Strong small-scale temporal bacterial changes associated with the migrations of bloom-forming dinoflagellates

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Abstract

Bacterial abundances in nearshore Mediterranean planktonic environments tend to change seasonally by 10-fold. Strong daily changes in bacterial abundance, at least as large as seasonal range, occurred in the presence of large dinoflagellate populations performing daily vertical migrations. The daily variability of heterotrophic bacteria was associated with the daily migrations of a bloom of *Alexandrium taylori* in La Fosca Bay, and *Gymnodinium impudicum* in Barcelona harbor. Bacterial abundance in surface waters can change daily as much as from 1 × 10^6 to 5 × 10^6 with apparent net change rates of 0.24 h⁻¹. We suggest that the migrating dinoflagellates create microstructures exploited by the bacteria, and that the large algal populations (>10^6 cells l⁻¹) make this microstructure visible with conventional sampling protocols. We also show evidence of the link between dinoflagellate abundance and relative bacterial activity in these waters, as measured by the percentage of bacteria with high nucleic acid content.

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1. Introduction

Large research efforts are centered in the understanding of the ecology of algae that produce harmful algal blooms (HABs), given their economic and sanitary impact (Anderson, 1997; Smayda, 1997). HAB events are thought to have strongly increased in frequency and importance in recent years (Millie et al., 1999; Garcés et al., 2000). Because of that, the interactions between the HAB and zooplankton or other components of the microbial food web are commonly studied from the perspective of the HAB species, i.e. whether zooplankton can impede the development or terminate the bloom (e.g. Turner and Tester, 1997; Calbet et al., 2003), or whether symbiotic bacteria might affect development or the dynamics of the dinoflagellates (e.g. cyst formation, Adachi et al., 1999), or their toxin production (e.g. Doucette, 1995; Doucette et al., 1998). While researchers have studied the toxic effects of the dinoflagellates on zooplankton...
(i.e. Frangópulos et al., 2000; Calbet et al., 2002), not much attention has been given to the effects of the HAB species on the free-living bacteria in the same waters.

There are reasons to expect that the presence of high dinoflagellate concentrations (a bloom can reach 10⁶ cells l⁻¹) should affect the dynamics of the bacterial community in the surrounding water, and more so if the dinoflagellate performs daily vertical migrations, as has commonly been shown (Eppley et al., 1968; Kamykowski, 1995; Kamykowski et al., 1998). Ecological factors promoted by the dinoflagellate that could influence the accompanying bacteria are: (a) the possible grazing by dinoflagellates directly on bacteria (Porter, 1988), or indirectly on bacterial predators (i.e., HNF, ciliates, Bockstahler and Coats, 1993; Li et al., 1996), (b) the daily variations in the organic matter and/or inorganic nutrient field caused by the daily light forcing on the migrating dinoflagellate population, or (c) the degradation of the dead or decaying algal cells (i.e. Vaqué et al., 1989). The interactions of a bloom-forming dinoflagellate with the autochthonous bacteria are not expected to be different from those between normal non-blooming algal populations and their bacterial neighbors, but they may appear exaggerated because of the large abundances reached by the blooming species. It is, thus, appealing to think that the study of the interactions between a dinoflagellate bloom and the natural bacterioplankton community might typify the processes commonly occurring between algae and bacteria (i.e. Cole, 1982), and illustrate the relationship of an almost monospecific algal community and its associated bacteria.

The aim of this study was to examine the spatial and temporal changes in bacterial abundance during bloom developments of HAB species in semi-enclosed areas of the Mediterranean coast. We also studied the daily changes in bacterial abundance, as they relate to the daily changes in the dinoflagellates. We highlight the fact that the common daily migrations of several dinoflagellate species generate daily changes in bacterial abundances much larger than the seasonal changes in total community bacterial abundance. To our knowledge, this is the first report in which such a strong impact of the dinoflagellates on total bacterial community is described. The reviews published to date on the relationship between HABs and bacteria (Doucette, 1995; Doucette et al., 1998) have not presented any indications of these effects. When we study the interactions between bloom-forming algae and bacteria from the point of view of the picoplankton, the presence and daily activities of the migrating dinoflagellate are the most important factor explaining the daily changes in bacterial properties.

