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# Sub meso scale phytoplankton distribution in the north east Atlantic surface waters determined with an automated flow cytometer

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#### Abstract

Phytoplankton cells in the size range  $\sim 1-50 \,\mu$ m were analysed in surface waters using an automated flow cytometer, the Cytosub (http://www.cytobuoy.com), from the Azores to the French Brittany during spring 2007. The Cytosub records the pulse shape of the

- optical signals generated by phytoplankton cells when intercepted by the laser beam. A total of 6 distinct optical groups were resolved during the whole transect, and the high frequency sampling (15 min) provided evidence for the cellular cycle (based on cyclic changes in cell size and fluorescence) and distribution changes linked to the different water characteristics crossed in the north east Atlantic provinces. Nutrient concentra-
- tions and mixed layer depth varied from west to east, with a decrease in the mixed layer depth and high nutrient concentrations in the middle of the transect as well as near the French coast. Data provided a link between the sub meso scale processes and phytoplankton patchiness, some abundance variations due to the cellular cycle can be pointed out. The high frequency spatial sampling encompasses temporal variations of the phytoplankton abundance, offering a better insight into phytoplankton distribution.

#### 1 Introduction

Phytoplankton encompasses thousands of species that develop by simple division at a rate of about once a day, thus potentially doubling their abundance every 24 h. The process is regulated by a succession of abiotic and biotic interactions specific to marine
environments. The phytoplankton size range is spread over 4 orders of magnitude. From a recent estimation, phytoplankton would represent 2% of the earth photosynthetic biomass, but they contribute to ~45% of the earth annual primary production (Field et al., 1998). Picophytoplankton cells are the most abundant, particularly in the oceanic oligotrophic provinces where their small size provides them a better buoyancy and accessibility to nutrients. The abundance of nano- and microphytoplankton highly depends on nutrient availability, the increase of which occurs after the shoaling of the

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winter mixed layer in early spring for example. Phytoplankton can occasionally behave as an inert tracer, depending on the hydrodynamism of their environment (Skellam, 1951). However, the situation is not as simple. Indeed, the abundance variability of phytoplankton, composed of drifting cells, is not only controlled by division processes,

- <sup>5</sup> but also by grazing, sinking, viral lysis, light and competition for nutrients, all depending on the scale and the strength of the surrounding physical processes (Fogg, 1991). This results in heterogeneous distributions with respect to both time and space, regarding abundances and assemblage composition. The actual phytoplankton data sets seldom regroup information on the spatial and temporal dynamics as well as on the morpho-
- logical and physiological status of the studied entities. As a consequence, the categorisation and explanation of the observed patchiness highly depends on the definition of the sampling strategy and may be different from one study to another because of inadequate sampling frequencies (Sherry and wood, 2001).

Phytoplankton spatial distributions are mathematically defined through spectral anal-<sup>15</sup> ysis (Gower et al., 1980), multifractal processes (Seuront et al., 1996), wavelet analysis (Henson and Thomas, 2007) and multipoint correlation (Garcia-Moliner et al., 1993). The aim of mathematical treatments is to define the structure of the phytoplankton patchiness and their dependence on physical or biological processes. They are running on data collected through teledetection (estimation of chl *a* concentration), automated

- fluorometry high frequency recording, spatial and temporal sampling involving pigment analysis and cell counts (either by flow cytometry or optical microscopy). These approaches need calibration of abundances and adjustment of temporal frequencies fitting biological processes in order to properly address in situ diversity and physiology status of the phytoplanktonic assemblages at sub meso scale to meso scale. It is im-
- <sup>25</sup> portant to accumulate as much information as possible on the origin and amplitude of the phytoplankton distribution variability in order to correctly define the processes that govern the phytoplankton distribution and to better understand the marine ecosystem functioning.

The north Atlantic Ocean spring bloom is one of the most impressive when observ-

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ing satellite images. This area is considered as a strong carbon sink (Takahashi et al., 2002), and the evolution of the north Atlantic bloom was subject to many studies (Ducklow and Harris 1993; Memery et al., 2005). Many processes can be involved in the origin, intensity and duration of the north Atlantic bloom (Siegel et al., 2002), and their abarraterization requires a set of accurate methods in order to collect represent

their characterisation requires a set of accurate methods in order to collect representative data. However, in situ observations in open waters covering both spatial and temporal scales of the phytoplankton dynamics and diversity are lacking.

In this paper, we describe the phytoplankton surface distribution determined with an automated flow cytometer (Cytosub, Cytobuoy b.v.; Dubelaar et al., 1999, Thyssen et

- al., 2008) during April 2007, at a sub meso scale sampling resolution (1–10 km), in the north-east Atlantic Ocean, along a transect from the Azores Islands (Portugal) to the French Brittany. This instrument collects the pulse shape of each optical parameter, enabling the cell discrimination into similar optical groups. Relationships were established between the different flow cytometric groups and the successive water characterstics
   crossed during the cruise and defined by their salinity, temperature and nutrient con-
- tent. The access to the sub meso scale variability gave accurate information on the phytoplankton distribution, which would have been critical to interpret otherwise.

#### 2 Material and methods

- 2.1 Sampling strategy
- Samples were automatically collected from the 14 to the 23 April 2007 during a cruise of the "Fetia Ura" sailing ship between Horta (38.6° N–28.6° W, Island of Faïal, Azores) and Lorient (47.6° N–3.6° W, French Brittany) (Fig. 1). Seawater was pumped from the ship central non toxic seawater supply at 1.5 m depth every 15 min during 3 min at 30 dm<sup>3</sup> s<sup>-1</sup>, filling a 1 dm<sup>3</sup> reservoir sampled by the Cytosub 2 min after the pump
   stopped in order to let air bubbles disappear. Another reservoir containing a Conductivity, Temperature Depth (CTD, Microcat SBE 37) sensor was fixed on the deck and

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#### simultaneously filled in order to determine in parallel the temperature and the salinity of the seawater analysed by the Cytosub.

