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Final Research Project

STUDY ON THE PREFERENTIAL VERTICAL DISTRIBUTION OF PHYTOPLANKTON IN ALFACS BAY (NW MEDITERRANEAN SEA)

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Abstract

Phytoplankton was sampled at various depths during 24 hours in the estuarine Alfacs bay (NW Mediterranean Sea, Spain) to determine the preferential vertical distribution of 8 species representative of microplankton assemblages at this location (4 dinoflagellates, 2 coccolithophorids and 2 diatoms). The hypothesis that diel vertical migrations are undertaken was tested for each target species. Results indicate that vertical distribution is primarily determined by the superimposition of two water masses (upper estuarine mixed layer and bottom marine layer) and by the oscillation of the pycnocline as an interface between them. Cell concentrations were maximal close above or below the pycnocline for most species. 2 out of 4 dinoflagellates showed evidence of upwards migration across the pycnocline at night, a pattern that contrasts with documented dinoflagellate diel vertical migration patterns in literature. 1 dinoflagellate, 2 coccolithophorids and 1 diatom showed downwards displacement of cells below the pycnocline at only one sampling time at night that coincided with a short-lived peak of fluorescence below the pycnocline. The potential effects of a surface freshwater runoff observed at night upon the aforementioned migrations and the importance of environmental gradients on the establishment of diel vertical migrations are discussed. It is hypothesized that the lack of a nutricline in the bay at this time of the year could have prevented dinoflagellate cells from displaying the nighttime descent typical of motile dinoflagellates in water-stratified environments.

Introduction

Phytoplankton constitutes a main component of the planktonic ecosytems with key roles in the trophic webs and carbon transfer. Thus, to understand the dynamics and the structure of planktonic ecosystems, it is important to know the spatio-temporal distribution of phytoplankton. The distribution of phytoplankton in marine ecosystems is highly dictated by the segregation of light and nutrients (Ryabov et al., 2010). Segregation in time strongly influences the ecological succession of different phytoplankton assemblages. Segregation in space (important in the vertical dimension) is related to the depth of the mixing layer and establishment of the pycnocline and may result in marked vertical gradients in the distribution of biomass and biological activity. In temperate seas, the pycnocline usually forms before the

production season in spring, when turbulence has calmed down and solar energy has heated sufficiently the superficial layers to provoke stratification. Mixing keeps primary producers above the pycnocline where light availability allows its growth from the nutrients, which in turn become progressively depleted. The pycnocline then becomes an interface where upwelled nutrients and light from surface meet. In open sea waters of an oligotrophic area such as the Northwestern Mediterranean, this interface can make way for a maximal accumulation of chlorophyll at a depth range of 20-70 m as early as February (Estrada et al., 1999), when polar seas are still at a production halt. Ultimately those phytoplankton species capable of occupying illuminated depths at day and nutrient-repleted depths at night would be favoured. Hence the evolutive advantage for phytoplankton with active swimming capacity (i,e, flagellates and ciliates) is manifest (Ault, 2000). Swimming speeds documented for various dinoflagellate species allow them to cover a vertical distance well superior to the depth of many coastal marine environments (Smayda, 2010).

The Alfacs bay, the southern estuarine bay of the Ebro Delta, located at the south of Catalonia (Spain), shares biological particularities with the adjacent Northwestern Mediterranean so far as the marine phytoplankton community is concerned (Delgado and Camp, 1987). It has an average depth of 3,13 m and it partly connects with the Mediterranean open sea to the south, although it is isolated over most of its 50 km^2 extension by a thin sandbar that originates from the Ebro deltaic depositional system. The prevailing conditions in the bay are that of a salt-wedge estuary, whereby the upper layer is constituted of semi-saline water and flows outwards, while the lower layer is of higher salinity and flows inward. The freshwater runoff to the bay comes mainly but not exclusively from irrigation water derived from the Ebro river and drained through the rice fields. Its volume varies from month to month, depending, in part, on agricultural activity. From January to April, the irrigation channels are usually shut for maintenance (Comunitat de Regants del Delta de l'Ebre), which increases the water residence time inside the bay. Since the irrigation water the bay receives is rich in nutrients, the winter shortage leads to a decrease in inorganic nutrients input to the bay (Llebot (2010) and Delgado and Camp (1987)). Other sources of nutrients, for example recycled organic nutrients or nutrients resuspended from the sediments, must be relied upon (Llebot et al, 2010). Nevertheless biomass appears to be relatively constant (weekly averages between 2 and 8 $\mu g \cdot L^{-1}$ through the year based on 14-year climatology, Llebot et al. (2011)), with the diatom bloom taking place in late-winter, prior to the opening of the channels (Llebot

(2011) and Delgado (1987)).

Fig. 1 – Location of the Alfacs Bay sampling site

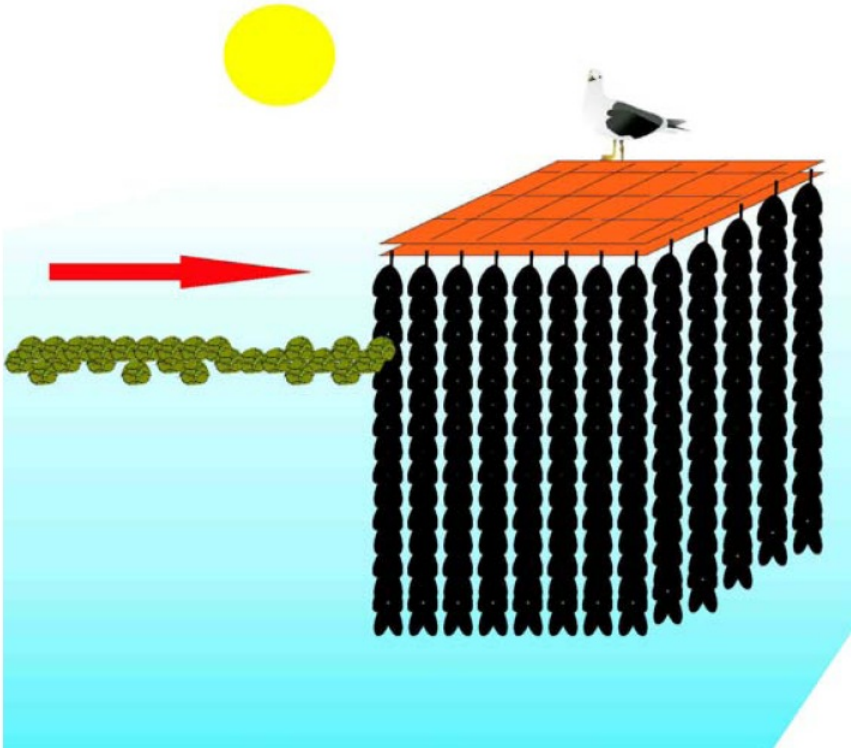

Fig. 2 – Outbreak of a harmful algal bloom and how it could possibly affect mussel culture without being detected by conventional sampling methods
(Source: Velo-Suàrez, 2009)

The phytoplankton production in the bay is high in comparison with the open Mediterranean waters. This has allowed establishment of aquacultural activities. However, the recurrent proliferation of harmful algal blooms (HAB) (e.g. Garcés et al. 1999, Loureiro et al. 2009) threatens the economical activities and the ecology of the bay. It is thus of particular interest to pin down what factors drive these ecological events. Knowledge of the phytoplankton vertical distribution may also help predict how a HAB outburst can affect commercial activities. For example, a concerning situation may arise where species producing toxins concentrate within a thin layer at a depth where mussel is cultivated but where monitoring design is insufficient (see fig. 2). Few studies (in part due to technical difficulties in sampling) have been led to investigate the preferential vertical distribution of phytoplankton assemblages in the Alfacs bay. Garcés et al. 1999 documented the vertical distribution of a harmful phytoplankton alga belonging to the *Karlodinium* genus. They did not however report

any diel migration tendency of this species at any part of the bay.

As explained in the opening paragraph, the benefits for phytoplankton algae capable of overcoming passive advection or sinking tendencies are manifold. Dinoflagellates can use their flagellate apparatus to swim actively while diatoms more passively inflate or deflate a gazeous vacuole to control their buoyancy (Mellard et al. 2011). Various laboratory and field experiments (e.g. Villarino et al. 1995, Ault 2000, Delgado 2009) demonstrate the existence of cyclical diel migrations in dinoflagellates. These cycles are traditionally explained as a combination of phototaxis, geotaxis or endogenous (metabolic cues) behaviours (Kamykowski & Yamazaki, 1997), though vertical nutrient gradients can also modulate dinoflagellate migrations (McIntyre et al. (1997). Despite the fact that too intense photo-active radiation (PAR) can lead to photoinhibition and induce positive geotaxis or negative phototaxis (i.e. Richter et al. 2002), a vast majority of studies (including all previously cited) reveal that dinoflagellates who migrate ascend to surface at day and descend to depths at night. Studies that report the opposite pattern are very rare (e.g. Tilzer 1973).

The objective of this research was to investigate whether the phytoplankton assemblages have a preferential vertical distribution in Alfacs bay, and if so, which biological and/or physical factors may be involved. In this study, intensive sampling during 24 hours was performed at a station located near the center of the bay and close to the mussel rafts. The hypothesis that diel vertical migrations take place in Alfacs bay was tested for some dinoflagellates as well as diatoms and coccolithophorids (i.e. the most relevant microphytoplankton phyla) among the most abundant in the bay during early spring. The experiment also was an opportunity to assess depth preferences of various species pertaining to different taxonomic groups and attempt to explain them in terms of niche ecology and evaluate the influenc of physical forcings.

Materials and methods

General design

Samples for the characterization of the microphytoplankton community were obtained at regular time (every 3h) and depth (every 0,5 m) intervals during 24 hours. CTD casts were made simultaneously in the whole water column (from 0 to 5,5 m). Between March 2, 2009, 12:50, and March 3, 2009, 13:00, a single station located in Alfacs bay at 750 meters off the coast (40º37,184' N, 0°39,799' E), was sampled for sea water approximately every 3 hours. Sampling times were 12:50, 16:00, 18:50, 22:05, 1:05, 4:05, 7:05, 10:15 and 13:00.

Phytoplankton samples

A water pump plugged to a plastic tube with length marks permitted water recollection from different depths. Samples were then immediately fixed with neutralized formaldehyde solution to a final concentration of 2% formalin which allows phytoplankton cells preservation for prolonged periods of time. Samples were kept dark at 4°C until processing. Because we were ultimately limited by time, only 0,5, 1,5, 2,5, 3,5, 4,5 and 5,5-meter depth samples were fully processed to determine phytoplankton abundance.

Fig. 3 – Examples of sedimentation chambers with different volume capacities (10 to 100 mL)

Physical and chemical parameters

CTD profiles were carried out simultaneous to the phytoplankton samples in order to monitor the sampling site physical conditions. Water temperature, salinity, density and fluorescence were obtained with high vertical resolution (every 10 cm). The depth of the pycnocline was defined as the middle point in the range of depths where density differential exceeded 0,15

$mg \cdot L^{-1} \cdot m^{-1}$. Fluorescence is calibrated to act as a proxy for *in situ* chlorophyll a concentration and serves as an estimate of total phytoplankton biomass. Water samples were also obtained to assess nutrients concentration (silicate, phosphorus and inorganic nitrogen forms) every m of the water column once on the first day and another time the second day.

Meteorological data

Meteological data (wind, temperature, PAR, precipitation) were obtained from a meteorological station run by Servei Meteorologic de Catalunya (http://www.meteocat.cat). The station is located 1 km from the sampling site.

Phytoplankton abundance estimation

The Utermöhl sedimentation technique (Utermöhl, 1958) was used for phytoplankton quantification. Preserved samples were gently mixed by turning the bottles a minimum of 50 times, and pouring its content into a 50-mL sedimentation cylinder mounted on a sedimentation chamber. Samples were processed after 18-24 hours of sedimentation.

