (Gasol and del Giorgio, 2000). Briefly, unfrozen samples were stained with Syto 13 (molecular probes), mixed with yellow−green latex beads (Polyscience) and run at low speed until 10.000 events were registered in a right-angle side scatter (SSC) versus green fluorescence (FL1, at 530 ± 30 nm) plot. The presence of nanoflagellates was detected by the observation of bacteria cytometer plots. These nanoflagellates were identified as autotrophic nanoflagellates (ANF) by their fluorescence characteristics in the flow cytometer and further epifluorescence microscopy. Subsamples were filtered through 0.6 μm black polycarbonate filters, and stained with DAPI (4, 6-diamidino-2-phenylindole) at a final concentration of 5 μg mL^{-1} (Porter and Feig, 1980). Observations were performed with epifluorescence microscopy (Olympus-BX40-5 102/E at 1000×) (Sieracki *et al.*, 1985) under both UV radiation and blue light, and the presence of ANF was confirmed by their red−orange fluorescence and plastidic structures. The quantification of ANF was performed by flow cytometry as described for bacteria. Virus-like particles were fixed with glutaraldehyde (0.5% final concentration), frozen in liquid-N_2 and stored at −80°. Unfrozen samples were diluted in TE buffer, stained with SYBR Green I for 10 min at 80°C in the dark, cooled down and estimated by cytometry as in Brussaard (Brussaard, 2004).

Statistical analysis

The differences in growth rates were tested by means of homogeneity of slopes (ANCOVA) (Zar, 1984). Analyses were performed with STATISTICA© 6 software.

RESULTS

Inorganic and organic nutrients

Phosphate concentrations (Fig. 1A) remained mostly constant, with lower values in incubations grown with L1/5 medium (L1/5+DOM treatment, and bacteria and virus control BV+DOM). Ammonium was low throughout the experiment (<1 μM) with a slightly decreasing trend over time for all treatments. Values in the L1+DOM culture (0.9−0.5 μM) were higher than in the other assays (Fig. 1B). Nitrate was mostly stable in L1+DOM and a general decline was observed in L1 (Fig. 1C). After an initial decrease of nitrate in L1/5+DOM and BV+DOM incubations, nitrogen followed a gradual accumulation. In the inorganic N-deficient treatment [(L1-N)+DOM], only the first two points are shown, as the remaining data were considered to be artefacts (TDN increased from 26 to 282 μM, a clearly impossible situation, so those data points were rejected as aberrant values).