#### 2.2 The Cytosub

The Cytosub was designed to analyse large phytoplanktonic cells (1 to  $1000 \,\mu m$  and a few mm in length) and relatively large water volumes (up to 4 cm<sup>3</sup> per sample). It was cable connected for energy supply and data transfer to a computer. The seawater was pumped to fill a sample loop before entering the flow cell in order to avoid external turbulences and run the analysis at atmospheric pressure. The sample flow was controlled by a peristaltic pump working at a rate of 8.3 mm<sup>3</sup> s<sup>-1</sup>. The instrument used  $0.2\,\mu$ m filtered seawater containing ~1% paraformaldehyde fixative as a sheath fluid. 10 The sheath flow rate was  $4800 \text{ cm}^3 \text{ s}^{-1}$ . The sheath fluid and the analysed seawater were mixed together at the output of the flow cell and filtered through a  $0.2 \,\mu m$  Polycap<sup>™</sup> AS Nuclepore cartridge in order to be recycled. In the flow cell, each particle was intercepted by a laser beam (Coherent solid-state Sapphire, 488 nm, 15 mW) and the generated optical signals were recorded. The light scattered at 90° (side scatter) and fluorescence signals were dispersed by a concave holographic grating and collected via a hybrid photomultiplier (HPMT). The forward scatter signal was collected via a PIN photodiode. The red (FLR), orange (FLO) and yellow (FLY) fluorescences were collected in the wavelength ranges 668-734, 601-668 and 536-601 nm, respectively.

- Data recording was triggered by the forward scatter signal. The shape of the signals 20 was encoded at a frequency of 4 MHz and data were saved in distinct 64 kbit grabbers before their transfer to a computer through the connecting cable. Particles flew at a rate of  $2 \text{ m s}^{-1}$  through the 5  $\mu$ m laser beam so that for instance the forward scatter signal shape of 1  $\mu$ m beads would be defined by ~12 points. More generally, particles
- flowing along their long axis (L ( $\mu$ m)), would have the shape of their forward scatter 25 signal defined by 2\*(5+L) points. The laser alignment and calibration processes were done before and after the cruise using Beckman Coulter Flowcount<sup>™</sup> fluorospheres (10 µm).

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#### 2.3 Cytometric softwares

Cytoclus software (version 2004, Cytobuoy b.v.) was used to analyse the data collected by the Cytosub. Clusters were selected by taking into account the amplitude and the shape of the different signals. In addition to 5 average signal heights for forward scatter (FWS), sideward scatter (SWS) and for three fluorescence signals: red (FLR), orange (FLO) and yellow (FLY), some simple mathematical models were assigned to each signal shape: inertia, fill factor, asymmetry, number of peaks, length, apparent size (FWS size) (Dubelaar et al., 2003). All these values are summarised in cytograms that facilitate the identification of clusters of cells sharing similar optical properties derived
from those mathematical models.

#### 2.4 Statistical analysis

Statistical analyses were performed on R freeware (http://cran.r-project.org/). A non parametric local weighted polynomial regression calculation (Cleveland and Devlin, 1988) script (function LOESS) was applied to the signal of the cell abundances, their red fluorescence and their forward scatter dynamics in order to collect the smoothed signals. The polynomial was fit using weighted least squares, giving more weight to points near the point whose response is being estimated and less weight to points further away. A user-specific input to the loess calculation is possible, and is called "span". The span determines how much of the data is used to fit each local polynomial. The

<sup>20</sup> span varies from 0 to 1; 0 resulting in a non smoothed signal. To extract the variability (low span) of the signals from the trends (high span), low span loess calculations were subtracted from the high span loess calculation. On those differences, the autocorrelation script (function ACF) was used to provide evidence of periodicities in the resulting data set by calculating the correlation of the time series against a time-shifted version <sup>25</sup> of itself.

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#### 2.5 Nutrients analysis

Nutrient  $(NO_3^-, NO_2^-, PO_4^{3-}, Si(OH)_4)$  analyses were processed using 20 cm<sup>3</sup> seawater samples collected every 4 h from the 1 dm<sup>3</sup> reservoir and transferred into polyethylene flasks directly frozen onboard. Analyses were performed using a Technicon Autoanalyser® according to Tréguer and LeCorre (1975). Detection limits were 50, 20, 20 and 50 nM for  $NO_3^-$ ,  $NO_2^-$ ,  $PO_4^-$  and Si(OH)<sub>4</sub>, respectively.

#### 3 Results

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3.1 Hydrology

A total of 691 samples were collected during the cruise. Average distance between <sup>10</sup> successive samples was of 1.84±0.07 km. The ship track (Fig. 1) was superimposed on the map of the average mixed layer depth (MLD) as calculated by the MERCATOR ocean group (http://www.mercator-ocean.fr/) between the 14 April and the 25 April 2007.

Temperature ranged between 13.19 and 16.04°C with an average value of 14.43±0.61°C. Diel oscillations were observable with a temperature decrease before dusk. The maximum temperature variation between day and night was of 20% and occurred between the 21 and 23 April 2007 (between 10° W and 5° W, Fig. 2a). The minimal temperature variation occurred within the period covering the night of 17 April 2007 and the day of 18 April 2007 (between 21.20° W and 18.5° W, Fig. 2a). Salinity decreased from the beginning of the CTD recording to the end (Fig. 2b). Values ranged between 34.05, near the French Brittany coast, and 36.05, in the north Atlantic open waters. Average salinity was 35.07±0.38. No diel oscillation in salinity was detected but a small increase of salinity was observed between 20.65° W and 19.45° W, with an average value of 35.97, corresponding to the area with the lowest temperature diel variation, also characterised by a low mixed layer depth of about 10 m (MERCATOR)

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ocean). The cruise track crossed 4 water types labelled M1, M2, M3 and M4 and distinguished on the basis of their temperature and salinity features (Fig. 3c). Their average hydrological values and nutrient contents are reported in Table 1. The initial fraction of the track where temperature and salinity samples were unavailable was labelled M0.