The present study is part of a wider research that characterized the whole microphytoplankton community. From that research (data not shown), we targeted 8 species belonging to 3 taxonomic groups whose abundances were determined in all samples. The species and the counting method used (sampled area and objective magnification) for each of them are provided in table 1. Conversion factors were used to transform gross cells counts into $cells \cdot L^{-1}$ figures.

Species	Sampled area	Magnification	Taxon
Prorocentrum micans	Entire plate	10x	Dino
Scrippsiella spp.	Entire plate	10x	Dino
Ceratium furca	Entire plate	10x	Dino
Alexandrium spp.	1 transect	20x	Dino
Syracosphaera pulchra	Entire plate	10x	Cocco
Calciosolenia brasilensis	1 transect	20x	Cocco
Thalassionema nitzchoides	Entire plate	10x	Diatom
Cyclotella spp.	10 fields	40x	Diatom

Table 1 – Sampled species and counting methodology used

Intrasample variability

We estimated the error associated to our estimations derived from the Utermöhl method. Four samples corresponding to time 22:05 were resedimented and recounts were made for 6 of the 8 species (*Syracosphaera pulchra* and *Alexandrium* spp. recounts could not be made for logistical reasons). This error was calculated for each species as the difference between both counts divided by their average

Phytoplankton abundance analysis

The abundance (cells·L^{-1}) results were graphically plotted against time of the day and depth with the help of Surfer 7.0 software. Results were also compared with physical (water temperature, salinity, density and fluorescence) time series. Additionally vertical profiles of chemical (nutrients) and irradiance (PAR) and meteorological time series (precipitation, winds) collected during the experiment were analysed to investigate further links.

To determine whether cells exhibited diel migration, we analysed the abundance distribution in 3 main layers of the water column. The definition of each layer was based on the location of the pycnocline. Namely, samples obtained at depths 0,5, 1,5 and 2,5 m were considered representative of the upper mixed layer. Samples obtained from 4,5 and 5,5 m depth were mostly below the pycnocline. Samples obtained from 3,5 m corresponded to intermediate water characteristics, somehow linked to the pycnocline for some sampling times, although they usually stood just above it. It was thus defined as the middle layer which receives influence from both the upper mixed and the bottom layer. For each layer cell concentration percentages expressed as a fraction of the whole water column were calculated for each species at each sampling time.

An additional tool to analyse cells vertical movements was the calculation of a variable that could be defined as the mean depth of cell occurrence. This variable was calculated as:

$$\Sigma(d*n_d) \times (\Sigma n_d)^{-1}$$

where d corresponds to sampling depth and n_d to the concentration of cells at this depth. The mean depth of cell occurrence was calculated for every species at every sampling time.

Results

Water physical parameters

Water temperature range was 11,8-12,8°C throughout the whole experiment (fig. 4). Highest temperatures were found at surface during the afternoon and early evening of the first day. By the morning of the second day, surface temperature had cooled sufficiently to produce thermal inversion (warmer bottom temperatures). Bottom temperatures ranged between 11,9 and 12,2°C and did not vary much between the first and second day.

Salinities varied between 33,4 psu at surface and 38,1 psu at bottom (fig. 5). Bottom salinities steadily increased in the course of the 24-hour experiment, going from 37,5 to 38,1 psu. Surface salinities registered a sudden fall at night observable in the upper 1,5 m but most pronounced in the upper 0,5 m. This could indicate freshwater runoff (see discussion)..

Density profiles indicated a range of values of 24,5-29,2 (σ_d) (fig. 6). A gradual ascent of the pycnocline occurred from 5,0 m at noon of the first day to around 3,5 m at night and then a descent to 4,5 m depth by morning of the second day.

The fluorescence maxima measured by the CTD instrument usually varied between 5 and 6 µg chl a·L^{-1} and were always close to the pycnocline (fig. 7). Outstandingly high values (>7,5 µg chl a·L^{-1}) were found between 22:05 and 1:05 in a portion of the water column located between 3,8 and 4,8 m depth. This corresponded to sub-pycnocline location at this time of the night. Considering the whole data set, the highest measurement was 11,5 µg chl a·L^{-1} (4,7 m, 1:05).

Weather conditions and nutrients

PAR maximum at water surface reached 500 µmol·m^{-2}·s^{-1} at 13:00 the first day but did not exceed 300 µmol·m^{-2}·s^{-1} the second day due to cloud cover. No extreme meteorological event occurred (maximum wind velocity was ca. 7 m·s^{-1}). Wind velocity only exceeded 5 m·s^{-1} between 9:00 and 13:00 the second day and no rainfall was registered during the experiment. Nutrient profiles revealed extreme scarcity of all measured inorganic forms, be it nitrogen,

phosphorus or silicate. Relatively higher concentrations of inorganic nitrogen were present above the pycnocline (sum of nitrites and nitrates between 6,0 and 7,9 µM at 0,5-3,5 m) than below (sum of nitrites and nitrates between 2,2 and 3,3 µM at 4,5-5,5 m). Similar concentrations of silicate forms were found above and below the pycnocline (range 1,0-1,3 µM the first day, range 0,5-1,4 µM the second day). Inorganic phosphate concentrations hardly exceeded the detection limit but seemed to be higher below (range 0,14-0,17 µM) than above (range 0,03-0,12 µM) the pycnocline.

Phytoplankton abundance

All targeted species were present at all depths above the detection limit. *Cyclotella spp.* were around 2 orders of magnitude more abundant than any other sampled species with cell concentrations over 1,000,000 cells·L^{-1} but it is also the species with the smallest biovolume. Daily averaged cell concentrations for all other species ranged between 7 cells·L^{-1} for *Ceratium furca* at 5,5 m to 7786 cells·L^{-1} for *Thalassionema nitzschoides* at 5,5 m. There was a general cell abundance boost between 19:00 and 22:05 the first day as all species underwent cell concentration increment (up to 2-, 3- or 4-fold). 4 out of 8 sampled species were most abundant at 13:00 the second day. Movement of cell concentration hotspots suggest that many species crossed the pycnocline during the night when it was situated relatively high in the water column (3,5 m)

Prorocentrum micans (fig. 8)

The dinoflagellate *P. micans* displayed good spatial and temporal omnipresence. Its concentration ranged between 480 and 6900 cells·L^{-1} throughout all 42 depth and time samples. Maximum was found at either 4,5 or 5,5 m depth, depending on time of the day. 5,5 m maximum were noted mostly at day time (12:50 and 16:00 the first day and 4:05, 10:15 and 13:00 the second day) while 4,5 m maximum occurred at night mostly (18:50, 22:05 the first day and 1:05 and 7:05 the second day). Concentrations were low above the pycnocline (3,5 m) and even lower at surface (0,5 m) , except for night time (between 22:05 and 4:05) where a concentration hotspot (2300 to 3800 cells·L^{-1}) developed between 0,5 and 1,5 m. Overall abundances were lowest before dawn (16:00-18:05) and before dusk (4:05-7:05).

Scrippsiella spp. (fig. 9)

This genus could not be visually identified to the level of species with light microscopy.

Scrippsiella spp. concentrations showed important temporal variability (for a same depth, concentrations varying from 100 cells·L^{-1} to over 2500 cells·L^{-1}). Concentrations were high throughout the whole column between 22:05 and 1:05, with 2 concentration hotspots clearly segregated, one at bottom (3,5-5,5 m) and the other at surface (0,5 m). Cell numbers were unusually high at 13:00 the second day (maximum of 2700 cells/L at 3,5 m), except at 5,5 m where they were similar to previous sampling times. Concentrations at other times of the day were lower and peaks were usually located between 4,5 and 5,5 m.

Ceratium furca (fig. 10)

The dinoflagellate *C. furca* was the least abundant of the sampled species. Peak abundances were usually found around 3,5 m (maximum = 1520 cells·L^{-1}). Cell numbers were relatively low at 0,5 m and virtually null at 5,5 m. Before sunset (16:05-18:50) of the first day, most cells were found at a higher height in the water column (1,5 m and 2,5 m). At 1:05, maximal cell concentration was located at 4,5 m, below the pycnocline. This is the only time this species showed a concentration peak below the pycnocline, though all around high cell concentrations were also found everywhere except for 5,5 m depth at 13:00 the second day.

Alexandrium spp. (fig. 11)

This genus could not be visually identified to the level of species with light microscopy. *Alexandrium* spp. showed maximal concentrations every sampling time between 1100 and 7500 cells·L^{-1} in a depth range between 1,5 and 3,5 m. Between 4,5 and 5,5 m, few *Alexandrium* spp. were present, especially the first day.

Syracosphaera pulchra (fig. 12)

The coccolitophorid *S. pulchra* had maximal occurences between 2,5 and 3,5 m (marked preference for 3,5 m) that ranged at all sampling times between 960 and 5800 cells·L^{-1}. As was the case with *Scrippsiella spp.*, concentrations were relatively high at 1:00 and very high at 13:00 the second day. 5,5 m occurrences were very scarce throughout the whole 24-hour period. An interesting phenomenon occurs at the 1:05 sampling time, where the 4,5 m depth shows one of the highest concentrations registered for this species, a situation that contrasts with all other sampling times where 4,5 m concentrations are low. This was the only sub-pycnocline hotspot observed during the 24-hour period.

Calciosolenia brasilensis (fig. 13)

C. brasilensis temporal and spatial distributions showed various similarities with its coccolitophorid companion *S. pulchra*. Cell counts typically gave high values between 1,5 m and 3,5 m and at 1:05, 10:15 and 13:00 the second day. Although highest cell concentrations ranged between 3000 and 9000 cells·L^{-1} throughout nearly the whole experiment, the highest concentrations (over 13000 cells·L^{-1}) was measured for 4,5 m at 1:05, despite the fact that 4,5 m evaluations at other times of the day never exceeded 1600 cells·L^{-1} . This 4,5-meter sub-pycnocline maximum coincides with a maximum in fluorescence measured by the CTD at that time. Abundances at 5,5 m depth were generally low.

Thalassionema nitzschoides (fig. 14)

The distribution of colonial diatom *T. nitzschoides* showed a sharp vertical gradient. Cell concentrations increased sharply between 3,5 m and 4,5 m. This species was concentrated principally below the pycnocline with maxima between 4900 and 11000 cells·L^{-1} occuring either at 4,5 m (between 13:00 the first day and 4:05 the second day) or 5,5 m (between 7:05 and 13:00 the second day). Near surface (0,5 m), cell concentrations never surpassed 920 cells·L^{-1}. Total biomass seemed lowest at night time (between 18:50 and 7:05).

Cyclotella spp. (fig. 15)

This genus could not be visually identified to the level of species with light microscopy. The small-sized colonial *Cyclotella* spp. diatom showed peaks between 1,700,000 and 3,000,000 cells·L^{-1} located between 0,5 and 4,5 m depth. Its abundance at 0,5 m depth was highly variable, going from very high (above 2,300,000 cells/L at 16:00 and 7:05) to 2 or 3 times less (below 900,000 cells / L at 22:05, 1:05 and 4:05). Abundance at 4,5 m depth was equally variable and showed a mirror trend. That is, where 0,5 m abundance was high, 4,5 m abundance was low, and *vice versa*. The high 4,5 m depth concentrations occurred at night and seem to indicate that cells descended across the pycnocline. A similar across-pycnocline nighttime descent of cells was already mentioned for *C. furca*, *S. pulchra* and *C. brasilensis*, though in their case it had only occurred at the 1:05 sampling time. *Cyclotella*'s abundance at 5,5 m depth was always relatively low (below 620,000 cells·L^{-1}).