2. Material and methods

We studied two areas of the NW Mediterranean coast, where we have described recurrent summer blooms of the dinoflagellate species Alexandrium taylori in La Fosca Bay (Garcés et al., 1999; 2000) and Gymnodinium impudicum in the Barcelona harbor (Vila et al., 2001). La Fosca bay (41°51′N and 3°8′E), located on the Catalan Costa Brava, is approximately rectangular, measuring ~500 m × 300 m with the opening facing southeast. The average and maximum depths are 3 and 7 m, respectively, with a fairly uniform and gentle slope between 2 and 7 m (for details, see Garcés et al., 1999). Tides are irrelevant in this area of the Mediterranean, and the water was calmed during the daily cycles studied here. During the summer of 2000, three fixed stations over 1 m depth, located 5 m away from the water break point and separated approximately by 15 m were sampled every 3–4 days at around GMT noon during the bloom period (from June to September). The samples were collected in 11 bottles and brought immediately to the laboratory, where they were appropriately fixed (see below). When cell density values were higher than 10⁵ cells l⁻¹ (and water discoloration occurred), sampling was carried out every 2 h during a 24-h period in 1996 (August 19–20) and in 2000 (July 25–26) to describe the daily changes in the population of the dinoflagellate and bacteria. These “daily” samplings were performed at the central sampling station. Seawater samples for the description of the vertical migration of G. impudicum were collected in surface waters of the Barcelona harbor in July 1998, when a bloom of the non toxic, chain-forming, red tide dinoflagellate G. impudicum was taking place. G. impudicum was studied in an enclosed population inside two transparent Plexiglas columns (6 cm of diameter and 2 m height). The Plexiglas columns were filled with the natural water in
the Barcelona harbor at 6:00 GMT, and placed in the harbor so that the cells could freely migrate, but advection was prevented. We started sampling at 8:00 GMT for the following 36 h, using a syringe with an extension tube to sample inside the columns.

Chlorophyll a (Chl) was measured fluorometrically with a turner design fluorometer after filtration of 60 ml of water through Whatman GF/F glass fibre filters and extraction in 90% acetone. Phytoplankton samples from La Fosca (150 ml) were preserved with formaldehyde (1% final concentration, 1996) or Lugol iodine (2000), while 25 ml samples from the Barcelona harbor were taken in duplicate (outside the columns) and preserved with Lugol iodine solution. In both harbors were taken in duplicate (outside the columns) and preserved with Lugol iodine solution. In both cases, an aliquot was settled overnight in 10–50 ml counting chambers and an appropriate area of the chamber then scanned for phytoplankton enumeration at 63–400× magnification using a Leica–Leitz DM-IL inverted microscope. Heterotrophic nanoflagellate abundance was determined by epifluorescence microscopy after DAPI (Porter and Feig, 1980) staining of 20–30 ml, that had been filtered through 0.6 μm Nuclepore filters and stained for 15 min with 5 μg ml⁻¹ of DAPI. For bacterial abundance and relative size by flow cytometry, a 1.2 ml subsample was preserved with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration), frozen in liquid nitrogen and stored at −70 °C. The samples were later unfrozen, stained for a few minutes with Syto13 (Molecular Probes) at 2.5 μM and run through a flow cytometer. We used a Becton & Dickinson FACS Calibur bench machine with a laser emitting at 488 nm. Samples were run at low speed (approximately, 18 μl min⁻¹) and data were acquired in log mode until around 10,000 events had been recorded. We added 10 μl per sample of a 10⁶ ml⁻¹ solution of yellow–green 0.92 μm polysciences latex beads as an internal standard. Bacteria were detected by their signature in a plot of side scatter (SSC) versus green fluorescence (FL1). The fixed samples were diluted (2–4)x with MilliQ water, so that the rate of particle passage was kept below 500 particles per second and thus, coincidence could be avoided (Gasol and del Giorgio, 2000). We used the percentage of high nucleic acid content bacteria (%HNA) as a proxy for the activity structure of the bacterial community. The observation of Syto13-stained bacteria in the flow cytometer allows the clear separation of two subgroups, that we have called HighNA and LowNA bacteria (Gasol et al., 1999). Growing evidence suggests that, at least in coastal environments, the HNA bacteria are the active members of the community (Gasol et al., 1999; Servais et al., 1999, 2003; Lebaron et al., 2001, 2002) while the LDNA bacteria include “bacterial ghosts” and other inactive bacteria (Gasol et al., 1999). Apparent net growth rates (ANG) were computed as ANG = ln(1 + (C₁ − C₀)/C₀)/dt, and apparent doubling times (T) as T = ln(2)/ANG, where C₁ and C₀ are the final and initial concentrations of bacteria in the time interval dt.