- MERCATOR model data obtained day after day yielded MLD values of 20–50 m for M0 and M1. Deepest values were calculated inside of M1, reaching ~50 m at 20.5° W, with surrounding values of ~25 m. In contrast, M2, M3 and M4 were characterised by shallower MLD, reaching ~10 m depth (data not shown).
- NO<sub>3</sub><sup>-</sup> concentrations varied from the detection limit up to  $3.95 \,\mu$ M with an average value of  $1.67 \pm 1.14 \,\mu$ m (Fig. 3a). The highest concentrations were observed within M1 and M2 and in the coastal waters M4 (Table 1). PO<sub>4</sub><sup>3-</sup> values varied between 0.07 and 0.42  $\mu$ M with an average value of 0.2 $\pm$ 0.08  $\mu$ m (Fig. 3b). The highest concentrations were observed as well in M1, M2 and in M3 (Table 1). Redfield NO<sub>3</sub><sup>-</sup>/PO<sub>4</sub><sup>3-</sup> ratio (Redfield, 1963) was <16 during the whole transect except near the French Brittany coast within M4, where it reached values of 30 (data not shown). Si(OH)<sub>4</sub> concentrations ranged between 0.2 and 2.4  $\mu$ m with an average value of  $1.05\pm0.4 \,\mu$ M (Fig. 3c) with an average value maximal inside M1 (Table 1). It is noteworthy that within M1 where diel temperature and salinity variations were the lowest, nutrient concentrations dropped down at about 21° W, and particularly that of SI(OH)<sub>4</sub> (Fig. 3).
- 20 3.2 Cluster resolution

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Six clusters were resolved over the whole transect as illustrated on Fig. 4b, c and d. Table 2 describes the average size and ratio (FLR/FLO and FWS/FLR) values characterising the cells of each cluster as defined on Fig. 4. Average cell length in the clusters varied from <1  $\mu$ m for C2 up to maximal values of 50  $\mu$ m for the biggest cells within C5. C2 and C6 clusters exhibited FLR/FLO ratios approximately three times lower than those of the other clusters. Abundances were maximal for C1 and C2 (average value  $15.9 \times 10^3 \pm 15.4 \times 10^3$  cells cm<sup>-3</sup> and  $39.4 \times 10^3 \pm 34.2 \times 10^3$  cells cm<sup>-3</sup>, respectively) and minimal for C5 (average value  $168 \pm 181$  cells cm<sup>-3</sup>).

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#### 3.3 Abundance, FLR and FWS spatial and temporal dynamics

The 6 defined clusters exhibited a high variability in abundance (Fig. 5), red fluorescence (Fig. 6) and forward scatter (Fig. 7) from the Azores up to the French Brittany. These figures were obtained by respectively plotting average abundance, 5 FLR and FWS values per cell for each cluster over the whole transect. Open circles correspond to values averaged over 24 h successive periods. C1 abundance increased from west to east, reaching maximal values of  $110 \times 10^3$  cells cm<sup>-3</sup> in the eastern part of M3 and inside M4 (Fig. 5a). C2 cluster exhibited two important abundance peaks, reaching  $\sim 150 \times 10^3$  cells cm<sup>-3</sup> inside M0 (25.8° W) and inside M2 (15.0° W). Abundance values inside M1 were in average three times lower than in M2 10  $(21.4 \times 10^3 \pm 7.8 \times 10^3 \text{ and } 57.3 \times 10^3 \pm 46.9 \times 10^3 \text{ cells cm}^{-3}$ , respectively) and twice lower in M3  $(32.7 \times 10^3 \pm 14.8 \times 10^3 \text{ cells cm}^{-3})$  than in M2. C2 cluster was undetectable inside M4 (Fig. 5b). FWS and FLR of C2 cells exhibited a decrease through M0 but a sharp increase was observed before entering M1 waters. FLR kept decreasing between M1 and the end of the transect (near 5° W). Cluster C3 reached its maximal abundance inside M1 (average value  $8.3 \times 10^3 \pm 2.5 \times 10^3$  cells cm<sup>-3</sup>) and presented its lowest abundance values in both sampled coastal zones, more particularly near the French coast (average value inside M4 was 765.5 $\pm$ 287 cells cm<sup>-3</sup>; Fig. 5c). Cluster C4 abundance had peaks inside M2, M3 and M4 (average values:  $3.9 \times 10^3 \pm 2.7 \times 10^3$ ,  $3.6 \times 10^{3} \pm 2.4 \times 10^{3}$  and  $3.8 \times 10^{3} \pm 2.4 \times 10^{3}$  cells cm<sup>-3</sup>, respectively; Fig. 5d). FLR 20 (Fig. 6d) and particularly FWS (Fig. 7d) averaged values were lower inside M1 and M2 than elsewhere. C5 cluster was abundant near both coastal areas and particularly inside M4 ( $304\pm398$  cells cm<sup>-3</sup> in M4 and  $206\pm138$  cells cm<sup>-3</sup> in M0; Fig. 5e). FLR and FWS of C5 cluster were particularly high inside M2 (Figs. 6e and 7e). C6 cluster was essentially observed inside M2 and M3 (average values: 299±384 and 608±667 cells cm<sup>-3</sup>, respectively); it was nearly undetectable in M4 and remained below the detection level elsewhere (Fig. 5f).

The short-term variability of abundance, FLR and FWS of the 6 clusters derived from

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loess treatments as detailed in Materials and Methods were submitted to autocorrelation calculations. Figure 8 illustrates such a data handling on abundances of clusters C1 and C3. In panels (a) are displayed the long-term trend and the weakly smoothed signal. In panels (b) are plotted the corresponding short-term variability obtained by
 <sup>5</sup> subtracting the long-term trend to the weakly smoothed signal displayed in panels (a). Panels 8c illustrate the autocorrelation calculations on the abundance short-term variability.

The two first maximal significant autocorrelation values (first negative for  $r^1$  and first positive for  $r^2$ ) of the abundance, FWS and FLR short-term variability for the 6 clusters are reported in Table 3. The autocorrelation calculation for the C1 abundance shortterm variability was significant over the whole sampling period with a lag of 13:30:00. Its FWS autocorrelation calculation was significant for the whole sampling period with a lag of 15:30:00 and only significant and negative for FLR at a lag of 06:30:00 (expressing half of a significant cycle of 13:00:00, Table 3). Consequently, abundance, FWS and FLR of C1 cluster varied cyclically twice a day, with an increase in the after-

- noon and in the early morning (Figs. 5a, 6a and 7a). The autocorrelation calculation for the C2 abundance, FWS and FLR yielded significant values with lags of 19:00:00, 23:00:00 and 21:00:00 respectively (Table 3), expressing an approximately daily cycle for those three variables. Abundance variations provided evidence for a second
- and shorter cycle of approx. 04:00:00 when the span used to define the difference between the general trend and the signal is sharper (span difference=0.3–0.05, auto-correlation significant value=–0.312 with a lag of 04:15:00 followed by –0.499 with a lag of 10:30:00, which corresponds to the main signal as defined in Table 3), FWS and FLR autocorrelations did not show a shorter cycle. But at ~10° W, C2's FLR and FWS increase occurred during the day, and not in the early evening as in M1 and M2.