Vertical migrations

A simple statistical test was applied to verify whether species displayed a diel vertical migration pattern during the course of the experiment, 3 water column divisions of ecological relevance were defined with the help of the density data (see materials and methods). The upper mixed layer definition comprised depths of 0,5, 1,5 and 2,5 m. The bottom layer definition comprised depths of 4,5 and 5,5m. The middle layer defintion comprised the 3,5 m depth. We first tested whether there was a significant difference between light (12:50, 16:00, 10:15 and 13:00) and dark period (18:50, 22:05, 1:05, 4:05 and 7:05) in the average cell concentrations of either species at either layer. Only one species at one layer showed a significant difference. This was *T. nitzschoides* whose abundance in the bottom layer was higher during the light than during the dark period (Student test, p = 0,0497). However, since this did not concurrently go with lower light-period surface abundance (instead it was higher), this cannot be interpreted as evidence that *T. nitzschoides* displayed diel migration.

An additional mean to investigate cells vertical movements consisted in the following. For each of the defined layers, cell concentration percentages expressed as a fraction of the whole water column were calculated for each species. These 3 fractions were graphically plotted against time of the day (fig. 16). The relative partitioning of biomass between the 3 defined layers may provide a finer visualization of cell vertical movements, as the effect of horizontal advection and cell division events are hindered by the use of percentage (relative) values. Out of the 3 layers, the middle layer fraction showed the least consistent patterns, and due to its tendency to act as a “cell buffer” between the upper mixed layer and the bottom layer displayed hectic up-and-down variations over the sampling period. The bottom layer fraction is most interesting in the case of the bottom-dwelling species and will be discussed for *Thalassionema nitzschoides*. The temporal patterns of the upper mixed layer appeared to provide a good amount of information and will thus be described hereafter for each species under the abbreviated “surface fraction” term. All descriptions refer to fig. 16.

The surface fraction for *Prorocentrum micans* increased steadily from 20% at 12:50 to 40% at 4:05, and then slightly decreased during the beginning of the second day before going up again at the last sampling time of 13:00. The surface fraction of *Scrippsiella* spp. showed a similar pattern but changes are greater in magnitude with a range of 10-50%. Another

difference with *P. micans* is that its surface fraction peaks around the end of the day (18:50) instead of night time. The curve for this species closely resembles a sine curve that would have a period of ~24 hours. Minimum then would occur around the morning of the second day. *C. furca*'s and *Alexandrium* spp.'s surface fractions peaked between 16:00 and 18:50 (more than 80% of all column biomass found in the upper mixed layer) and another time around sunrise (over 40% and 60% for *C. furca* and *Alexandrium* spp. respectively). *C. furca* displayed greater shifts in the surface fraction from one sampling time to another than *Alexandrium* spp.

Surface fraction for both coccolithophorids showed a strong increase between 12:50 and 18:50 (from 40-50% to 70-80% of the whole column) and was then punctuated by a sharp drop between 18:50 and 22:05. This is similar to what is observed for *C. furca* and *Alexandrium* spp. During the night, biomass fraction decreased to below 40% and then by sunrise rose again.

Surface fraction for *Cyclotella spp.* was all around high and showed a trend similar to that of both coccolithophorids, rising during the first day, falling at night and then steadily rising again the second day. Surface fraction for *Thalassionema nitzschoides* was low at all times (inferior to 15%) and showed no trend. Its bottom layer fraction on the other hand showed a subtle decreasing trend during the 24-hour period. This becomes more obvious when the deepest sub-component (5,5 m) of the bottom layer is considered seperately, then we see that biomass fraction at that depth decreased regularly from 54% at 12:50 the first day to 34% at 13:00 the second day, indicating upwards movement of cells.

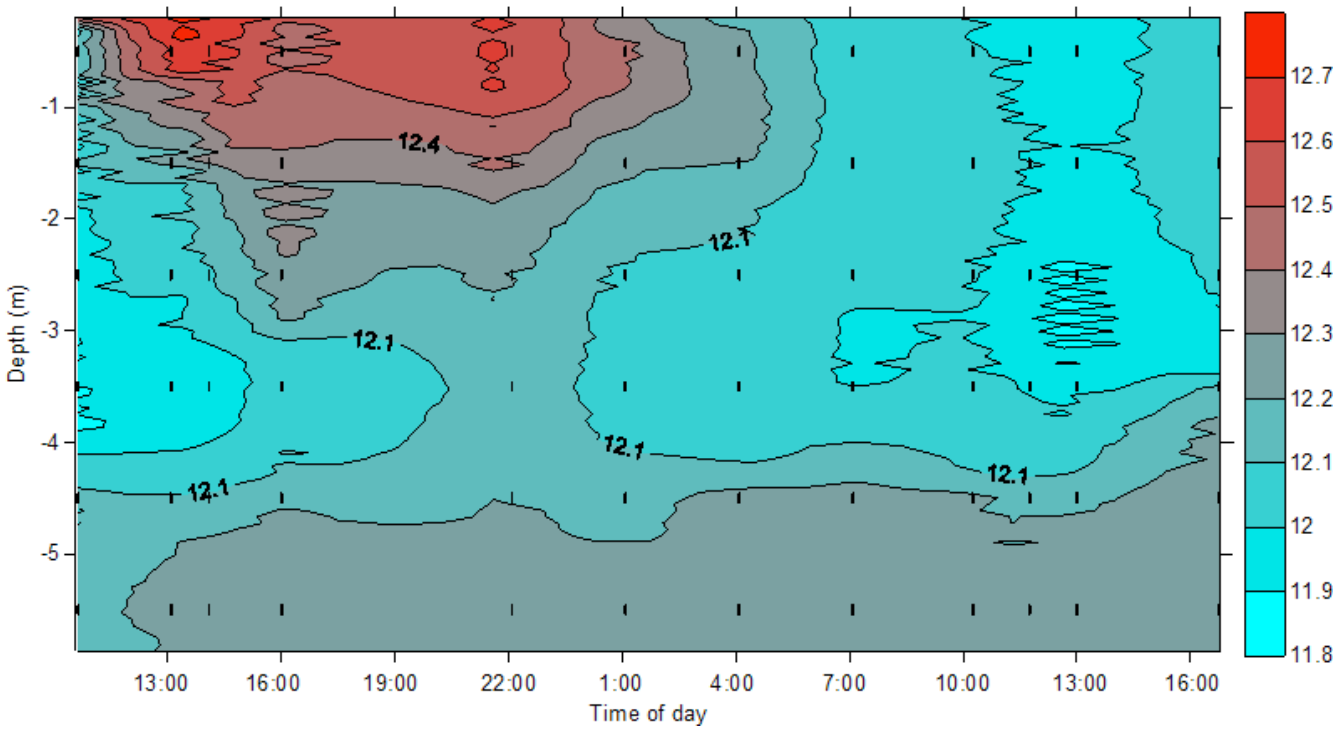

Fig. 4 – 24-hour evolution of temperature in the water column. Vertical bars indicate times of CTD cast

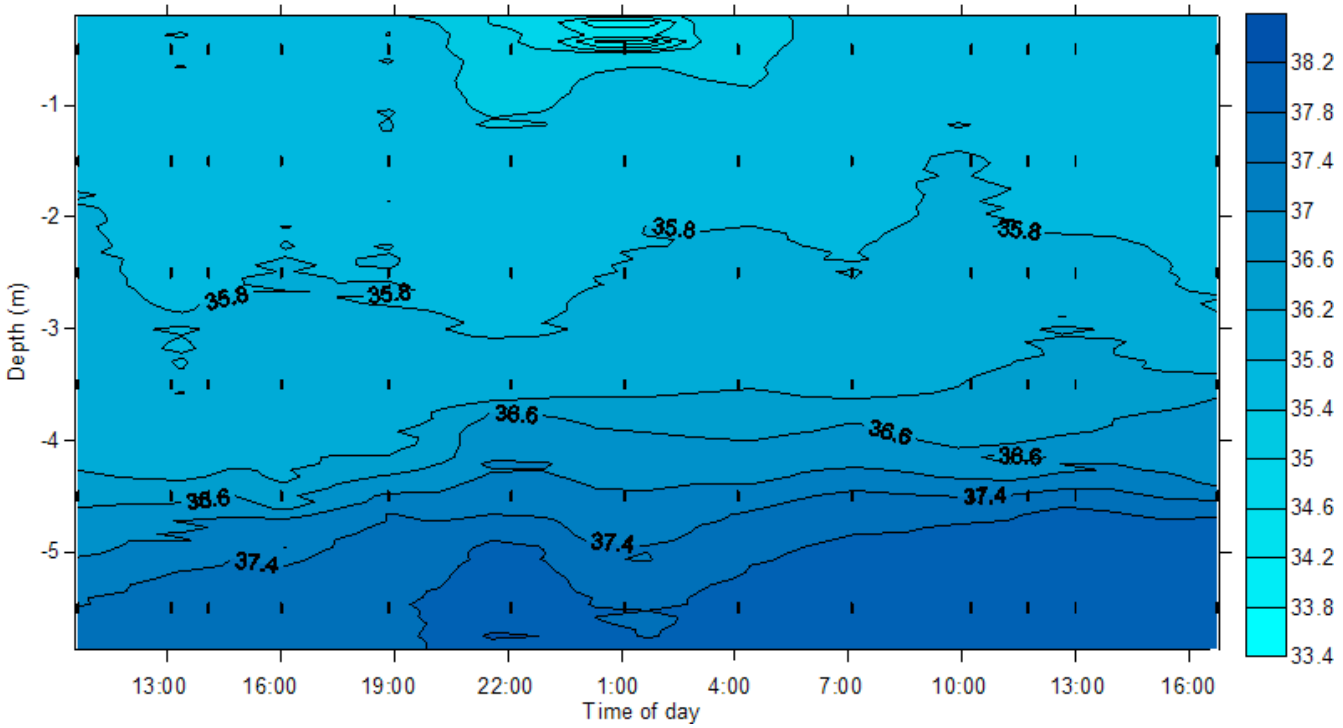

Fig. 5 – 24-hour evolution of salinity (psu) in the water column. Vertical bars indicate times of

CTD cast

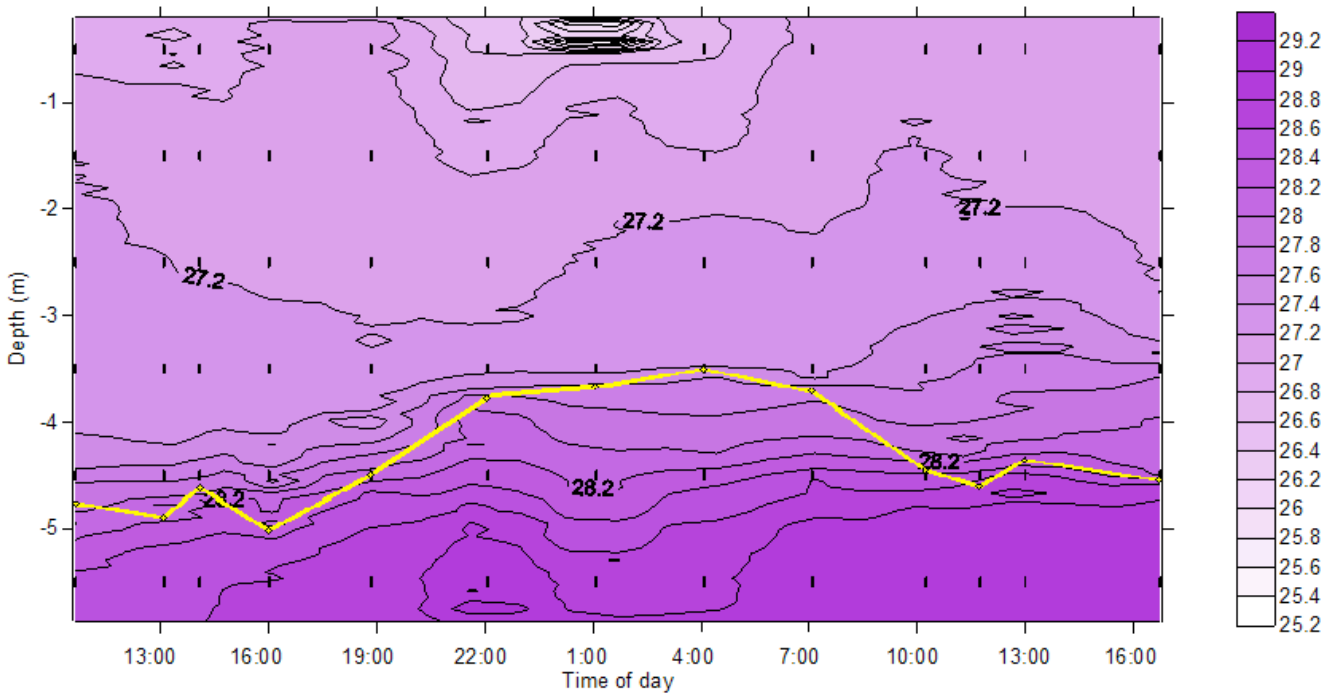

Fig. 6 – 24-hour evolution of density (σ_t) in the water column. Vertical bars indicate times of CTD cast. Yellow line shows the evolution of the pycnocline depth as defined in materials and methods