3. Results

3.1. Seasonal and spatial changes in bacterial abundance

Bacterial abundance changed year-round by a factor of ~10 during the summer of 2000 in La Fosca Bay (Fig. 1C), a typical representative site of NW Mediterranean coastal waters. On a first glimpse, bacterial abundance in La Fosca was not related to the development of the recurrent bloom of A. taylori during summer (N = 17, Pearson’s correlation R = 0.51, P = 0.03) that typically occurs from June to September (Fig. 1A, B and García et al., 1998, 1999). This bloom, which reached cell concentrations of up to 8000 cells ml⁻¹ in the summer of 2000, produced chlorophyll a values as high as 45 μg l⁻¹, while levels in early summer never exceeded 0.5 μg l⁻¹ (Fig. 1A). Similarly, there was no significant relationship between the abundance of heterotrophic nanoflagellates and that of bacteria (N = 16, Pearson’s correlation R = −0.16, P = 0.54, Fig. 1C). However, and perhaps surprisingly, an index of bacterial activity, such as the (percentage) of HNA cells, strongly covaried with dinoflagellate abundance (Fig. 1B). Log Alexandrium abundance and %HNA were well correlated (N = 19, Pearson’s R = 0.733, P < 0.0005), indicating that, even though bacterial abundance did not respond directly to the presence of the dinoflagellate, the relative composition of the community in terms of active and inactive cells did.

In the summer of 2000, we sampled three sites in La Fosca Bay spaced a few meters apart to check whether spatial variability was, or was not of the same order of
magnitude as temporal variability. Neither for *Alexandrium* nor bacterial abundance, was spatial variability stronger than temporal variability (CVs of 41% for the spatial variability and 43–56% for the temporal component in the dinoflagellate, and 19% for the spatial and 19–29% for the temporal component for bacteria). This indicated that whatever the spatial structure measured in the bay, it was not more relevant than the temporal factor in determining final abundances.

### 3.2. Changes in bacterial abundance during daily cycles

Daily changes in bacterial abundance varied as much as five-fold in a single day in the summer of...
1996 (Fig. 2A). In the summer of 2000, the daily change was slightly smaller (Fig. 2B). Apparent net growth rates computed from the data showed that the rates of variation of bacterial abundance throughout a daily cycle were much larger than the seasonal rates (Table 1). These rates converted to apparent doubling times are of ~2 days on average in Blanes Bay, 4-5 days in the seasonal study of La Fosca Bay, but as low as 5 (1996) or 7 (2000) h in the daily cycles.

In 1998, we sampled a population of G. impudicum that bloomed inside the Barcelona city harbor. This species reached concentrations of up to 3500 cells ml\(^{-1}\), when we studied its daily variations (see also Belviso et al., 2000). Gymnodinium migrated to the bottom of the water column during the dark hours and to the surface in the early morning (Fig. 3). Bacteria, again, exhibited strong daily changes, with rates of apparent change in the order of magnitude of

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those detected in La Fosca Bay (Table 1). We computed an average apparent doubling time of 9 h, slightly lower than for bacteria in La Fosca. The changes are important not only as changes in bacterial abundances, but also in the relative activity of the members of the community, as seen in the %HNA values: bacteria were more active at night, when the abundances were smaller (Fig. 3). Both in La Fosca Bay and Barcelona harbor, bacterial numbers were below the daily averages during the night and above the daily averages during the day (Fig. 4), even though the cycles were different.

3.3. Bacterial relative activity

While seasonally bacterial relative activity, as measured by the %HNA bacteria, covaried with dinoflagellate abundance (Fig. 1), this did not seem to be the case when inspected at the daily variation scale (Figs. 2 and 3). The percentage of HNA bacteria, however, varied daily as much as 20% in the 1996 La Fosca cycle and, most commonly, 10–12% in 2000 in La Fosca and 1998 in Barcelona harbor. There was an inverse relationship between total bacterial abundance and %HNA. The maximum %HNA bacteria was observed either at the abundance minimum (in the Barcelona harbor, Fig. 3) or a few hours before the maximum bacterial abundances (in La Fosca Bay, Fig. 2).