Considering values averaged over 24 h successive periods, FLR of C3 cluster was rather stable (Fig. 6c) whereas FWS increased along the transect (Fig. 7c). The cycle frequency of those parameters varied during the cruise. From the departure up to 23.6° W, autocorrelations of abundance, FWS and FLR were significant with lags of

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13:45:00, 15:30:00 and 15:30:00 respectively (Table 3). Between 22.6° W and 14° W, autocorrelations were significant with lags of 13:30:00, 28:00:00 and 25:30:00 respectively, indicating that abundance periodic variation had a frequency twice that of FWS and FLR. Between 14° W and 3.75° W, the autocorrelation value of the abundance FWS
 and FLR was significant with the same lag (23:00:00).

Autocorrelation calculation supported significant periodic properties for C4 abundance, FWS and FLR with lags of 14:00:00 (only the negative autocorrelation was significant twice, the first one at 07:30:00, meaning that the positive value of the cycle would be at 14:00:00, and the second one at 21:30:00, confirming the periodicity of the signal), 22:45:00 and 23:45:00, respectively (Table 3). As for C3, FLR and FWS expressed a diel periodicity whereas the abundance periodicity was twice a day, this

expressed a diel periodicity whereas the abundance periodicity was twice a day peculiarity being maintained over the whole transect.

Autocorrelation calculations provided evidence of a periodicity for C5 cluster abundance with a lag of 20:30:00 (Table 3) but failed for FLR and FWS. Only a negative autocorrelation was obtained with a lag of 36:15:00 for FWS and a lag of 13:30:00 for FLR.

C6 cells, although only observed in M2, M3 and M4, expressed a 23:00:00 periodicity for their FLR signature and a 33:15:00 for their abundances and only a negative autocorrelation value for their FWS variability at a lag of 20:00:00.

#### 20 **4 Discussion**

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#### 4.1 Hydrological background

The cruise track crossed three Atlantic provinces (Longhurst, 1995): (i) the North Atlantic East Province SubTropical Gyre from the Azores to approximately 17–18°W, corresponding to M0 and M1 water type areas; (ii) the North Atlantic Drift Province <sup>25</sup> up to approximately 5°W, corresponding to M2 and M3 water type areas; and (iii) the North Atlantic Shelves Provinces, corresponding to M4 water mass area.

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A strong southward geostrophic current was observed between 40° N-45° N at 24° W by Paillet and Mercier (1997), generating a permanent frontal area more specifically located by Paillet and Arhan (1996) at 41–42° N. This frontal area may correspond to the one observed in 2001 during the spring POMME experiment (Fernandez et al., 2005). The increase of NO<sub>3</sub><sup>-</sup> observed between M0 and M1 is reminiscent of the one reported by Fernandez et al. (2005) who found during spring, low surface nutrient values (~1  $\mu$ m) south of this front and high values (close to 7  $\mu$ M) north of it. Siegel et al. (2002), described two regimes north and south of 40° N explaining the origin and the intensity of the phytoplankton bloom. South of this area, the bloom is theoretically limited by light but also by the nutrient availability which is linked to the intensity of the winter mixing, while in the north the bloom development depends on the achievement of the Sverdrup's critical depth, which is mainly a light accessibility process.

4.2 Cluster resolution

The automated resolution of 6 clusters was made possible by recording the shape of
<sup>15</sup> 5 optical parameters giving access to the cell morphological variability (Jonker, 1995; Dubelaar and Gerritzen, 2000). The high sampling frequency and the permanent features of the observed clusters increased the accuracy of the method even if for less populated clusters, the direct counts were not always complying with the 3% tolerated variability (Thyssen et al., 2008). The 6 clusters were nearly the same as those
<sup>20</sup> observed in previous studies (M. Zubkov, personal comm.) as it is the case for ultraphytoplankton clusters definition using bench top flow cytometers. The average cell size in the observed clusters ranged from less than <1 μm up to 50 μm.</li>

C1 cells corresponded in terms of size (~2–3  $\mu$ m, Table 2) to the picoeukaryote community whose average abundance value was 15.9±15.4×10<sup>3</sup> cells cm<sup>-3</sup> (Table 2).

<sup>25</sup> In the study of Zubkov et al. (2000), the abundance of picoeukaryote phytoplankton varied from  $\sim 10 \times 10^3$  to  $\sim 15 \times 10^3$  cells cm<sup>-3</sup> with a peak of  $\sim 25 \times 10^3$  cells cm<sup>-3</sup> at 50° N. During 2001 POMME spring study (18° W, 38–44° N), surface values varied between  $\sim 10 \times 10^3$  and  $\sim 50 \times 10^3$  cells cm<sup>-3</sup> (Fernandez et al., 2007). In spring 2004,



picoeukaryiote-phytoplankton abundance peaked ( $\sim 14 \times 10^3$  cells cm<sup>-3</sup>) in the North Atlantic East Province at 40° N and made a major fraction of total phytoplankton in terms of abundance (Tarran et al., 2006).

- C2 cells had a strong orange fluorescence signature (Table 2) and their small size makes them looking like some phycoerythrin-containing picocyanobacteria (Sherry and Wood, 2001) such as *Synechococcus*. The abundance of C2 cells during this study matches the observed *Synechococcus* abundances reported for surface samples collected from 22 April to 26 May 1997 in the north Atlantic between 35 and 45° N: ~20×10<sup>3</sup> to 200×10<sup>3</sup> cells cm<sup>-3</sup> (Zubkov et al., 2000; Fig. 5b).
- The sum of all the other largest cells (C3, C4, C5 and C6) reached averaged values of 6.9×10<sup>3</sup>± 4.3×10<sup>3</sup> cells cm<sup>-3</sup>, in between the surface abundance (14×10<sup>3</sup> cells cm<sup>-3</sup>, unpublished data) of nanoeukaryote in spring 2001 within the POMME study area (18° W, 38–44° N), and surface abundances (max. 2.2×10<sup>3</sup> cells cm<sup>-3</sup>) of nanoeukaryote in spring 2004 (Tarran et al., 2006). In terms of biomass, Dandonneau et al. (2004) reported that in the North Atlantic Drift Province, most of the phytoplankton and surface abundance (max. 2.2×10<sup>3</sup> cells cm<sup>-3</sup>) of the phytoplankton area constant.
- was composed of nanoplankton (more than 70% of the biomass even during the spring bloom) and of microplankton (30–50% during April 2000 and April 2001).