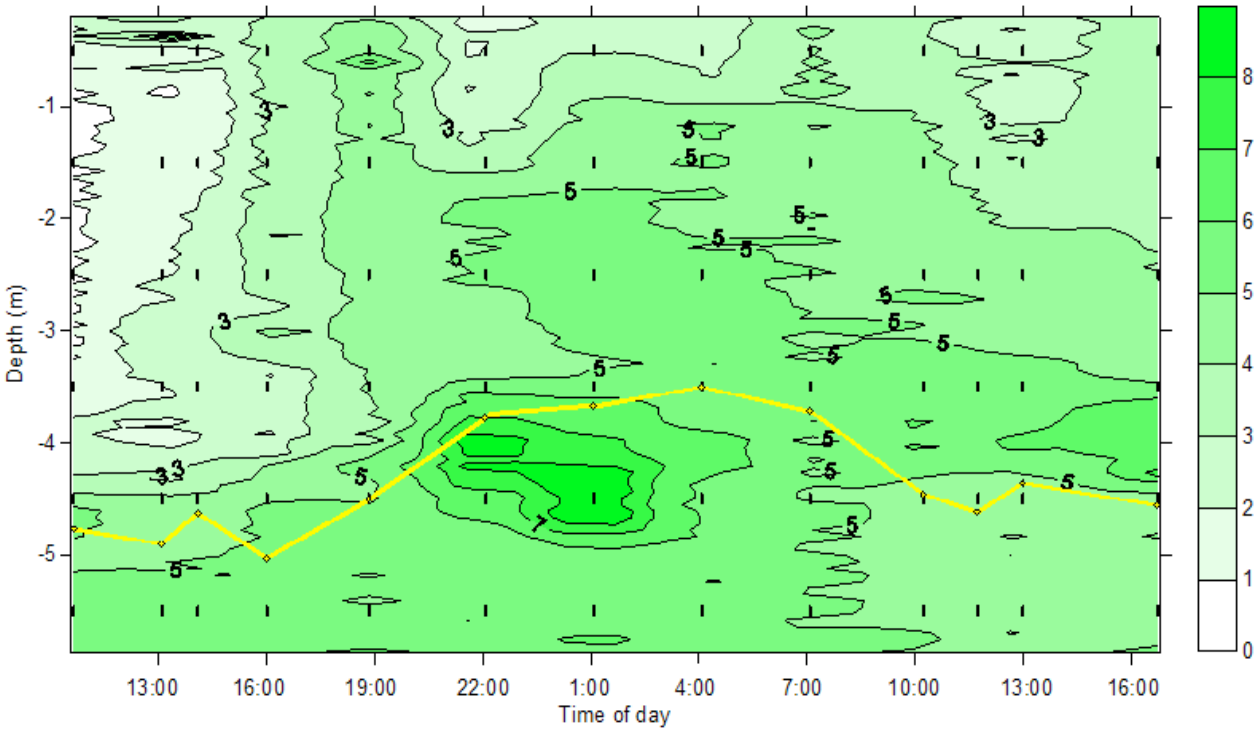

Fig. 7 – 24-hour evolution of fluorescence (µg chl a·L^{-1}) in the water column. Vertical bars

indicate times of CTD cast. Yellow line shows the evolution of the pycnocline depth as defined in materials and methods

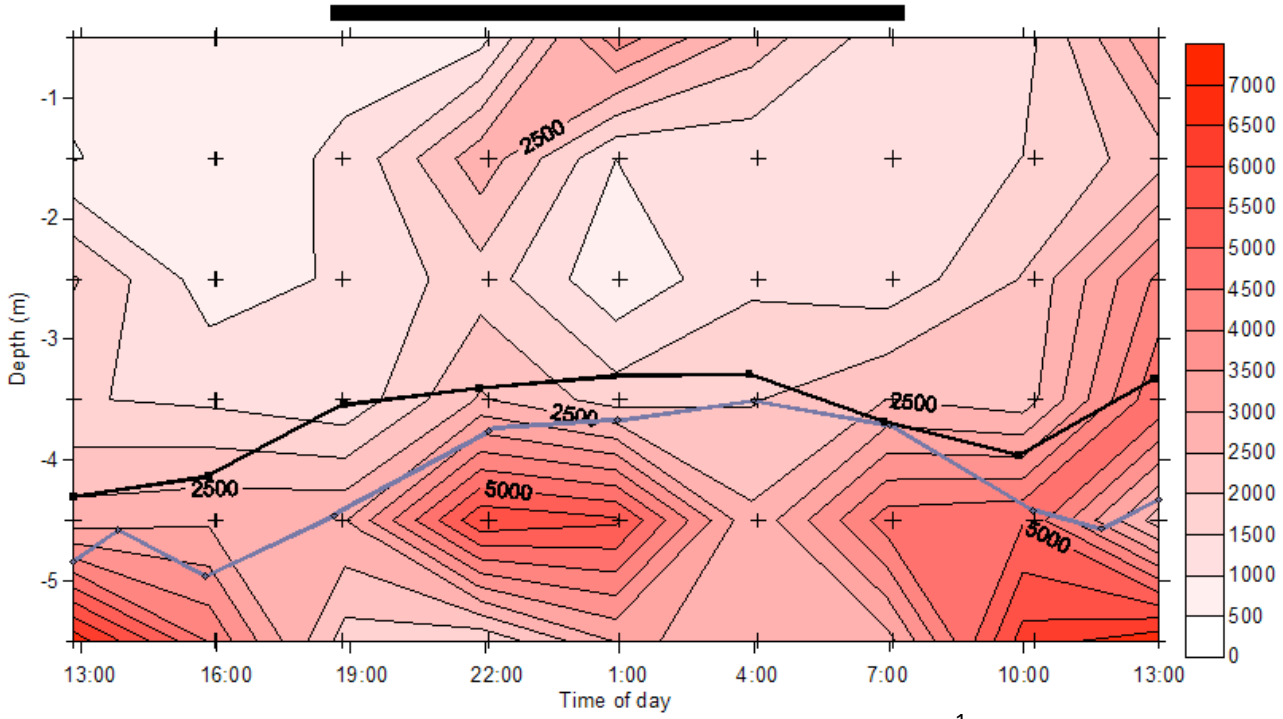

Fig. 8 – 24-hour evolution of *P. micans* cell concentrations (cells·L^{-1}). Crosses indicate sampling points. Black and blue lines show the evolution of the mean depth of cell occurrence and of the pycnocline depth respectively. Black bar above figure denotes the dark period.

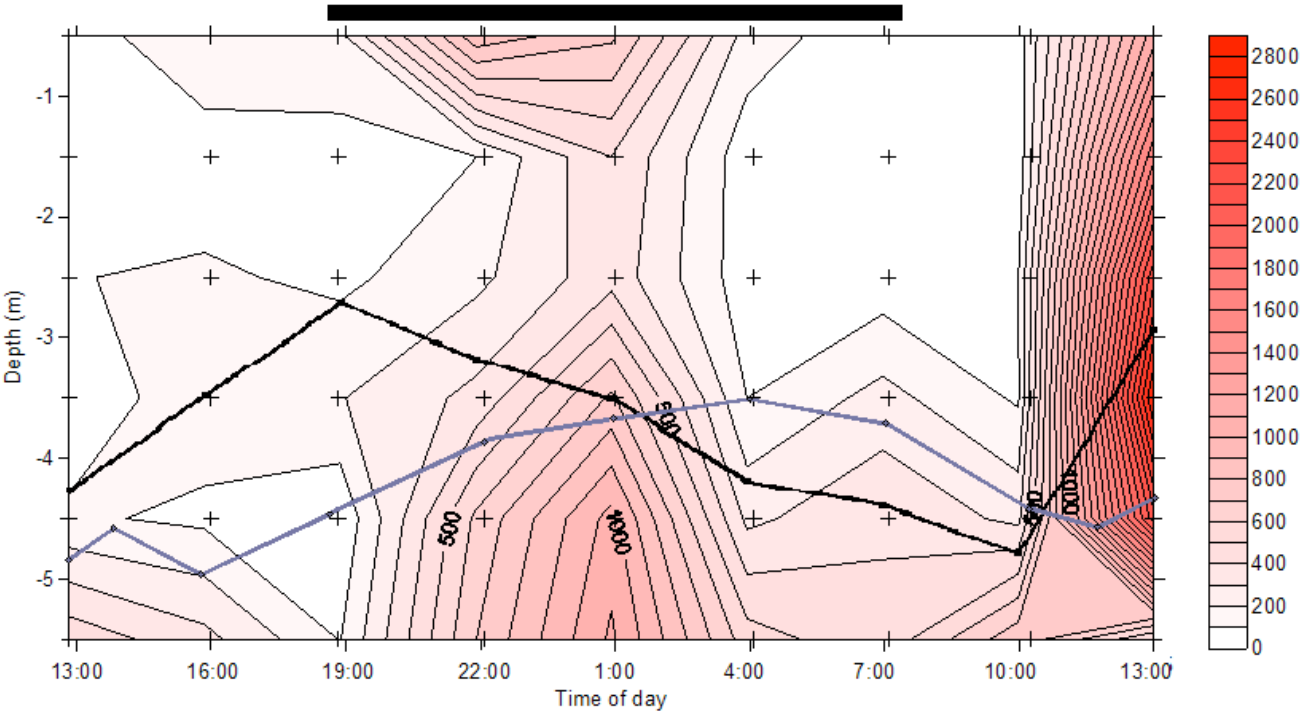

Fig. 9 – 24-hour evolution of *Scrippsiella* spp. cell concentrations (cells·L^{-1}). See fig. 7 for explanations