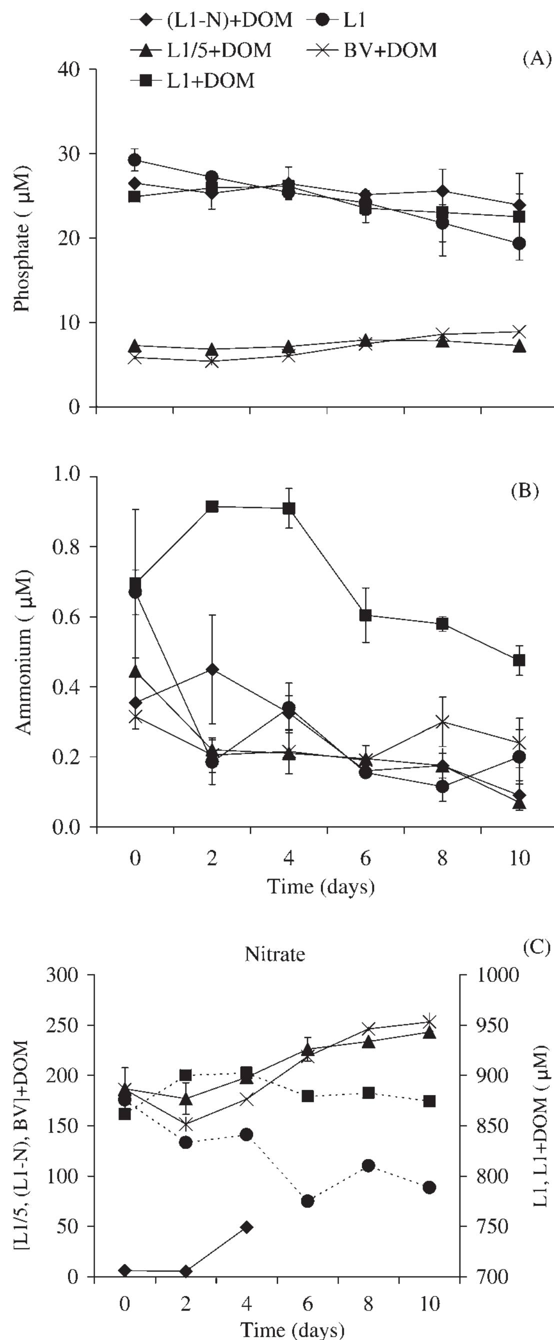

Fig. 1. Evolution of dissolved inorganic phosphorus (**A**), ammonium (**B**) and nitrate (**C**); treatments represented with dashed lines correspond to the second *Y*-axis (right side) scale. Error bars correspond to standard deviation. L1 and L1/5 are growth mediums; BV+DOM stands for bacteria+virus bioassay (see Method section).

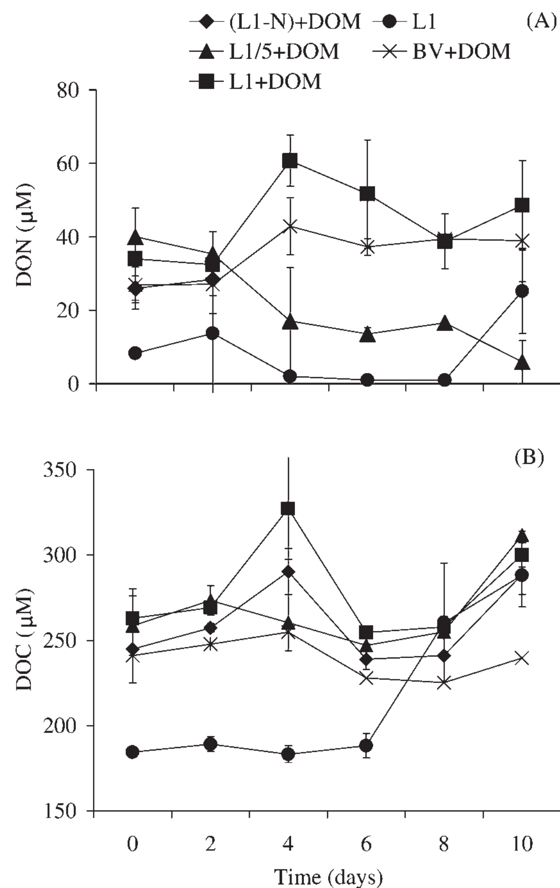

Fig. 2. Changes in dissolved organic nitrogen (DON) (**A**) and dissolved organic carbon (DOC) (**B**). Error bars correspond to standard deviation.

Regarding the organic pool, DON varied differently according to the incubation (Fig. 2A). A significant decrease was observed in L1/5+DOM treatment (from ~40 μM down to ~5 μM) leading to a utilization of ~63 μM in 10 days, whereas from the nitrate data, an uptake of ~58 μM can be estimated. Thus, a total of 121 μM of *N* was used in 10 days. In accordance, the nitrogen demand for *A. catenella* growth estimated from N cell content (Collos *et al.*, 2004) ranges from 25 to 125 μM. In (L1-N)+DOM, only the first two data points are shown, the others were not include due to aberrant TDN values. In L1+DOM, DON peaked twice (days 4 and 10). Finally, in the bacteria and virus control (BV+DOM), DON was generally constant with a peak observed on day 4. DOC had a more homogeneous variation, with L1+DOM and (L1-N)+DOM reaching higher concentrations by mid (day 4) and the end (day 10) of the experiment. DOC was mostly stable in L1/5+DOM and BV+DOM with an increase after day 8 in L1/5+DOM, whereas in L1 values increased sharply after day 6. The extracted HMWDOM used for enrichment had an organic molar C:N ratio of 16.