4.3 Cluster dynamics

The cluster dynamics involved two different spatial scales: (i) the meso scale corresponding to water mass changes, and (ii) the sub meso scale at which cluster dynamics was driven by small physical processes under the strong influence of the cell cycle occurring at a daily scale (time for the ship to cover about 150 km).

The sub meso scale (1–10 km), where space and time are strongly linked, approximately corresponded to 1 to 4 samples, or 15 min. to 1 h at the average speed of the ship. At this scale, clusters exhibited strong cell cycle signatures through FLR and FWS parameters, evidenced on their abundance dynamics and clearly confirmed by



the autocorrelation calculation. Abundance variations, FLR and FWS diel variability

were consistent with cell cycle in numerous studies (Jacquet et al., 2002; Binder and Durand, 2002 and references therein). During the whole study, the cell abundances were affected by the stage of the cell cycle at which sampling occurred. The cell cycle seemed to play a great role in the patchiness observed at sub meso scale. The other patchiness sources would be linked to other small spatial temporal control factors such

- patchiness sources would be linked to other small spatial temporal control factors such as interrelation between species, grazing, viral lysis, migration and turbulence. Considering meso scale, the distribution of the clusters, the cell pigment content and cell size (FLR and FWS, respectively) varied with water mass properties such as nutrients, salinity and temperature. When addressing the spatial distribution of phytoplankton
   assemblages at sub meso scale, it seems quite impossible to define a sampling frequency shorter than the smallest cell cycle that may lead to a correct representation of
- quency shorter than the smallest cell cycle that may lead to a correct representation of this distribution.

Cells in C1 cluster were about  $2.5\,\mu m$  in size and their FLR average value was 5 times lower than that of cells in C3 cluster in spite of their similar size (Table 2). C1 cluster divided approximately twice a day and most of the observed abundance peaks 15 were consistent with this rate. The variation of the global FWS and FLR signals was linked to the crossed water types. For instance, the decrease of FWS and FLR inside M1 can be related to a photoperiod decrease resulting from strong vertical mixing while consistently, the extent of the diel temperature variations was low compared to that observed in M3 and M4. However, the cell cycles were not affected by vertical mixing, 20 in agreement with Jacquet et al. (2002) who reported that strong physical perturbations did not modify the picoeukaryote flow cytometric FLR signature of the cell cycle. Daily average C1 abundances were maximal inside M3 (Fig. 5b, open circles), corresponding to low nutrient values (mainly NO<sub>3</sub><sup>-</sup>, Fig. 3a), and minimal inside M1 and M2 where nutrients and certainly turbulence were high. Indeed, the mixed layer depth was still 25 deep at those latitudes compare to northern areas of the north east Atlantic (Fig. 1). In addition, the intensity of winter mixing south of 40° N, part of the North Atlantic East

Province SubTropical Gyre, is the main source of nutrient input in surface waters by the advection of deep and nutrient rich waters (Siegel et al., 2002) as observed for

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M1 and M2. C1 cells were more abundant in areas with low nutrient content (or non turbulent waters) as commonly observed for picoeukaryotic cells, which was not the case for C2, C3 and C6 cells (Fig. 5b, c, and f). But, C1 abundances were low inside M0 with relative low nutrient concentrations, and high in M4 with relative high nutrient concentrations, suggesting that nutrients were not the principal abundance regulation factor.

Following Nyquist sample theory (Nyquist, 1928) who defined the minimal sampling frequency of a system to be at least twice its highest frequency, in order to sample C1 cells correctly, it would be necessary to make one sample at least every 6 h, or at the average speed of the boat, every 43.2 km. Anyway, this sampling distance may not resolve the sub meso scale processes that would require a higher sampling frequency. Cells of C2 cluster were about 1  $\mu$ m in size and were characterised by high orange fluorescence (FLO). Their abundance was maximal inside of M0 and of M2 and they were not detected near the French coast (Fig. 5b). Such a strong variation in abundance was reported by Martin et al. (2005) for *Synechococcus* cells, with a 50 fold increase 12 km apart. The diel cycle of cells belonging to C2 was similar to the one

- observed for *Synechococcus* in the north Atlantic Ocean (Olson et al., 1990; Wyman, 1999) and the north-western Mediterranean Sea (Jacquet et al., 1998), with an increase of FWS and FLR in the early evening leading to night division. However, the diel
- <sup>20</sup> cycle of C2 cells seemed to shift between M2 and M3. Indeed, despite the strong autocorrelation of abundance, FLR and FWS (Table 3), the period during which FWS and FLR increased, changed inside of M3. The diel cycle remained similar, but occurred earlier (maximum FLR and FWS during daylight), meaning that C2 cells were not synchronous over the track covered by the ship. Such a phase shift was observed for *Syne*-
- chococcus in the surface waters of the equatorial Pacific Ocean (Vaulot and Marie, 1999). The autocorrelation value of abundance dynamics was maximal at 19:30:00, very close to the 18:00:00–19:00:00 periodicity calculated for *Synechococcus* by using Fourrier analysis (Jacquet et al., 1998). M0 appeared as a peculiar environment since a broad peak of abundance was observed during daytime, in contrast with the main

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peak of abundance inside M2 that occurred during night-time, just after the assumed cell division, and no specific FLR or FWS decrease were observed (Fig. 5). In parallel, FLR and FWS values of C2 cells were the lowest in M0 during daytime. Would C2 cells be represented by *Synechococcus* species, their photoprotection properties (Vaulot