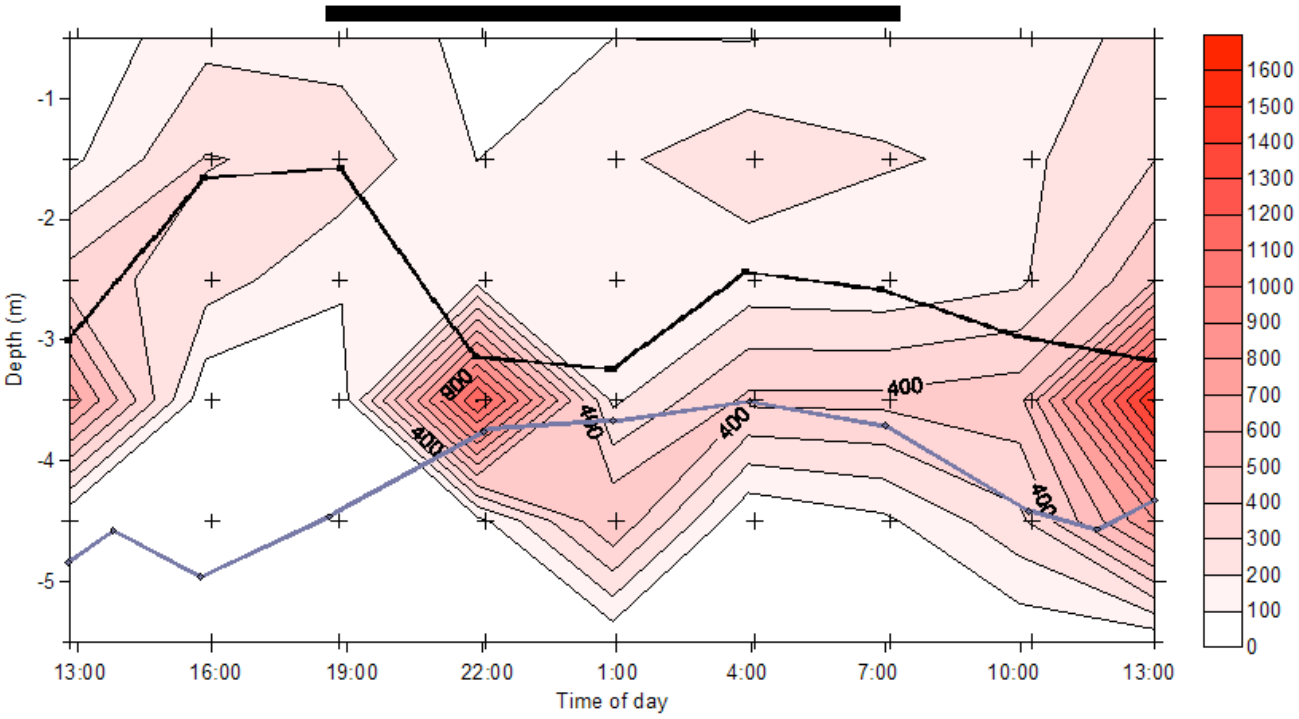

Fig. 10 – 24-hour evolution of *C. furca* cell concentrations (cells·L^{-1}). See fig. 7 for explanations

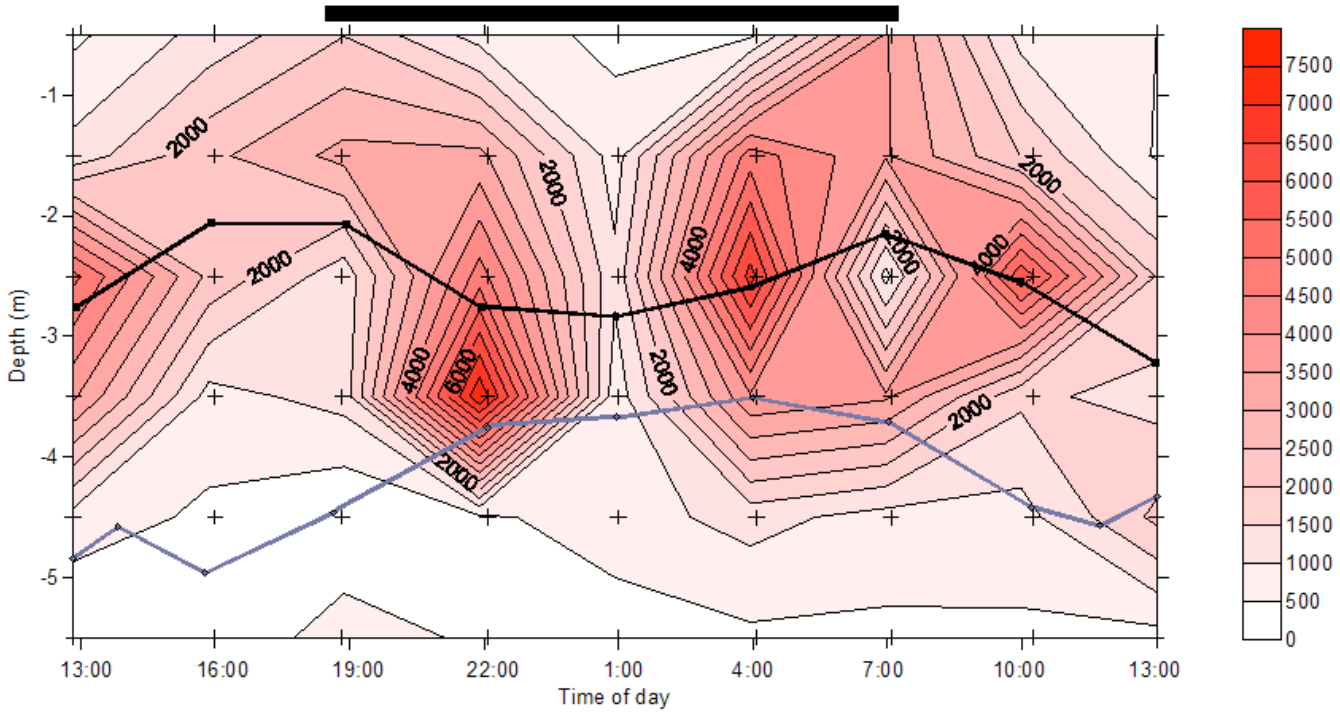

Fig. 11 – 24-hour evolution of *Alexandrium* spp. cell concentrations (cells·L^{-1}). See fig. 7 for explanations

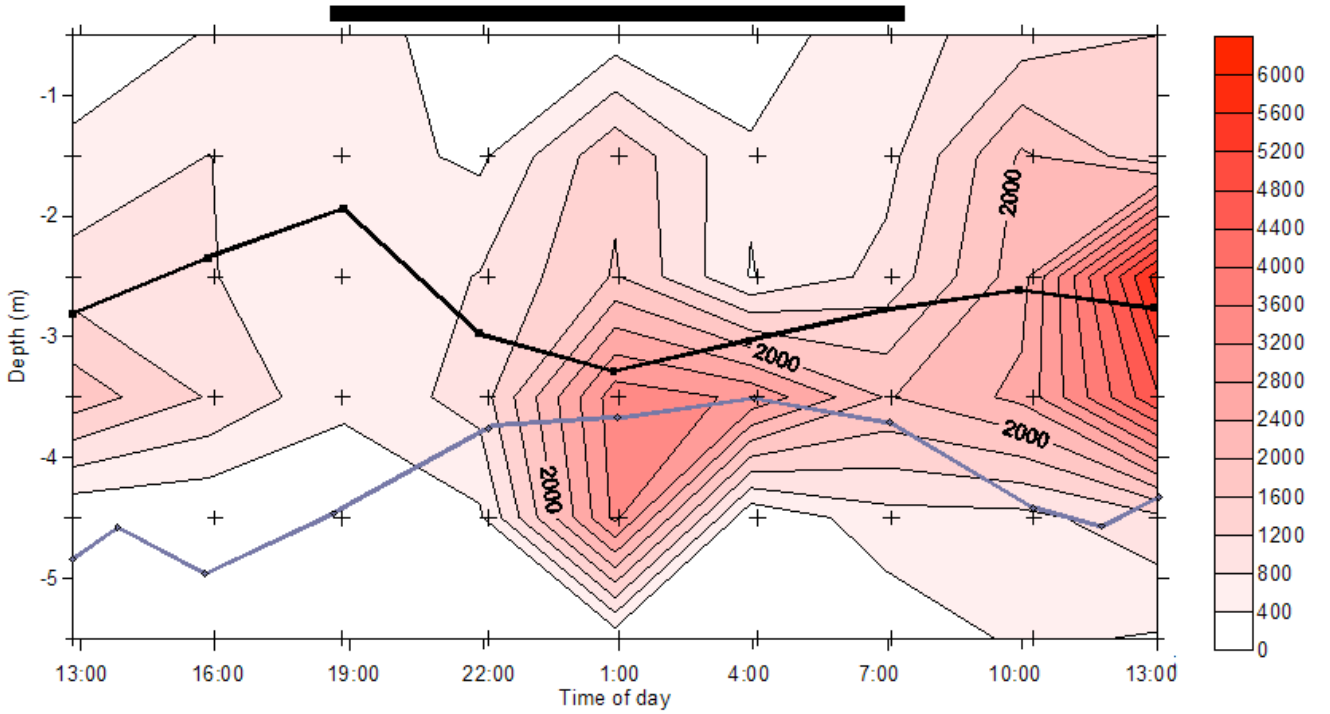

Fig. 12 – 24-hour evolution of *S. pulchra* cell concentrations (cells·L^{-1}). See fig. 7 for explanations

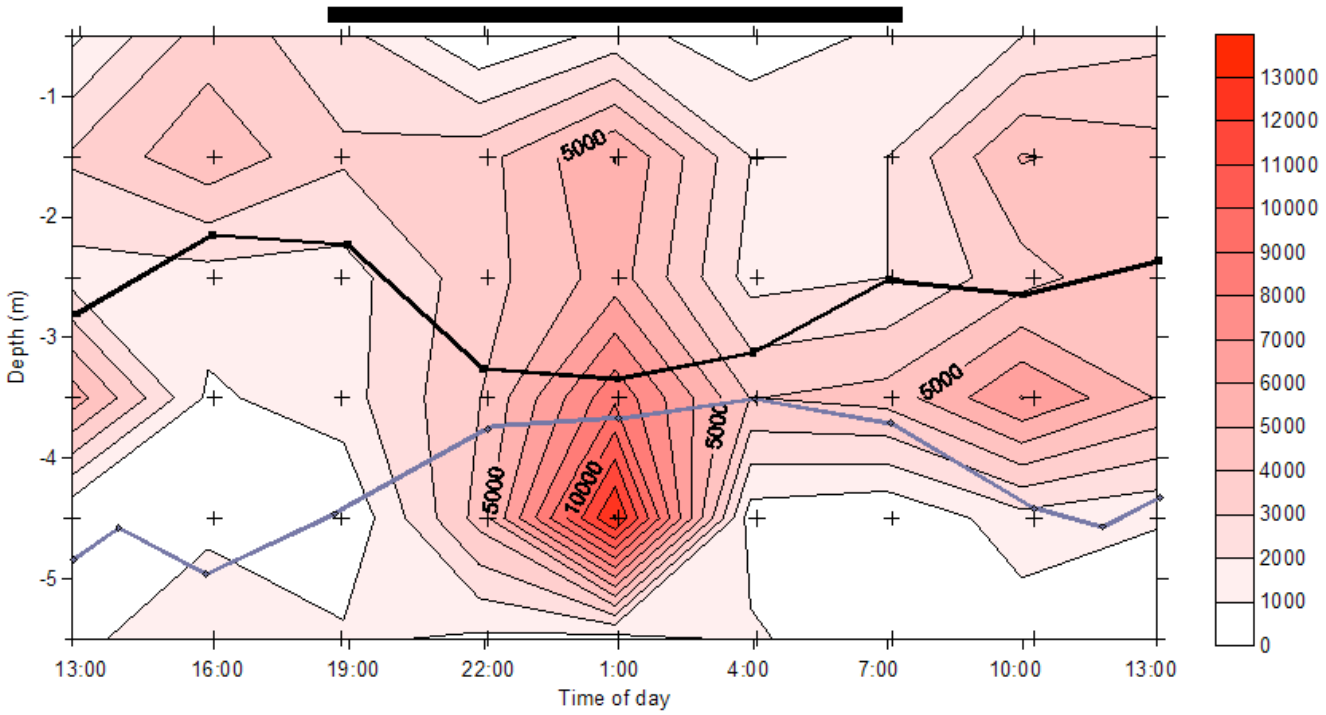

Fig. 13 – 24-hour evolution of *C. brasilensis* cell concentrations (cells·L^{-1}). See fig. 7 for explanations