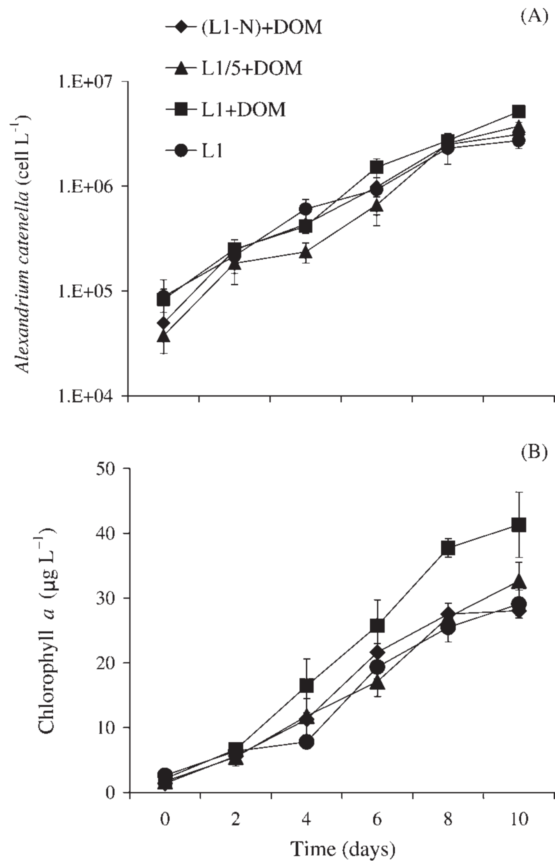

Fig. 3. *Alexandrium catenella* (**A**) and chlorophyll *a* (**B**) concentrations. Error bars correspond to standard deviation.

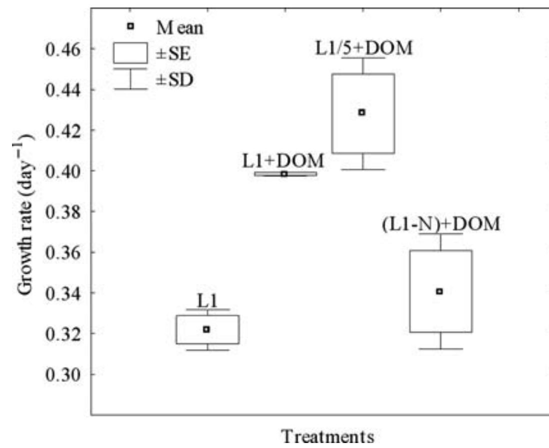

Fig. 4. Box-whisker representation of *Alexandrium catenella* growth rates from different treatments.

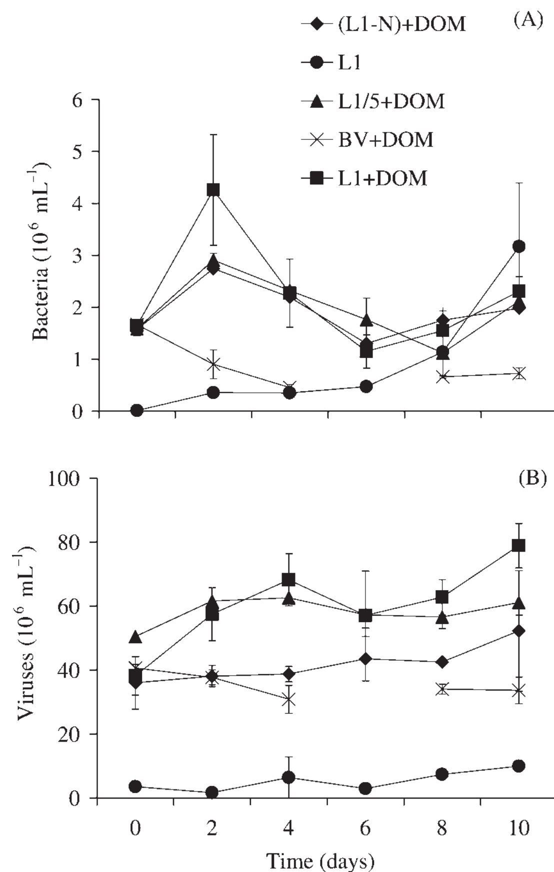

Fig. 5. Bacteria (**A**) and viruses (**B**) concentrations. No data were available for day 6 of BV+DOM control. Error bars correspond to standard deviation.