- and Marie, 1999) could not account for the large FLR decrease during daytime in M0 since the large mixed layer depth of M0 implies a rather low exposure to maximum light. Such a decrease in FLR and FWS did not occur so dramatically for the other clusters (Figs. 6 and 7). Jacquet et al. (2002) reported that an increase in pigment content for *Synechoccocus* could be linked to an increase in nutrient concentrations. In this study,
- <sup>10</sup> a nutrient increase occurred inside M1 but FLR of C2 cells increased nearly 24 h before the ship entered M1. From M1 to M4, the daily averaged FLR decreased continuously, which may be explained by a change in phenotype of the phycoerythrin containing picocyanobacteria as observed in many cases for *Synechococcus* when reaching coastal zones (Wood et al., 1998).
- <sup>15</sup> Cells of C3 cluster exhibited a high periodicity with an abundance increase twice as fast as the FLR and FWS signatures up to the middle of M2 waters (i.e. 14° W, Table 3). This may be linked to thermal water mass advection occurring between day and night, with no effect on cell cycle. Indeed, M1 may be the most dynamic area since the ship track may have crossed the north east frontal region as discussed previously. Around
- 41° N–21° W where C3 cells were the most concentrated, the MLD went from deep to shallow within a small distance (Fig. 1), and nutrient concentration showed a little decrease (Fig. 3). The C3 phytoplankton cells seemed to be under favourable development despite the little nutrient depletion, and salinity signature suggests that we may have crossed an eddy at a specific stage of the north Atlantic bloom evolution (Karrash
- et al., 1996; Garçon et al., 2001; Fernandez et al., 2005). The periodic variation of FLR and FWS increased in M1 and M2 with respect to M0 (from 15:30:00 in M0 to 25:30:00 and 28:00:00 in M1 and the first part of M2, Table 3), as for the daily average FLR and FWS amplitude (Figs. 6c and 7c) suggesting that in some favourable areas, the cells maintain high abundances with a small size and a low pigment content, coupled to a

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decrease in growth rate. In the Bay of Biscay (Fig. 1), C3 abundance decreased but remained strongly influenced by the cell cycle as suggested by the frequency of FLR and FWS peaks that occurred with some delay with respect to the peak of abundance (Figs. 5c, 6c and 7c, Table 3).

- C4 cells belonged to small nanoplankton (4 μm, Table 2); their FLR and FWS decreased in M1 and M2 (Figs. 6d and 7d) that may be related to the mixing processes as observed for C1 and C3 but lasting much longer for C4 cells since the increase in daily averaged FLR and FWS only occurred inside M3. C4 abundance dynamics clearly illustrate the difference in information brought by high and low frequency sam-
- pling. The periodicity of C4 abundance was twice as fast as the assumed cell cycle derived from the FLR and FWS cyclic signatures (Table 3). In addition, the amplitude of the abundance cyclic variation increased up to 4 times inside M2 and M3, without affecting the average daily value (Fig. 5d). This may result from different biological parameters such as diel vertical migration, cyclic grazing or cyclic virus lysis that would
- have affected C4 cells during the whole cruise, but not C3 cells. C4 cluster dynamics would have been impossible to describe if the sampling frequency were not appropriate to the specific abundance cycle of approx. 14:00:00. Following Nyquist theory (Nyquist, 1928), the maximum sampling interval to account for the C4 abundance periodicity would be 07:00:00, still too large to account for an accurate sub meso scale
   observation.

C5 cells were mostly present near the coasts (Fig. 5e) and their larger size implies a strong morphological diversity. High FLR and FWS signals (Figs. 6e and 7e) were observed inside M2 although C5 cell abundance was weak (around 100 cells cm<sup>-3</sup>), suggesting a cluster composition different from the one near the coasts rather than some physiological variation.

The abundance of C6 cells, characterised by a high FLO signal like C2 cells, varied periodically between the detection limit and  $2 \times 10^3$  cells cm<sup>-3</sup>. The abundance of C6 cells together with their FLR sharply decreased near the French coast but was elevated inside M2 like the abundance of C2 cells. C6 cells could be coccolithophorids, though

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their abundance was higher than that reported ( $\sim$ 440 cells cm<sup>-3</sup>) by Tarran et al. (1996) in the studied area.

At the global ocean scale, north of 40° N, the development of the north Atlantic spring bloom goes northward, following the establishment of the Swerdrup's critical depth (Swerdrup, 1953). However, south of 40° N, its development depends on nutrient availability linked to the winter mixed layer depth (Siegel et al., 2002). At the meso scale level, the situation appears less simple. Indeed, Karrasch et al. (1996) observed that the north Atlantic bloom formed a patchwork representing different development stages within meso scale features that may be over or under estimated due to a lack in sub meso scale observations.

In our study, we observed a series of different cell groups, each composed of similar cell morphotypes, depending on the crossed water types. M0 waters were part of the northern area of the North Atlantic Subtropical East Gyre, mostly oligotrophic. Abundances were generally low but C5 cells were more abundant in M0 than in the middle

- of the transect. C3 cells appeared the most adapted to highly turbulent areas such as M1 waters that were on the edge of the two north east Atlantic Provinces. However the maximum concentration for C3 cells was observed within a specific area inside M1 that is reminiscent of some isolated eddy interior. In the northward adjacent M2 water mass, C2 and C4 cells were at their highest abundances. The Bay of Biscay
- <sup>20</sup> waters (M3) were certainly at an advanced stage of the north Atlantic bloom, with a shallow mixed layer depth and low nutrient concentrations. Those waters were particularly suited for C1 small cells, C4, C5 and C6 cells. Abundances reached high values as observed for previous spring blooms (Tarran et al., 2006). M4 coastal waters were specifically rich in NO<sub>3</sub><sup>-</sup>, favouring the large C5 cells, but also C1 and C3 cells.

#### 25 5 Conclusions

The automated high frequency sampling conducted with the Cytosub from the Azores up to the French Brittany during April 2007 singled out the importance of a high fre-

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quency sampling, both in space and time, in agreement with the fluorometry study of Rantajarvi et al. (1998). Sub meso scale processes are certainly affected by micro scale processes. Modelling phytoplankton distribution in the marine ecosystem is better achieved by considering its sub meso scale than its meso scale distribution, be-

- cause the sub meso scale distribution takes into account the natural cell cycle. Thus, the cell cycle is one of the major factors responsible for patchiness, certainly on the same level as grazing and turbulence. Further works on ecosystem dynamics should consider high frequency sampling as a necessary procedure to account for spatial heterogeneity and short-term variability.
- Acknowledgements. We thank the captain and crew of the Fetia Ura ship as well as the teenagers and the instructors of the "Deferlante". The work was supported by the "Cytometry In Situ" (CYMIS) contract between the Centre National de Recherche Scientifique (CNRS) and the City of Marseille, partially supported by "l'Agence de l'Eau Rhône Méditerranée Corse", by the "Deferlante" association and the "Seanergies Oceanes" company that allocated the ship.

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**Table 1.** Water type average characteristics, defined by their temperature-salinity properties (Fig. 2).