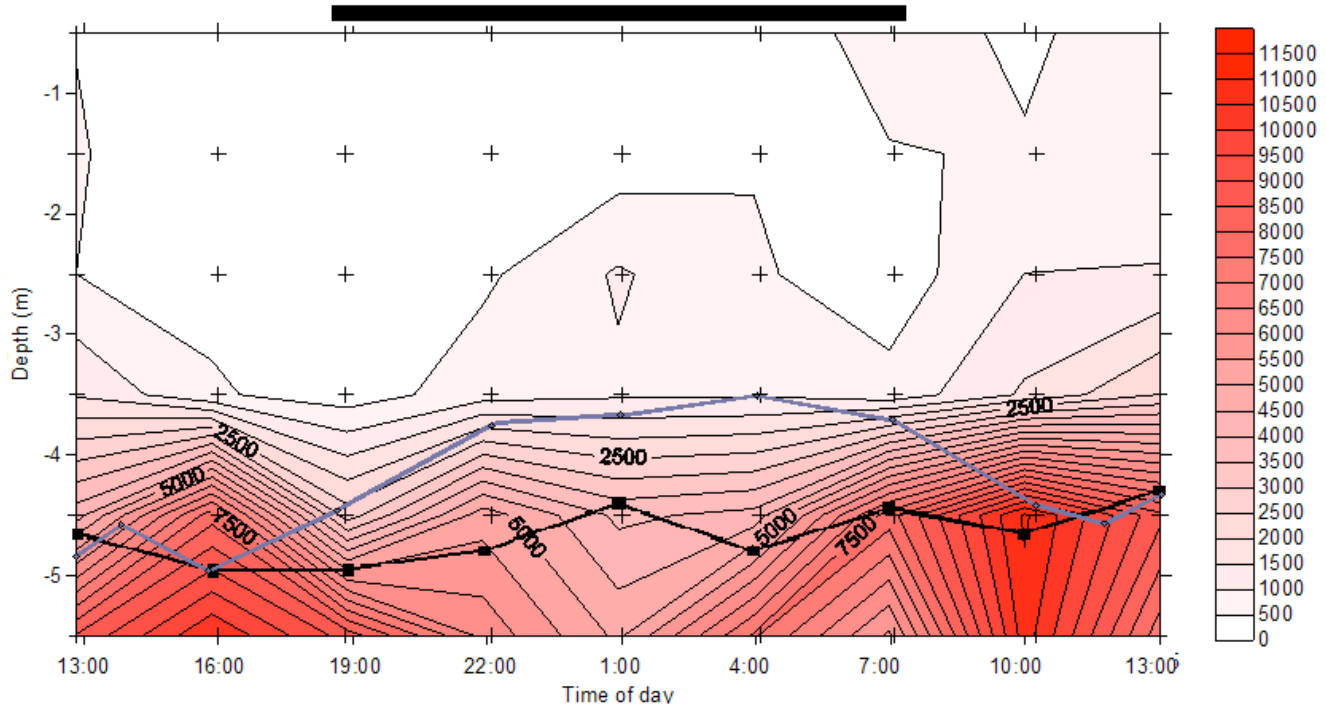

Fig. 14 – 24-hour evolution of T. nitzschoides cell concentrations (cells·L^{-1}). See fig. 7 for explanations

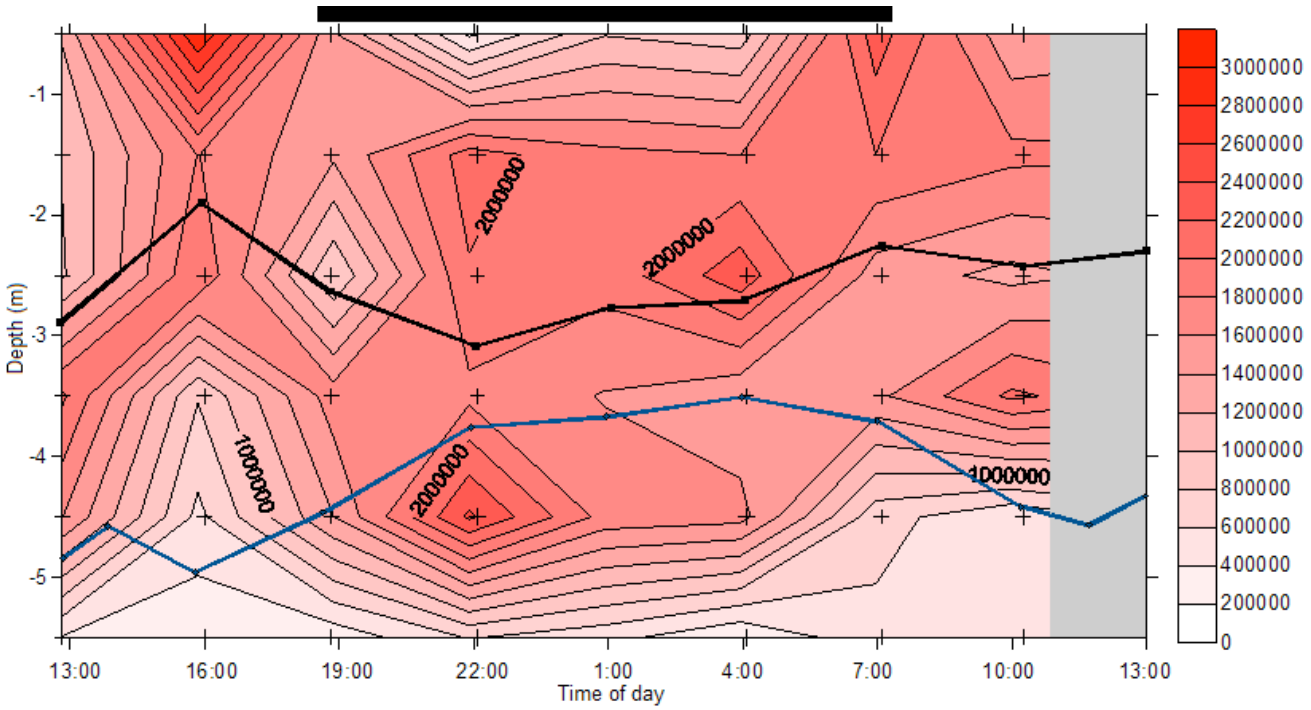

Fig. 15 – 24-hour evolution of Cyclotella cell concentrations (cells·L^{-1}). Values for the 1:05 profile correspond to interpolation between the 22:05 and 4:05 values. See fig. 7 for explanations

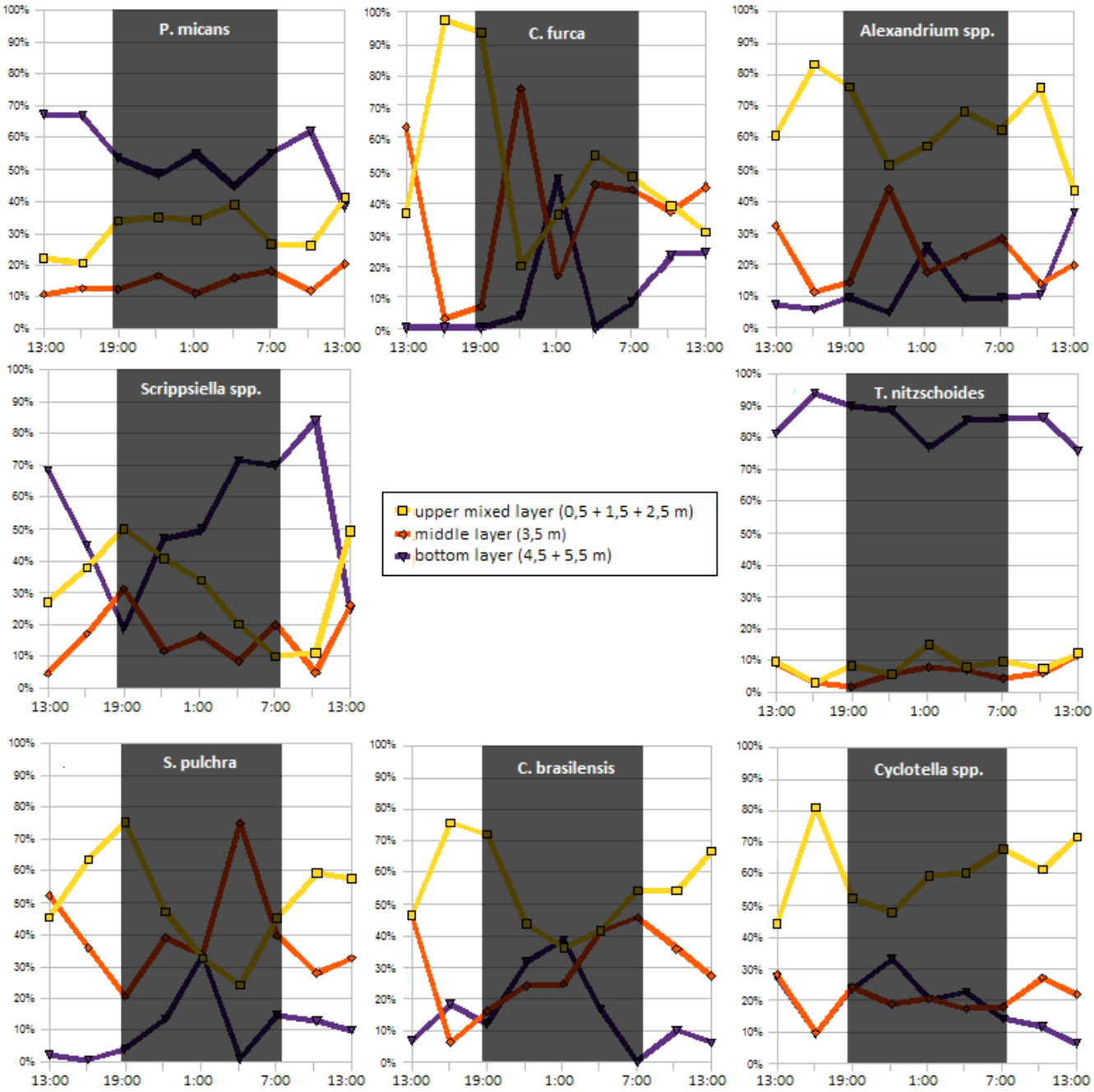

Fig. 16 – 24-hour evolution of the relative cell concentrations fraction (%) of the 3 layers defined in materials and methods. Graphs were ordered to facilitate comparison between same-taxon species. Shadow corresponds to the dark period.

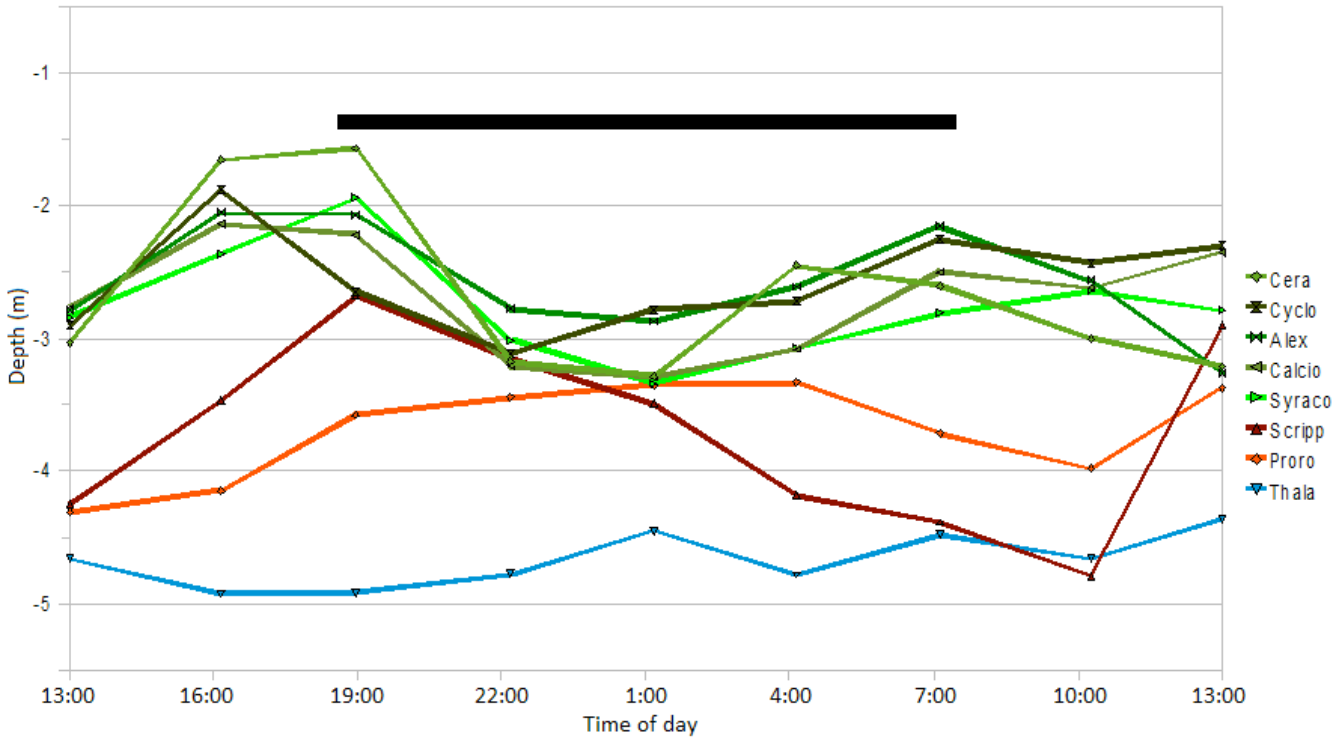

Fig. 17 – 24-hour evolution of the mean depth of cell occurrence as defined in materials and methods. The legend was ordered from highest to lowest based on the depth at 16:00 sampling time. Dark bar above graph denotes the dark period.

