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Spatio-temporal patterns in phytoplankton assemblages in inshore–offshore gradients using flow cytometry: A case study in the eastern English Channel

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ABSTRACT

A pulse-shape recording flow cytometer (CytoSense©) was applied to the monitoring of changes in phytoplankton distribution along an inshore–offshore transect across the eastern English Channel (EEC), on 13 occasions during the main productive period of the year. Amongst the eight phytoplankton groups discriminated, picophytoplankton (picoeukaryotes and *Synechococcus* spp.) and *Phaeocystis globosa* nanoflagellates were the main contributors to total phytoplankton abundance, while Diatoms-like, Coccolithophores, and Cryptophytes represented each one less than 5%. High spatial resolution revealed important changes on relatively short distances. Moreover, a general decrease of Diatoms-like, *P. globosa* haploid cells, Coccolithophores, and picoeukaryote abundance was evidenced from inshore to offshore waters, associated with an increase of *Synechococcus* spp. abundance. Seasonal variability accounted for 71% of phytoplankton abundance changes. Compared to previous studies in the area the CytoSense allowed highlighting new players during the winter–spring–summer phytoplankton succession: (i) high abundance of *Synechococcus* spp. and picoeukaryotes I in winter and of *Synechococcus* spp. also in the summer, (ii) a transient abundance peak of picoeukaryotes II, and (iii) high abundance of Coccolithophores and Cryptophytes during the wax of *P. globosa* bloom and in the summer. The relationships between environmental variables and phytoplankton assemblages indicated that nutrients and the daily light intensity were the most important parameters in structuring the winter–spring–summer transitions.

1. Introduction

Phytoplankton microorganisms, both prokaryotic and eukaryotic, are responsible for about 45% of annual global photosynthesis, and their community structure plays a key role in the link between biogeochemical cycles (Falkowski, 1994; Falkowski et al., 1998; Gregg et al., 2003) and trophic web dynamics (Cloern, 1996). Therefore, it is crucial to address the general pattern of their temporal and spatial variability, and the factors controlling the observed changes in the phytoplankton community structure.

Previous studies on phytoplankton composition in the mesotrophic and well-mixed coastal waters of the eastern English Channel (EEC), based upon microscopic counts, showed that colonial diatom abundance, which gradually decreases towards offshore waters (Dupont, 1980; Hédin-Bougard, 1980), forms the bulk of phytoplankton biomass except during the spring bloom of the Prymnesiophyte *Phaeocystis globosa* (Breton et al., 2000; Gómez and Souissi, 2007; Grattepanche et al., 2011a, 2011b; Lamy et al., 2009; Lefebvre et al., 2011; Schapira et al., 2008). Pigment signatures confirmed these results showing the dominance of *P. globosa* and the lower contribution of other taxa such as Cryptophytes, Chlorophyceae and Cyanobacteria, despite their occurrence throughout the year (Breton et al., 2000).

Although light microscopy allows identification and enumeration of microphytoplankton and in particular diatoms, it has several drawbacks: (1) it makes difficult or impossible to obtain an accurate identification and quantification of small cells (<5 μm), (2) the requirement of fixatives to preserve samples may cause bias by modifying the shape and also the integrity of the naked organisms (e.g. Breteler, 1985; Zarauz and Irigoien, 2008). Pigment analysis by HPLC has often been used to complement light microscopy and to reveal the phytoplankton composition, especially of small sized phytoplankton groups. However, some marker pigments (e.g. fucoxanthin) are present in several phytoplankton classes and some classes present various pigment signatures, such as Prymnesiophytes (Jeffrey and Wright, 1994). Recently, molecular analysis made it possible to reveal the whole eukaryotic in the EEC but this innovative technique has the major drawback of being semi-quantitative (e.g. Christaki et al., 2014). Besides, there is not yet the

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possibility of applying sequencing in studies that focus on high spatial or temporal resolution and real time monitoring of phytoplankton dynamics. Compared to a conventional FCM, the CytoSense (CytoBuoy©) pulse-shaped recording flow cytometer (FCM) allows the analysis of larger volumes of water samples and can detect a larger size range of cells and/or colonies, from ~1 μm to ~800 μm. The CytoSense can discriminate particles on the basis of their optical properties (Dubelaar et al., 1999), including size, shape, and fluorescence derived parameters (Rutten et al., 2005; Thyssen et al., 2008a, 2008b). Another advantage of the CytoSense is its capacity for a rapid enumeration of live samples allowing the possibility of performing a relative high spatio-temporal resolution.

Recently, the use of the CytoSense flow cytometer during a survey carried out in the EEC (Bonato et al., 2015) highlighted the presence of picophytoplankton in relatively high abundance in offshore waters, which had been overlooked in previous studies using light microscopy and/or pigment signatures (Breton et al., 2000). These observations were also confirmed through sequencing studies in the area (Christaki et al., 2014; Genitsaris et al., 2015). Moreover, this previous study showed that phytoplankton abundance and composition varied greatly over short distances as small as ~10 km in the French part compared to the English one (Bonato et al., 2015), probably caused by a mosaic of different freshwater signatures along the French coast. However, no attempts have been made to relate this spatial variability to that of the major environmental factors driving phytoplankton variability such as nutrients and light. Finally, the relative magnitude of spatial variability compared to the temporal one was not evaluated.