		Average values±standard deviation						
		Temperature (°C)	Salinity (psu)	NO <sub>3</sub> (μM)	PO <sub>4</sub> (μM)	$SIOH_4 (\mu M)$		
Masses	M0	_	_	0.61±0.26	0.14±0.03	1.18±0.17		
	M1	14.87±0.28	35.92±0.04	$2.35 \pm 0.04$	0.23±0.04	1.36±0.35		
	M2	13.93±0.29	35.80±0.04	$2.62 \pm 0.50$	$0.28 \pm 0.05$	1.15±0.47		
ter	M3	14.50±0.50	35.68±0.02	$0.54 \pm 0.63$	0.19±0.09	0.77±0.38		
Water	M4	$15.67 \pm 0.25$	$34.39 \pm 0.45$	3.15±0.72	0.11±0.04	$0.52 \pm 0.41$		

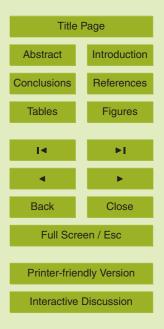
# **Table 2.** Average values of cell abundances from the whole dataset and of flow cytometric parameters for the 6 resolved clusters. FLR:FLO is the red fluorescence (a.u.) on orange fluorescence (a.u.) ratio and FWS:FLR is the forward scatter (a.u.) on red fluorescence ratio. Due to the very high variability of the abundances, the standard deviation is larger than the average value which does not mean that abundances would be negative.

		Cluster C1	Cluster C2	Cluster C3	Cluster C4	Cluster C5	Cluster C6
	Estimated length ( $\mu$ m)	2.7±0.8	0.9±0.7	3.1±0.7	4.2±1.8	23.9±31.3	9.5±2.0
values± deviation	Forward scatter size ( $\mu$ m)	2.4±0.7	0.8±0.6	2.6±0.6	2.6±1.1	9.8±7.2	7.7±1.8
e valu I devi	FLR/FLO	6.5±3.45	$1.61 \pm 0.51$	7.38±3.06	6.59±1.12	5.91±1.45	1.61±0.5
Average standard	FWS/FLR	0.77±0.46	0.09±0.12	1.43±0.91	1.78±0.89	0.7±0.33	1.34±1.09
A, Sta	Abundances (cells.cm <sup>-3</sup> )	15930±15405	39441±34242	4076±3088	3088±2225	168±181	430±584

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**Table 3.** Autocorrelation of the signal of abundances, FWS and FLR for each cluster obtained following the procedure illustrated on Fig. 8. (Low loess-High loess) values (called span in the loess calculation) are user dependent, and give access to the small scale variability without the influence of the trend at a larger scale, and of the extreme values at a scale of successive samples.  $r^1$  and  $r^2$  are the two first negative and positive maximal significant autocorrelation values given at a sample lag corresponding to the hours.

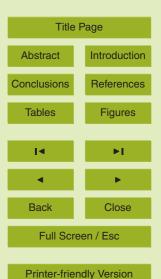
	CLUSTERS	C1	C2		C3		C4	C5	C6
				28.6° W to 23.6° W	22.6° W to 14° W	14° W to 3.75° W			
Low loes	s-High loess (span)	0.4–0.08	0.4-0.08	1–0.1	1–0.1	1–0.1	0.1–0.05	0.6–0.1	1–0.1
	<i>r</i> 1	-0.552	-0.661	-0.289	-0.649	-0.532	-0.322	-0.331	-0.522
	hours	06:00	10:30	06:30	06:30	10:45	07:30	08:45	18:15
ABD	r2	0.221	0.323	0.258	0.344	0.239	-0.294	0.103	0.257
	hours	13:30	19:00	13:45	13:30	23:00	21:30	20:30	33:15
Low loes	s-High loess (span)	0.6-0.08	0.4-0.08	1–0.1	1-0.1	1–0.1	0.6-0.05	1–0.1	1–0.1
	<i>r</i> 1	-0.46	-0.489	-0.269	-0.558	-0.622	-0.477	-0.286	-0.549
	hours	07:15	09:15	12:00	14:15	10:30	12:15	36:15:00	20:00
FWS	r2	0.26	0.325	0.309	0.456	0.358	0.219	-	-
	hours	15:30	23:00	15:30	28:00	23:00	22:45	-	-
Low loes	s-High loess (span)	0.6-0.08	0.4-0.08	1–0.1	1–0.1	1–0.1	1–0.1	0.6–0.1	0.6–0.1
	<i>r</i> 1	-0.379	-0.491	-0.501	-0.509	-0.691	-0.427	-0.361	-0.358
	hours	06:30	09:45	09:15	10:30	11:30	13:45	13:30	09:45
FLR	r2	-	0.278	0.432	0.377	0.616	0.229	-	-0.235
	hours	-	21:00	15:30	25:30	23:00	24:15	-	23:00

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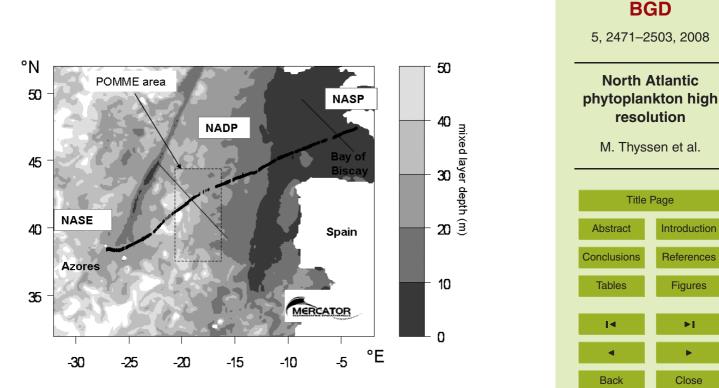
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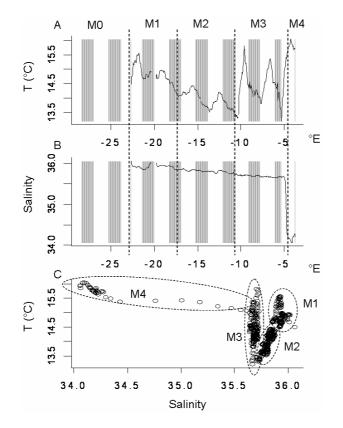


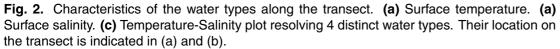
**Fig. 1.** Sampling area. The ship track is superimposed to the averaged mixed layer depth map from the 14 April to the 25 April 2007, derived from the MERCATOR model of the MERCATOR Ocean's group. The main Provinces defined by Longhurst, 1998, and the POMME study area (Memery et al., 2005) are mentioned.