Biotic data

Alexandrium catenella cells were maintained in exponential growth by semi-continuous incubation. The maximum value was reached in L1+DOM (5.1×10^6 cell L^{-1}) (Fig. 3A). Chlorophyll *a* (Chl *a*) (Fig. 3B) increased steadily in every bioassay with higher concentrations in L1+DOM (maximum: 41 ± 5 μg L^{-1}). The slopes of the regression lines of ln cell abundance versus time (growth rate) were significantly different at $P = 0.048$ ($F = 2.94$, homogeneity of slopes test). The highest growth rates were observed in the L1/5+DOM culture (0.43 ± 0.03 day^{-1}) followed by the L1+DOM (0.40 ± 0.00 day^{-1}) bioassay (Fig. 4).

At time 0, bacteria and virus concentrations were lower in the control, indicating the presence of these microorganisms in the DOM extract used for the bioassays enriched with DOM. Bacteria increased after day 4 in the control medium (L1), reaching the highest number on day 10 ($3.2 \pm 1.2 \times 10^6$ cells mL^{-1}) (Fig. 5A). BV+DOM followed a descending pattern until mid-period, after which abundance did not vary. In the remaining sets, bacteria concomitantly peaked twice, at day 2 and day 10, with minimum values around day 6. Viruses were mostly constant increasing in the last days of the experiment. In L1+DOM, a two-step increase was observed from days 0 to 4 and 6 to 10 (Fig. 5B).

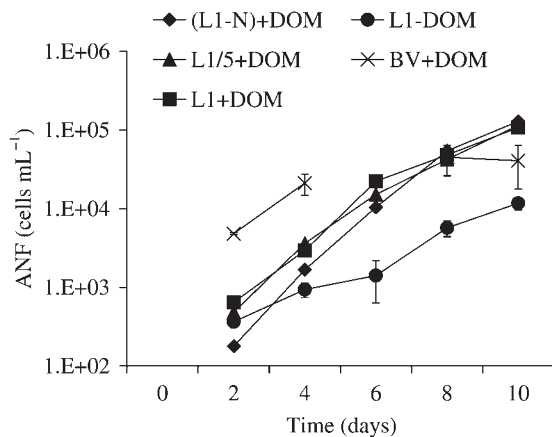

Fig. 6. Autotrophic nanoflagellates (ANF) concentration. No data were available for day 6 on the BV+DOM control. Error bars correspond to standard deviation.

The highest concentration of viruses was reached by day 10 in this experimentation set ($78.9 \pm 6.9 \times 10^6$ mL^{-1}).

The examination of bacteria flow cytometric plots discriminated the presence of an additional population in the incubation sets after day 2, which was not detected by previous inspection of *Alexandrium catenella* cultures by optical microscopy due to the low concentration of these elements at the beginning of the experiment. This population was identified as ANF according to the green (FL1, 530 nm) and red (FL3, 670 nm) fluorescence emissions and by the observation of DAPI-stained samples under epifluorescence microscopy. In general, ANF followed an increasing pattern during the whole experiment except for BV+DOM where concentrations remained stable by the end of the experiment (Fig. 6). ANF were nevertheless higher at day 2 in BV+DOM likely because of the pre-filtration step used for the preparation of BV+DOM control, which excluded *A. catenella* cells and therefore the possible competition for nutrient sources favouring the initial development of ANF.

DISCUSSION

Biotic and trophic interactions

Inorganic phosphorus data seem to plausibly exclude P-limitation growth conditions. We will therefore focus on N nutrient changes. The gradual consumption of nitrate was mainly observed in L1 treatment (where DOM was not added) suggesting that in this case the basic nutrient source was of inorganic nature.