Discussion

Reliability of the Uthermöhl method

The accuracy of the cell counts obtained with the Uthermöhl method relies on a number of conditions (in this section we purposely disregard variabilities coming from the field sampling itself, however important they may be). These are, a) that the sample is well homogenized prior to pouring into the sedimentation chamber, b) that all cells have sedimented during the sedimentation period and c) that they have done so uniformly over the plate, not clumping into patches or adhering to the column walls. An additional condition d) is that the sample has undergone no deterioration between the moment it is collected at sea and the moment the counting is made. This latter issue is of special importance here as cell concentration assessment for two of the profiles (1:05 and 13:00 the second day) has been done nearly 2 years earlier than for the rest; this will be discussed in more detail later on.

To assess error, 4 of the samples pertaining to the 22:05 profile were processed twice (see materials and methods). Hereafter are enumerated different sources of error and how they are supposed to impact differently for different species (summary provided in table 2).

	Sources of error				
Species	n is small	Area sampled	Colonial behaviour	Confounding shape	Error (%)
P. micans	-	-	-	-	14
Scrippsiella spp.	+	-	-	+	65
C. furca	+	-	-	-	25
Alexandrium spp.	++	+	-	+	?
S. pulchra	-	-	-	-	?
C. brasilensis	++	+	-	-	58
T. nitzschoides	-	-	+	+	53
Cyclotella spp.	-	++	+	+	69

Table 2- Sources of error affecting cell concentration assessment. The importance of each source of error is indicated for each species: not very important (-), important (+) and very important (++)

Not surprisingly, species that involved the least microscope observations showed great relative errors. This kind of error (where n is very low) affected primarily *Calciosolenia brasilensis* and *Alexandrium* spp., because these species were assessed through a single transversal section of the whole sedimentation plate and actual counts never exceeded 19 by sample. The relative error averaged over all 4 recounts for *C. brasilensis* was 58%.

Another source of error is caused by the colonial behaviour of species, namely *Thalassionema nitzschoides* and *Cyclotella* spp. The fact that individual cells and not colonies were counted inflate the observations without reducing error, giving a false sense of security about what is to be regarded as sufficiently high n value. In the case of *T. nitzschoides* (whose colonies are usually formed by half a dozen cells, sometimes as much as 21), this became particularly obvious when recounts were made in samples where they were few (i.e. 1,5 and 2,5 m). For example, where the initial count of the 1,5 m sample tallied to 12 individuals, 48 cells were identified in the repeat (119% error). On the other hand, the relative errors for 4,5 m and 5,5 m samples (with n > 200) were both <11%; the relative error averaged for all 4 samples was 53%. *Cyclotella* spp. was more problematic as high relative errors (over 60%) were observed even though n was high (over 200). This might be due to 2 additional sources of error: one is the representativity of the area sampled and the other is the capacity of the observer to discriminate species.

As the entire plate was scanned in order to assess *Prorocentrum micans*, *C. furca*, *T. nitzschoides*, *Scrippsiella* spp. and *Syracosphaera pulchra* cell concentrations, the problem of lack of representativity of the area sampled affected exclusively the other 3 species. These are *Alexandrium spp.* and *C. brasilensis*, for which a transect (roughly $1/20^{th}$ of the plate) was scanned, and more importantly *Cyclotella* spp., for which 10 visual fields (a mere $1/280^{th}$ of the plate) were scanned. It was a recurrent constatation that species do not always tend to spread uniformly over the plate. Big species like *C. furca* showed propensity to settle on the periphery of the plate area, while *Cyclotella* spp. colonies were often found clinging to each other, resulting in a few abnormally inflated counts within a single visual field. The relative error averaged over all 4 samples for *Cyclotella* spp. was 69%, and as stated earlier was high even though n was high.

One last source of error is the subjective capacity of the observer to discriminate cells. This

error affects all species to varying degrees. For an unexperienced observer (as was the case in this study), this error is bound to decrease with time, as habits and visual cues are acquired to help discriminate quickly and correctly. Some species, like *Scrippsiella* and *Alexandrium* spp., adopt relatively generic shapes that make them easily confused with other same-taxon species for an untrained eye. *T. nitzschoides* can be confused with other same-genus species while *Cyclotella* spp. can be confused with (or even hidden by) detritus. In the case of *Scrippsiella* spp., the relative error averaged over all 4 samples was quite high at 65%. It is perhaps an exageration of the actual error if it were to be evaluated for other samples though, because the 22:05 profile samples that were selected for error assessment were the very first samples to be processed and counted under the microscope. In order words, valuable experience had been acquired between the moment the 22:05 profile samples were processed and the moment further samples were processed.

P. micans and *C. furca*, being relatively little affected by either of the above-mentionned error sources, fared well during the repeats with averaged relative errors of 14% and 25% respectively. It is reasonable to think that *S. pulchra*'s error would have been low too had it been assessed, not being strongly affected by either source of error.

Whether high relative errors translate into wrong interpretations of spatio-temporal patterns depend on the clarity and resolution of said patterns. *T. nitzschoides*' high relative error for instance is advantageously compensated by the presence of a clear and sharp pattern between the lower and upper depths that is observable at all sampling times. *Cyclotella* spp. on the other hand is present at high concentrations throughout much of the water column, and recurrent errors might blur what would otherwise stand out as subtle but actual spatio-temporal differences.

As stated earlier, times 1:05 and 13:00 were sampled 2 years before the rest and different sampling areas and observer were employed. This makes temporal patterns overlapping these two precise times prone to distortion and caution must be taken in interpretating them. Although the fixative was chosen on the basis of prolonged cells preservation capacity, cells can dissolve or change their morphology over long periods of time. For instance, loss of cell integrity were noted for two genera (*Chaetoceros spp.* and *Pseudonitzchia spp.*) that were not included in our study.

Scrippsiella spp. cell concentrations for time 13:00 were outrageously high when compared with the rest of the experiment and especially with previous sampling time (10:15). *Cyclotella* spp. counts for times 1:05 and 13:00 were nearly 2 orders of magnitude lower than for all other sampling times and for this reason were excluded from any analysis involving absolute values and only retained for relative vertical distribution analysis. Unlike for *Scrippsiella* spp., in the case of *Cyclotella* spp. it cannot be argued that cells had denatured over time, because the earlier counts are the ones yielding the lowest estimations. Only a major discrepency between methodologies employed can be invoked. It is important to stress that even when a rigorous and constant methodology is employed, counts obtained with the Uthermöhl method still remain prone to high error.

How did species distribute vertically? Were migrational patterns observed?

In general, all the species were found with more or less abundance in all sampled depths at least in a part of the 24 h of our study. However, each species appeared to display preferential locations within the water column, and furthermore, such location varied over time. Such patterns may be explained by a combination of the physiological preferences of the organisms for light, nutrients or entrained diel life cycles (e.g. encystments, sexual reproduction) with a superimposed forcing of the water motion.

As said in the introduction, the relative segregation in time and/or space of light and nutrients are major drivers of the phytoplankton dynamics. Thus, we present first a snapshot of how light and nutrients may have affected the vertical distribution of the phytoplankton in our study in general. During the study period, the PAR levels in surface waters were around 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ decreasing down to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ around the pycnocline and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ near the bottom (M. L. Artigas, pers. comm.). The second day of our experiment was cloudy, so those values were about 50% lower. These ranges are much lower than those found in summer (ca. 2000 mmol at surface), so photoinhibition would not be expected to happen. Light limitation in turn would only be expected at 5,5 m depth. Our data suggest that *Thalassionema nitzschioides* and *Prorocentrum micans* grew at relatively low light levels. Indeed the patchy *P. micans* surface abundance corresponded to the night period. In contrast, *Cyclotella* spp. and *Calciosolenia brasilensis* thrived under higher light intensities. The rest of the studied species (*Alexandrium* spp., *Ceratium furca*, *Scrippsiella* spp.,

Syracosphaera spp.) experienced a wide range of medium to high light intensity exposure.

Nutrient levels were relatively low during the studied period and when compared to the rest of the year (Loureiro et al. (2009), Llebot et al. (2010)). The low values of silicate (< 1 µM) concurrently to nitrate concentrations ca. 5 µM suggest that diatoms had grown at the expenses of the available silicate in the previous days. Furthermore, near the bottom, silicate was close to exhaustion, likely consumed by *T. nitzschioides*. Inorganic phosphorus, which is often limiting in the bay, was slightly higher in the bottom (likely due to recycling and/or resuspension processes) than in surface waters, but the differences were not important. This element experiences a high biological turnover in the water column. Thus, we do not think that the small differences in inorganic phosphorus could have constituted a major forcing in the vertical discrimination of the species. Having said that, however, organic nutrient sources (e.g. Loureiro et al. 2009, Llebot et al. 2010) not considered here could have played a role. This can be explored in further studies.

Hydrographic properties during our study indicate the presence of two main water masses: a mostly marine layer near the bottom and an estuarine layer above, separated by an oscillating pycnocline. The upper layer would correspond to the mixing layer which experienced greater variability in regards to day:night temperature shifts and to the sporadic freshwater supply at surface in the middle of the night. The pycnocline was used to define a potential barrier for the phytoplankton species inhabiting the two layers. We remark that *Alexandrium* spp., *Ceratium furca*, *Calciosolenia* spp. *Syracosphaera* spp. and *Cyclotella* spp. would be part of the phytoplankton assemblage characterizing the upper layer, although they too were present in the marine layer (albeit marginally). In contrast, *Thalassionema nitzchioides* would mostly characterize the marine bottom layer, despite also being found in upper layers. *P. micans* and *Scrippsiella* spp. would be present in the two assemblages. It should be noted that 6 out of 8 species repeatedly showed maximum or close-to-maximum cell concentrations in the middle layer (i.e. around the pycnocline), highlighting the importance of a density gradient in the establishment of a biomass maximum. Fluorescence data support this interpretation.

The fluorescence maximum observed below the pycnocline near midnight occurs simultaneously to the input of fresh water to surface, yet there is no indication that both events are linked. More likely, the formation of high chlorophyll maximum at this depth would

be a cyclical phenomenon that involves the inherent bay dynamics. A fluorescence maximum is observed around this depth practically every night in the bay (M.L. Artigas, pers. comm.). Our data suggest that *P. micans*, *C. furca*, *S. pulchra*, *C. brasilensis* and *Cyclotella* spp. have contributed to this fluorescence increase by either ascending towards the pycnocline (*P. micans*) or descending through the pycnocline (all others). Of course more species than those included in our study must also have contributed. Processes such as *in situ* growth combined with advection from elsewhere (tidal currents for example) can explain the observed fluorescence increment. Active migration of some or all of the species could play a part and superimpose over these processes too.