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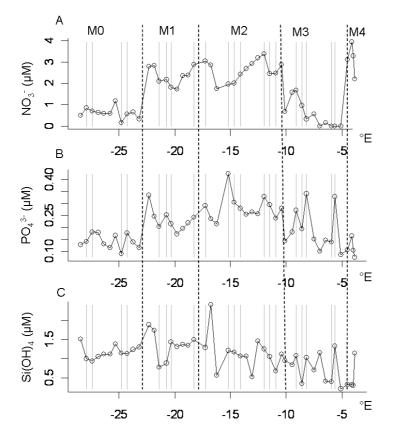
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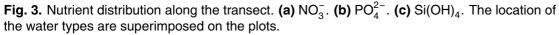
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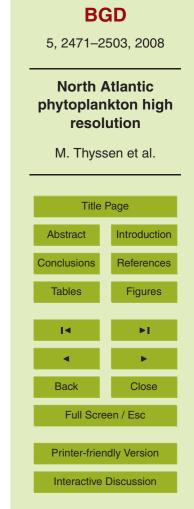




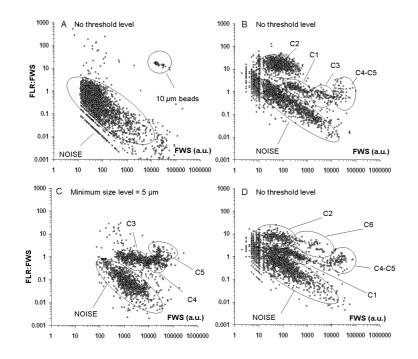












**Fig. 4.** Cytograms illustrating the cluster resolution. **(a)** FLR:FWS ratio (Red Fluorescence: Forward scatter) versus FWS for the analysis of  $0.22 \,\mu$ m filtered seawater supplemented with  $10 \,\mu$ m beads. No threshold level was applied so that the instrument noise could be recorded separately. **(b)** Same cytogram as in (a), related to the analysis of a pumped seawater sample. Four clusters were defined and labelled C1, C2, C3 and C4. (See Table 2 for the average values of their flow cytometric features). **(c)** Same cytogram as in (a) and (b) related to the analysis of a pumped seawater sample where a threshold was applied to the FWS signal in order to analyse a larger volume address larger cells like those in C3, C4 and C5. D. Same cytogram as in (b), illustrating the resolution of C6.

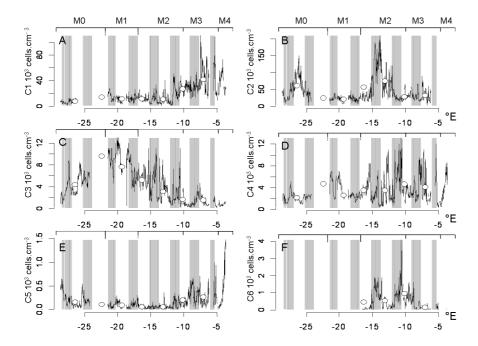
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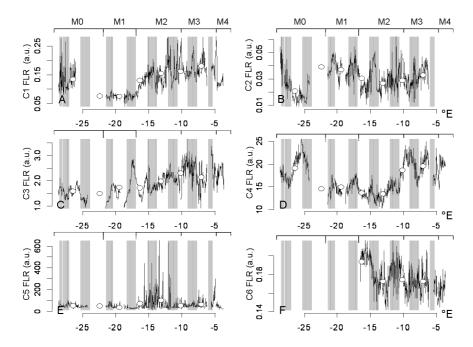
**Fig. 5.** Distribution of cell abundances for each resolved cluster along the ship track. White dots are the daily average value of abundances. Day and night periods are indicated with white and grey boxes respectively. The different water types are specified on top of each plot.

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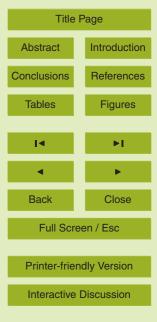




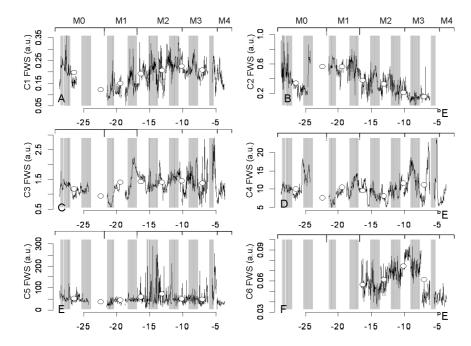
**Fig. 6.** Spatial variation along the ship track of the cell average red fluorescence (FLR) for each cluster. White dots are the daily average value of the cell average FLR. Day and night periods are indicated with white and grey boxes respectively. The different water types are specified on top of each plot.

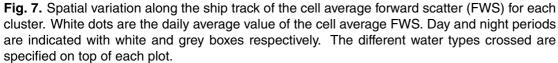
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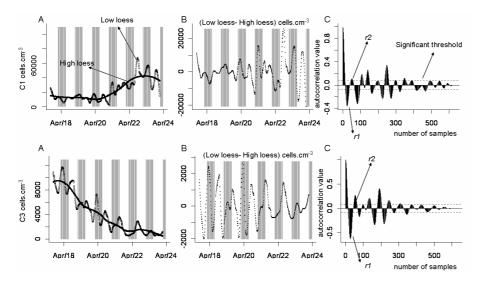


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**Fig. 8.** Illustration of the procedure to get the autocorrelation values  $r^1$  and  $r^2$  mentioned in Table 3. (a) Superimposition of a high loess process and a low loess process calculated on the original abundances dynamics (Fig. 5) for C1 and C3 cluster. The span used to define the high and the low loess are mentioned in Table 3. (b) Difference of both loess calculations in order to extract the short scale variability (low loess process) from the overall trend (high loess process). (c) Autocorrelation results of the signal obtained in (b).  $r^1$  and  $r^2$  are the two maximal significant values. The dotted line is the limit of significance of the autocorrelation.

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