4. Discussion

The seasonal changes in bacterial abundance in La Fosca Bay are of the magnitude expected in non-tidal coastal areas of the NW Mediterranean. Vaqué (1996) studying Blanes Bay, approximately 20 km south of La Fosca Bay, measured concentrations ranging from...
2 × 10^5 cells ml^-1 in winter up to maximal values of 1.4 × 10^6 ml^-1 in summer. The range was smaller in the Medes Islands, approximately 20 km north of La Fosca Bay: from 3 to 9 × 10^5 cells ml^-1 (Ribes et al., 1999). Further North, in Villefranche-sur-mer, bacterial abundance ranged seasonally between 2 and 7 × 10^5 cells ml^-1 (Ferrier-Pagès and Rassoulzadegan, 1994). These values contrast with the daily changes that we report: bacterial abundance can vary as much as five-fold in a single day, as found in the summer of 1996 (Fig. 2). These daily changes are of the same magnitude as the changes measured year-round in the Medes Islands example cited above. They are also more important than the small-scale temporal changes recorded in Blanes Bay. Furthermore, the doubling times calculated from the daily changes (Table 1) would be unrealistic rates for coastal bacterial communities, which normally double in 14–35 h (e.g. Ferrier-Pagès and Rassoulzadegan, 1994). The daily bacterial rates are, however, not greater than those of Alexandrium, that had apparent doubling times of around 1 h (Table 1).
We observed strong changes in bacterial relative activity during the daily cycles. It is tempting to suggest that the lower %HNA coinciding with the maximal dinoflagellate cell density (e.g. Fig. 2) is related to the demonstrated capability of some dinoflagellates to produce antibacterial metabolites that would prevent or retard bacterial attachment and activity, particularly when in stationary growth (e.g. Trick et al., 1984). However, the variations in %HNA that we have identified can be related and/or be confounded with the daily variations in specific bacterial activity shown in NW Mediterranean waters (where the (percentage) of nucleoid-containing cells were lower in the morning than during the day; Gasol et al., 1998), and also in the Baltic (Hagström et al., 2001). With the limited information that we have, we cannot discriminate whether the variations in %HNA shown are related strictly to the daily bacterial cycle, or to different types of bacteria (LowNA, HighNA) that possibly appear/disappear with different daily patterns (e.g. Zubkov et al., 2004).

It seems safe to assume that the daily changes in bacterial abundance are related to the changes in dinoflagellates, which perform daily vertical migrations from the surface sediment to surface waters (Garcés et al., 1999), typical movements of blooming dinoflagellates (e.g. Kamykowski et al., 1998). We can divide the possible interactions between the migrating dinoflagellates and bacteria into three types: (i) the daily changes in bacterial abundance could be related to daily variations in grazing pressure, (ii) to changes in the attachment of bacteria to flagellates, or (iii) to changes in the organic matter microenvironment consequence of the light-dependence of dinoflagellate photosynthesis. We explore these possibilities in the following paragraphs.

(i) Relatively strong daily changes in bacterial abundance have been associated with changing protozoan predation pressure in some freshwater environments (Psenner and Sommaruga, 1992), but the changes reported in those studies are below a 2x variation between the maximum and minimum daily concentration (see also Riemann et al., 1984). For HNF to have a role in the daily changes of bacteria in La Fosca Bay, they should be feeding at rates similar to those of growth (i.e. Table 1). Thus, average hourly rates of 0.07–0.12 (Table 1), with HNF abundances around 1 x 10^3 ml^-1 (Fig. 1), would translate into unrealistic (e.g. Vaqué et al., 1994) protozoan grazing rates (for 1 x 10^6 bacteria and 1 x 10^3 HNF ml^-1, between 70 and 200 bacteria HNF^-1 h^-1). While Legrand and Carlsson (1998) concluded that there was no evidence of bacterial phagocytosis in Alexandrium, feeding of dinoflagellates on small ciliates and flagellates is commonly reported (Bockstahler and Coats, 1993; Jacobson and Anderson, 1996). While migrating dinoflagellates could be feeding on the bacterivorous protists, it is not likely that the reported daily changes in bacterial abundance are related to that pattern, particularly because in the 1996 daily cycle the bacterial maxima followed the dinoflagellate maxima (Fig. 2); in 2000, the bacterial maxima preceded that of the dinoflagellate; and in Barcelona harbor (Fig. 3) bacteria and dinoflagellate seemed to covary.