The C:N ratio obtained for the concentrated HMWDOM (C:*N*=16) is concurrent with DOM of predominately autotrophic origin and is within the range of oceanic DOM (Biddanda and Benner, 1997). The accumulation of organic matter is commonly related to the decoupling of production and consumption/degradation mechanisms (Biddanda and Benner, 1997) such as (i) increased release of organic exudates not paired by microbial uptake, (ii) the refractory degree of the organic pool, (iii) reduction of decomposition processes by limitation factors (e.g. nutrients) and (iv) regulation of the bacterial population through grazing and viral lysis (Williams, 1995; Sondergaard *et al.*, 2004). In this study, the build-up of organic matter by day 4 was coincident with an increase in virus concentrations associated with a decrease of bacteria (condition 4). By the end of the experiment, *A. catenella* cells were likely attaining a late phase of the exponential stage, which is generally associated with an increase of exuded material (Nagata, 2000) suggesting that condition (i) could be responsible for the increment observed in the organic pool at this time.

We detected the presence of ANF in bioassays after day 2. The potential interference of these elements in our investigation was low and the experiment was still conducted. Estimates of chlorophyll content from cell diameter (Montagnes *et al.*, 1994) indicate that those ANF contributed about 1/40 and 1/100 of *A. catenella* chlorophyll at the beginning and end of the incubations, respectively. Thus, they were neglected in the following mass balance calculations.

The exponential growth of *A. catenella* in medium without mineral N but with organic N is no definite proof of direct use of DON in a medium also containing bacteria. However, taking into account that ammonium concentrations represent a balance between mineralization and uptake, the low ammonium concentrations (0.1 to 0.4 μM) measured throughout the experiment in the (L1-N)+DOM medium as well as the ammonium uptake kinetics of *A. catenella* strain VGO565 (Jauzein *et al.*, 2008a) indicate that the observed growth rates could not be supported by ammonium alone. For example, from the highest ammonium concentration (0.4 μM) observed, a maximum growth rate of about 0.03 day^{-1} can be calculated for the (L1-N)+DOM series. For the other series, the nitrate levels could support the observed growth rates. Therefore, this leads us to the conclusion that DON must be used directly by *A. catenella* for realizing the observed growth rates in the medium without nitrate. Furthermore, the higher growth of *A. catenella* in DOM enriched incubations relative to L1 suggests a favourable interplay between inorganic and organic nutrients when both sources are available, which was already observed in some strains of this

species (Jauzein *et al*., 2008b). Such a pattern is usually associated with heterotrophic (Davidson *et al*., 2007) and mixotrophic organisms (Jacquet *et al*., 2002).

Notwithstanding nutrient-sufficient cells grown in enriched L1 medium (L1+DOM) reached higher abundances and Chl *a* levels, L1/5+DOM *Alexandrium catenella* had a higher growth rate. This was also the series in which the DON decrease was the largest (Fig. 2A). The growth rates observed in the present study (0.32 ± 0.01 day^{-1} in the control and up to 0.43 ± 0.03 day^{-1} in L1/5+DOM) are higher than those reported by Carlsson *et al*. (Carlsson *et al*., 1998) in a similar experiment on the same species (0.21 day^{-1} in the control versus 0.27 day^{-1} with DOM added). The DOM concentrations were lower in our case, from 10 (natural) to 30 μM DON (after enrichment) versus 25 (natural) to 40 μM DON in Carlsson *et al*. (Carlsson *et al*., 1998), and from 170 (natural) to 250 μM DOC versus 110 (natural) to 850 μM DOC in Carlsson *et al*. (Carlsson *et al*., 1998). This difference could be attributed to the marine and predominant autotrophic nature of DOM used in the present investigation, apparently more favourable than the higher levels of DOM of terrestrial origin (Carlsson *et al*., 1998). Nevertheless, the relative increase of growth rates in the treatments with added DOM was similar (28% in our study versus 29% in Carlsson *et al*., 1998). In both cases, no ammonium accumulation occurred (0.1–0.9 μM) in our study versus undetectable to 0.8 μM in Carlsson *et al*. (Carlsson *et al*., 1998). Also, no apparent ammonium excretion was observed during DON use, either by bacteria or *A. catenella*, as reported (Jauzein *et al*., 2008b).