Within the upper mixed layer, the vertical and diurnal variability of some species may appear tightly linked to the water motion generally speaking, but once again we cannot discard biological processes. The physiology of the organisms may be sensitive to very small-scale gradients of the physical parameters or to the light:dark variability. Motile organisms in particular have the capacity to respond to those environmental changes and could have crossed the (potential barrier) pycnocline during certain periods of our study. The low turbulent energy dissipation rates estimated by a microscale profiler, 10^{-6} and 10^{-8} m^2 s^{-3} (M.L. Artigas, pers. comm.), would have been compatible with the swimming speeds of dinoflagellate species (see discussion below). Thus, their observed variability could be interpreted in terms of vertical migrations, although the whole duration of our experiment (24 h) and the difference in irradiance between the first and the second day hampers our capacity to directly link the migrations to a diel cycle. As a final note to this preambule, if we cannot disregard *in situ* growth of the species as a causal agent for observed increases in cell numbers, neither can we disregard grazing as a cause for cell decreases observed.

Having said that, our results indicate that density gradients play a more important part than light in determining the species general distribution. As noted earlier, the pycnocline represents a layer of higher cell concentration and an important change is observed in the relative dominance of species when passing from one side to the other. The one species with preferential bottom habitat (*T. nitzschoides*) decrease in abundance above the pycnocline, 2 species (*Scrippsiella* spp., *P. micans*) usually (but not strictly) decrease too, and all other 5 species increase in abundance. Physical parameters of the water column seem to modulate the species vertical distribution. Species inhabiting at different layer depths appear to be

influenced by advection patterns altogether different. This is evidenced by the mean depths of cell occurrence (see fig. 17) which vary differently between different depth groups. On the one hand, the bulk of *T. nitzschoides* cells oscillate minimally, being constricted between the sea bottom and the pycnocline. On the other hand, the vertical movement of all five species from the upper mixed layer is likely linked to physical events as they affect all species in a similar fashion.

Two species (*P. micans* and *Scrippsiella spp.*) cannot straightly be grouped with the upper mixed layer or the bottom layer assemblage. Although both concentrate most often below the pycnocline (and *P. micans* cells always flirt very close to it), they are also capable of crossing the pycnocline as evidenced by their aggregating near surface during the night. It is not evident if one or a combination of factors would regulate more specifically the vertical movements of the two dinoflagellates. *P. micans*' aggregation pattern loosely points to a scenario where some (but not most) cells pass through the pycnocline by night fall and colonize the upper mixed layer. Nevertheless, the fraction of biomass found in the upper mixed layer never outnumbers that found below it (maximum reached is 41%), so it cannot be said that there is massive cell migration, but rather that a dispersion of some of the cells above the pycnocline is observed. These observations are markedly different from those of Eggersdoffer (1991) and Ault (2000) whose studies describe precise and generalized diel vertical movements for *P. micans* cells in laboratory settings. Another respect in which our results differ from theirs, but also from an overwhelming majority of the litterature on dinoflagellates alike, is that migratory behaviours is almost always characterized by an ascent at day and a descent at night, a strategy that is hypothesized to maximize photosynthetic yield. Only with too-high irradiance settings did *P. micans* avoid the upper 0,5 m layer, again for the sake of high photosynthetic yield (PAR above 300-500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ can harm cell functions and provoke photoinhibition (Figueroa et al. (1998), Eggersdorfer (1991), Richter et al. (2002))). In our case however it cannot be argued that photoinhibition explains the night-up and day-down pattern, because irradiance was low and cells did not only avoid the upper 0,5 m of the water column but the whole upper mixed layer during the day.

As for *Scrippsiella* spp., during our study there were times when much of the biomass was found in the higher half of the upper mixed layer and other times when it would accumulate below the pycnocline. This is a surprising result as *Scrippsiella hoeigei* has been cited for its

apparent lack of diel vertical movements (Olli et al., 1998) while *Scrippsiella trochoidea* in the field did migrate but remaining exclusively above the pycnocline (Figueroa et al., 1998). Yet during our experiment *Scrippsiella* spp. inhabited sub-pycnocline depths a lot of the time.

Due to *Scrippsiella*'s diel cycle being dephased with the daily irradiance cycle (fig. 16), it is far from obvious that it may be linked to phototaxis (be it positive or negative). Phototaxis is perhaps the most often cited mechanism for dinoflagellates diel vertical movements, but environments undergoing high hydrodynamic disturbance sometimes fail to display diel cycles (i.e. Alexander and Imberger (2009), Olli et al. (1998)). During the course of our experiment turbulence was weak and stratification was marked, hence physical forcings alone could not have shuffled the water column sufficiently to mask diel vertical movements.

Another factor to take into account is nutrients. Although it is only implicitly inferred in most studies, the reason dinoflagellates aspire to migrate downwards at night rather than stay afloat is to gain access to the pool of nutrients located below the pycnocline. Controlled experiments with *Alexandrium tamarensis* have demonstrated that, withdrawing the light factor, cells migrate in the direction of positive nitrogen gradient (Macintyre et al., 1997). But undertaking migration has a metabolic cost and should be done only if the cost is compensated by the benefit of replenishing the celular nitrogen pool (i.e. when the gradient between the N-depleted mixed layer and the N-repleted sub-nutricline layer makes migration worthwhile). The Alfacs bay does not comply with this condition during the period of experiment. Its upper mixed layer is in fact slightly more replete in nitrites and nitrates than the layer below the pycnocline, although all nutrient levels were low as discussed earlier. Phosphates on the other hand seem slightly more abundant at depths, although concentrations hardly surpass the detection limit. In any case nitrogen is usually the more limiting factor at the Alfacs bay during spring (Delgado & Camp (1987), Llebot et al. (2010), Loureiro et al. (2009)). Dinoflagellates in the Alfacs bay would gain no advantage from migrating downwards during the night if they only did it for nitrogen. This could explain why this migrational pattern did not form part of our observations.

It is an interesting observation that both diatoms apparently exclude each other from their own preferred residence depths. *Cyclotella* spp. was found in the upper mixed layer and to a lesser extent around pycnocline depth, while *T. nitzschoides* was found at the bottom layer

and to a lesser extent around pycnocline depth. The superimposition of 2 different water masses discussed earlier provides a convincing physical explanation for this mutual exclusivity, but this situation is also reminiscent of one where mutual niche exclusion takes place. Due to the lack of evidence for environmental cues orientating *P. micans*' and *Scrippsiella*'s migrations, and given that hydrodynamics alone cannot account for their displacements and that both species show unique migratory trajectories (see fig. 17), then both species could be envisaged to gain some advantage with their behaviour through niche exclusion. Olli et al. (2008) observed that 3 dominant autotrophic species of a coastal ecosystem adjusted their migrations so as to avoid each other and limit competition for resources. Here *P. micans* was overall the dominant dinoflagellate species in the upper mixed layer, this made it a potentially important competitor for *Scrippsiella* spp.

Literature reports diel vertical migrations in *Ceratium furca* and species of the genus *Alexandrium* (i.e. Delgado et al. (1998), Figueroa et al. (1998)). Our observations do not exclude the possibility for migration in these species but do not support the existence of diel cyclicity. Furthermore, the temporal evolution of their vertical distribution seems to converge towards that of *Syracosphaera* spp., *C. brasilensis* or *Cyclotella* spp., all of whom are deprived of flagellate apparatus. Although physical forcing in the delta could have hindered actual diel migratory movements by these two dinoflagellates, as discussed earlier turbulence was low throughout the sampling period. More likely, environmental gradients such as that in light and nutrients were too weak to contribute to a sustained displacement of cells towards a given taxis. In the absence of environmental gradients, endogenous cues (the «biological clock») can still contribute to the displacement of cells in the water column. However for *C. furca* and *Alexandrium* spp. this did not happen. It could be that environmental gradients had been weakened for a prolonged period of time already. In a controlled experiment, 5 days of steady ambient light conditions were sufficient to break Alexandrium's endogenous migratory rythm (Delgado et al., 1998).

It is interesting to note that Figueroa et al. (1998) had observed in the estuarine Ria de Vigo that *C. furca* massively swam beneath the pycnocline at night, while our results suggest that he only does during a short time. For *Scrippsiella trochoidea* on the other hand, they observed movement restrained to the upper layer, while we observe on the contrary across-pycnocline upwards movement of Scrippsiella spp. In a certain way our results do not

contradict theirs though, at least in the sense that in both cases *Scrippsiella* spp. displayed higher tendency for surface than C. furca at night. Furthermore, various studies cite other *Scrippsiella* species as lacking diel vertical migration patterns (Vilarino et al. (1995), Olli et al. (1998)). Thus our incapacity to support other authors' observations could be due to our grouping of various *Scrippsiella* species under a single label.

The shallow depth of the bay (6 m) decreases our capacity to detect migration, since cells have little room to move up and down and the boundary effect (air/water and water/ground interfaces) is felt over a larger portion of the water column than would be in a deep-sea location. Limitation of the available methods for sampling too undermines this capacity. Most field studies investigating dinoflagellates diel vertical migrations take place in marine or lacustrine locations of greater depths. The Alfacs bay is a relatively complicated estuarine location so far as its hydrodynamics are concerned (Camp & Delgado, 1987). Although channels are usually shut at this time of the year, an auxiliary channel was actually open during the sampling period (Comunitat de Regants del Delta de l'Ebre). Freshwater input to the surface layers at night time likely came from the channels. When channel water flows freely to the bay, a gradient in nutrients and phytoplankton biomass forms along its transversal section, with inshore water being richer in both (Delgado, 1987). Any discharge of irrigation water while the experiment was led could have changed water properties (nutrients, salinity) and provoked advection or triggered escape responses on the part of some phytoplankton.

The knowledge of sinking and swimming velocities for all studied species allow to put in perspective the relevance of vertical movements discussed so far. All four targeted dinoflagellate species have swimming speed sufficient to cross the whole 6-meter water column in a 12-hour period (Smayda, 2010). Turbulence was low for the whole water column. Therefore, if cells respond to simple environmental gradients such as happens with positive phototaxis, this should result in diel vertical migration. Since this was not the case in our study it must be speculated that other controlling factors were at play.

Conclusion

Two phytoplankton assemblages corresponding to distinct vertical distribution were distinguished at the sampling site. The assemblage of *Cyclotella* spp., *C. brasilensis*, *S. pulchra*, *C. furca* and *Alexandrium* spp. was associated with a shallow water mass, the upper mixed layer, whereas *T. nitzschoides* was associated with a deeper water mass, the bottom layer. The vertical extent of Prorocentrum's distribution responded to variations in the pycnocline depth. But additionally, this species and Scrippsiella spp. didn't exclusively reside in the bottom layer but periodically developed a separate concentration peak well above the pycnocline. This was interpreted as evidence for active swimming undertaken by some (but not most) cells of these species through the pycnocline. The apparent diel cyclicity of their vertical movements could not be associated with any taxis or environmental stimulus. It is suggested that the absence of a nutricline and the shallow depth of the bay could explain the deviation from the usual paradigm of surface-at-day and depth-at-night diel vertical movements. Additionally, niche speciation could be envisaged as an alternative advantage that both species draw from their different migratory patterns. Diel vertical migrations for the dinoflagellates *C. furca* and *Alexandrium* spp. was not obvious from our results. The present study was based on estimations of microphytoplankton cells abundances based on the available techniques, characterized by limited samplings (every 1 m, every 3h) in combination with the Utermöhl (1958) method. Altogether, the procedure has high associated error (over 50%) and low resolution compared to the technologies that allow to characterize physical parameters with high spatio-temporal resolution. A clear improvement in the sampling methods for biological parameters, is thus required. Such tools could allow to understand how phytoplankton respond to small scale variation of environmental variables. For instance, it could shed light on which factors most strongly rule diel vertical migrations under natural conditions.

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