(ii) The rates of apparent change reported in Table 1 are much greater than expected in a coastal bacterial community (which tend to be in the range of 0.02–0.05 h^-1). These rates would represent extremely high bacterial productions, and it is most likely that it is not that bacteria were being produced and consumed at these high rates, but that they appeared/disappeared from surface waters (those sampled) at these rates.

Given that we measured bacterial abundances with a flow cytometer and, consequently our bacterial numbers represent the concentrations of free-living bacteria, one could postulate that daily attachment/unattachment patterns could explain the data we report. However, the samples were vortexed before counting so that any bacteria loosely attached to particles would be counted as free-living. Bacteria could, instead, be attached to the surface of the migrating dinoflagellates and, thus, move in association with the algal cells. If those bacteria were mainly LowNA, this would explain the daily variation in %HNA that we report. While a given algal cell has commonly between 5 and 20 attached bacterial cells when in full growth (Vaqué et al., 1989), they might harbor as many as 100 cells per algae in lab cultures (Doucette, 1985) or during senescence (Rausch de Traubenberg and Soyer-
Microscopical examination yielded a value of ~100 bacteria per *Alexandrium* in the La Fosca samples, but we could not detect any relevant daily variations in this number. Furthermore, not even a daily change (attachment/unattachment) of 100 bacteria per dinoflagellate per day could explain the apparent bacterial net growth rates of Table 1: *Alexandrium* changed in La Fosca in 1996 from a minimum of 10 to a maximum of 2000 cells ml$^{-1}$ (Fig. 2). This concentration would translate to a value of $1.8 \times 10^5$ bacteria per milliliter, if we assume that there were 100 bacteria per flagellate. This value, scaled to the $10^6$ bacteria per milliliter present in the bay (Fig. 1), would yield a rate of change of 0.014 h$^{-1}$, that does not compare to the values of Table 1.

An alternative possibility that our observations do not allow testing, is that a gyrotaxis-like (Kessler, 1985) downward movement of the algal population could potentially force bacterial cells in the proximity of the algae to perform daily migrations being transported with the flagellated algae. The phenomenon of gyrotaxis has been observed in cultures of *A. taylori*, and potentially occurs in situ. At the population level, it has been suggested that gyrotaxis may contribute to the formation of shear-mediated cell clusters in natural water columns that increase passive descent velocities by 10-fold (Mitchell et al., 1990). This phenomenon could help the downward movement of the cells in the water column and contribute to the “disappearance” of bacterial cells from surface.

We believe that the most likely explanation for the daily changes in bacterial abundance associated with changes in the presence of migrating dinoflagellates has to do with the varying environmental conditions in the sampled surface waters caused by the presence/absence of large quantities of algal cells performing photosynthesis during light hours, and excreting DOC during only a fragment of the daily cycle. Dinoflagellates would thus create microenvironments, which would attract motile bacteria, as suggested by Bell and Mitchell (1972) and later demonstrated by Blackburn et al. (1998). Up to 70% of the bacteria can be motile in nearshore marine environments (Grossart et al., 2001), and the strong changes in the nutrient field created by the moving dinoflagellates could be exploited by these motile bacteria. Thus, the presence and dynamics of a dinoflagellate bloom would simply be an amplified view of the microheterogeneity at the bacterial scale that different researchers, with different techniques, have measured (Mitchell and Furhman, 1989; Duarte and Vaqué, 1992). In particular, Seymour et al. (2000) using a pneumatically-operated multiple syringe system have shown that 40-fold variations in bacterial abundance can exist over distances as small as a few millimeters. This microscale variability, detectable only with special sampling devices, would appear detectable to us if amplified by the presence of the dinoflagellate, even if we had sampled at an inappropriate scale (i.e., one 1.5 ml sample). If the blooming dinoflagellates perform strong vertical movements, this would simply help create such heterogeneity.

Given that bloom dinoflagellates are common in the Catalan Mediterranean coast (Vila et al., 2001), and that there are no reasons to believe that it should be different elsewhere, we believe that the strong daily changes in bacterial abundance and activity reported could be general in coastal regions rich in natural or man-made, semi-enclosed bays where low turbulence and high nutrient levels facilitate dinoflagellate growth (i.e. Vila et al., 2001). Our data suggest that any seasonal study on the abundance and activity of coastal bacteria based on weekly, or even daily samples, might be spurious if, as shown, strong daily changes in bacterial abundance and activity are expected to be associated with the development of dinoflagellate populations.

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