Bacteria concentrations were generally lower in BV+DOM control where microalgae were absent. Indeed, fresh photosynthetic DOM is recognized as a substantial factor for bacterial development (Hopkinson *et al*., 2002). However, the decrease of bacterial abundance observed after day 2 in DOM enriched bioassays could be associated with nutrient limitation (Williams, 1995) and further competition mechanisms between *A. catenella* and bacteria (Davidson *et al*., 2007) as well as viral lysis (Fuhrman, 1999). In the L1 medium (control), bacterial growth rate (0.8 day^{-1}) was higher than that in Carlsson *et al*. (Carlsson *et al*., 1998) in their control (bacteria in natural seawater) (0.30 ± 0.07 day^{-1}), even though background DOM levels were higher in the latter. The difference could be due to the higher temperature in our study (20°C) in comparison with theirs (16°C). The increase of viruses in the final days of the experiments was concomitant with the increase of host-virus systems, herein represented by the increase in bacterial numbers (Bratbak *et al*., 1994).

Possible implications to the natural environment

Alexandrium catenella was subject to the enrichment of autotrophic DOM of diatom origin. Predominantly autotrophic DOM may be found in regions where primary production represents the basic source of organic matter such as offshore areas, upwelling regions with limited allochthonous inputs and other areas near pristine conditions.

Taking into consideration that (i) the export of DOM from coastal upwelling areas to the adjacent oligotrophic ocean represents a large part (35%–58%) of the local net production (Álvarez-Salgado *et al*., 2007) and that (ii) diatoms are the greatest primary producers during the active upwelling stage (Hutchings *et al*., 1995; Loureiro *et al*., 2005), we may hypothesize that diatom-autotrophic DOM exported from coastal upwelling centres to oligotrophic open waters could contribute to the triggering of *A. catenella* offshore blooms. Indeed, the contribution of advected nutrients, from upwelling areas to open shore waters, to the development of offshore blooms has already been recognized (McCreary *et al*., 1996). In the California upwelling system, *A. catenella* blooms appear to develop offshore being transported onshore during relaxation-favouring winds and/or downwelling conditions (Price *et al*., 1991; Anderson *et al*., 2008). On the south coast of Chile, also affected by periodic upwellings (Blanco *et al*., 2001; Escribano *et al*., 2004) and recurrent blooms of *A. catenella* (Clément *et al*., 2002; Fuentes *et al*., 2008), the hypothesis of blooms initiated offshore has also been raised (Molinet *et al*., 2003). Overall, although no specific investigations have been performed to directly assess the dynamics and initiation of *A. catenella* open coast blooms near upwelling areas, the implications of autotrophic DOM could be significant for bloom development.

Alexandrium catenella inshore outbreaks could also benefit from autotrophic DOM produced by previous diatom blooms. Few studies have addressed *in situ* population dynamics of these blooms. Nevertheless, both off the northern (Clément *et al*., 2002) and southern (Guzmán *et al*., 2002) coast of Chile diatoms generally precede the blooms of this dinoflagellate. In Japan, *A. catenella* proliferations are observed following diatom blooms during low inorganic nutrient conditions (Takeuchi and Yoshida, 1999). The ability to use the labile organic pool freshly produced by diatoms would complement the trophic needs of *A. catenella* conferring this species a competitive nutritional strategy favouring its development.

In a broader context, this study may complement the classical view of microalgal succession from diatom to dinoflagellate life-forms based on turbulence and inorganic nutrients factors (Margalef, 1978) by including

autotrophic organic matter of diatom origin as a potential surplus for the sustenance of mixotrophic dinoflagellates.

The complex biotic and trophic interactions associated with DOM utilization, including processes such as physiological adaptation, competition, predation, viral lysis, often confound a simple relation between microalgae and organic matter. Taking into account the growing information on the mixotrophic abilities of species forming HABs, we recommend that DOM be included as a variable in monitoring programs as well as in model approaches. Additionally, the integration of *A. catenella* proliferations into the context of previous bloom events as well as co-occurring species would help attain a better understanding of its associated biological and trophic background.

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