The present study aimed at complementing this recent high spatial resolution study during a short period of time (Bonato et al., 2015) by applying this same high spatial resolution approach on an ecological gradient and including the seasonal variability of phytoplankton. Important environmental factors driving phytoplankton variablity such as nutrients and light were also integrated in the present study. This was accomplished by monitoring changes in phytoplankton distribution along an inshore–offshore transect across the EEC, at high spatial resolution, and on 13 occasions during a winter to summer transition including the phytoplankton spring bloom period. Finally, multivariate statistical analysis was used in order to relate the main environmental parameters (temperature, salinity, daily light intensity and nutrients availability) and phytoplankton spatial and seasonal variability.

2. Material and methods

2.1. Sampling strategy and physico-chemical variables

Subsurface seawater (−2 m) samples were collected for FCM analysis, using a 8 dm^3 Niskin bottle onboard the "*Sepia II*" Station Research Vessel (CNRS INSU) on 13 occasions from February to July 2012, along an inshore–offshore transect of a total length of ~10 km, at high spatial resolution (9 samples; Fig. 1). Sea surface Temperature (SST, °C), and salinity (SSS) were measured at a depth of 2 m with a CTD Seabird probe (SBE 19) equipped with a PAR sensor (QSP 2300, Biospherical Instrument). The diffuse attenuation coefficient for downwelling irradiance (k, m^{-1}) was assessed from instantaneous vertical CTD profiles. The average subsurface daily light intensity (I, E m^{-2} d^{-1}) experienced by phytoplankton (over the last 6 days before the cruises) was estimated using the formula of Riley (1957):

$$I = \frac{I_0(1-e^{-kz})}{kz} \quad (1)$$

With z corresponding to the depth where phytoplankton was collected ($z = 2$ m), and I_0, the daily incident light estimated from global solar radiation (GSR, W m^{-2}) measured continuously with a time step of 5 min with a solar radiation sensor (Vantage Pro, Davis) mounted on the roof of our laboratory bordering the seashore, by the sampling area. Before calculating the coefficient, GSR was converted to PAR by assuming PAR being 50% of GSR and by considering 1 W m^{-2} = 0.36 E m^{-2} d^{-1} (Morel and Smith, 1974).

In addition, seawater samples for nutrient (silicate [DSi], phosphate [DIP], and nitrate + nitrite [DIN]) analysis were collected only at 5 stations over 9 (S1, S3, S5, S7 and S9). Nutrient concentrations (μM) were assessed according to the methodologies outlined in Aminot and Kérouel (2004), using the *Integral Futura* autoanalyser system (*Alliance* Instruments).

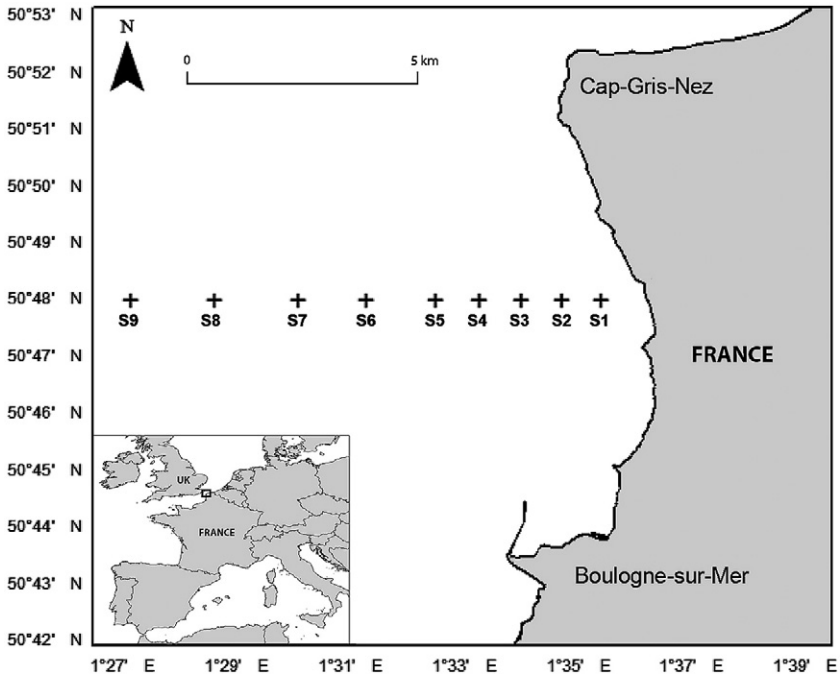

Fig. 1. Map of the study area showing the location of the 9 sampling stations in the eastern English Channel (Saint Jean Bay, near the Strait of Dover).