RELATIONSHIP BETWEEN PHYSICO-CHEMICAL CONDITIONS AND PHYTOPLANKTON VARIABILITY IN ALFACS BAY

Author: OIHANE MUÑIZ PINTO

Director: ELISA BERDALET I ANDRES
Additional supervision: NORMA ZOE NESZI

Centre: Institut de Ciències del Mar (ICM-CSIC). Departament de Biologia Marina & Oceanografia

Màster en Ciències del Mar: Oceanografia i Gestió del Medi Marí
September 2012
ACKNOWLEDGMENTS

I am grateful to the University of Barcelona and the ICM-CSIC for giving master's students the opportunity to get to know what working on a research project means. It has been a really positive and enriching experience.

I would also like to thank my supervisor Norma Zoe Neszi, for all that she taught me, and Elisa Berdalet for her help, support and knowledge.

And finally, I am really grateful to my parents and friends, for their patience and encouragement.
ABSTRACT

Understanding the spatio-temporal variability of phytoplankton in aquaculture areas is necessary for the appropriate management of natural resources and the prevention of noxious outbreaks. With this objective in mind, we conducted weekly samplings during the spring of 2011 in Alfacs Bay, a microtidal estuary in the NW Mediterranean and an important area of shellfish and finfish exploitation which is regularly affected by toxic outbreaks. The study was part of the ECOALFACS project (CTM2009-09581), and here we focus on the results obtained during 4 consecutive weeks (May 31st to June 21st). We describe the main water column properties (temperature, salinity and inorganic nutrient composition) and phytoplankton community composition.

CTD casts revealed that the water column was initially well mixed, subsequently stratified during the 2nd and 3rd weeks, and mixed again in the last sampling. Over the studied period, temperature increased but the salinity variation remained within the range characteristic of this embayment where freshwater supply occurs at surface from irrigation channels from the nearby delta rice fields. Inorganic nutrients were very low, specially phosphorus, which was close to the detection limit. Silicate tended to increase towards the second half of the studied period. Likely due to such limiting nutrient conditions, chlorophyll values remained pretty similar, with values no exceeding the 4 µg·L⁻¹. The organisms smaller than 5µm contributed by more than the 50% of the total biomass, specially towards the end of the studied period.

The succession of the main phytoplankton groups indicated that the spring diatom bloom was decaying during the 1st weeks of our study, with flagellates dominating over dinoflagellates and diatoms. The most relevant differences among the microphytoplankton organisms was due to particular dinoflagellate taxons.
RESUMEN

Es importante entender la variabilidad espacio-temporal del fitoplancton en zonas de acuicultura para una apropiada gestión de los recursos naturales y la prevención de brotes nocivos. Con este objetivo, llevamos a cabo muestreos semanales durante la primavera del 2011 en la bahía dels Alfacs, un estuario micromareal en el Mediterráneo noroccidental e importante zona de mariscos y explotación de peces que es regularmente afectada por brotes tóxicos. Este estudio es parte del proyecto ECOALFACS (CTM2009-09581), y nos centramos en los resultados obtenidos durante 4 semanas consecutivas (31 de Mayo - 21 de Junio). Describimos las principales propiedades de la columna de agua (temperatura, salinidad y composición de nutrientes inorgánicos) y la composición de la comunidad fitoplanctónica.

Los datos obtenidos con el CTD revelaron que, inicialmente, la columna de agua estaba mezclada, posteriormente estratificada durante la 2ª y 3ª semana, y mezclada de nuevo en el último muestreo. La temperatura aumentó durante el período estudiado, pero la variación de la salinidad se mantuvo dentro del rango característico de esta ensenada, debido a los aportes superficiales de agua dulce procedente de los canales de riego de los campos de arroz del delta. Las concentraciones de nutrientes inorgánicos fueron muy bajas, especialmente las de fósforo, que se encontraba cerca del límite de detección. El silicato tendió a aumentar en la segunda mitad del período estudiado. Los valores de clorofila se mantuvieron bastante similares, con valores que no excedieron los 4 µg·L⁻¹, probablemente debido a la limitación por nutrientes. Los organismos inferiores a 5 µm contribuyeron en más del 50% de la biomasa total, especialmente hacia el final del período de estudio.

La sucesión de los principales grupos de fitoplancton indicó que el bloom primaveral de diatomeas fue decayendo durante las primeras semanas de estudio, pasando a un dominio de los nanoflagelados sobre los dinoflagelados y las diatomeas. Las diferencias más relevantes entre los organismos microplanctónicos fue debida a taxones concretos de dinoflagelados.
RESUM

És important entendre la variabilitat espai-temporal de fitoplàncton en zones d’aqüicultura per a una apropiada gestió dels recursos naturals y per la prevenció de brots nocius. Amb aquest objectiu portem a terme mostres setmanals durant la primavera del 2011 a la badia dels Alfacs, un estuari micromareal en el Mediterrani nort-occidental i a més a més, una zona important de mariscs y explotació de peixos, la qual és regularment afectada per brots tòxics. Aquest estudí és part del projecte ECOALFACS (CTM2009-09581), i ens centrem en els resultats obtinguts durant quatre setmanes consecutives (31 Maig – 21 Juliol). Descrivim les principals propietats de la columna d’aigua (temperatura, salinitat y composició de nutrients inorgànics) y la composició de la comunitat fitoplanctònica.

Les dades obtingudes amb el CTD van deixar veure que, inicialment, la columna d’aigua estava barrejada, posteriorment estratificada durant la segona i la tercera setmana i barrejada de nou en l’ últim mostreig. La temperatura va augmentar durant el període estudiat, però la variació de la salinitat es va mantindre dins la franja característica d’aquesta badia, degut a les aportacions superficials d’aigua dolça procedent dels canals de reg dels camps d’arròs del Delta. Les concentracions de nutrients inorgànics van ser molt baixes, especialment les de fòsfor, que es trobava a prop del límit de detecció. El silicat va tendir a augmentar en la segona meitat del període estudiat. Els valors de clorofila·la es van mantindre bastant similars, amb valors que no es van extendre dels 4 ug·L⁻¹, probablement va ser degut a la limitació per nutrients. Els organismes inferiors a 5 µm van contribuir en més del 50% de la biomassa total, especialment fins al final del període d’estudi.

La successió dels principals grups de fitoplàncton va indicar que el bloom primaveral de diatomees va anar de cap a caiguda durant les primeres setmanes d’estudi, passant a un domini dels nanoflagelats sobre els dinoflagelats i les diatomees. Les diferències més rellevants entre els organismes microplanctonics va ser deguda a taxones concrets de dinoflagelats.
INDEX

1. Introduction ...................................................................................................................... 1
   1.1. Alfacs bay .................................................................................................................. 1
   1.2. Phytoplankton in Alfacs bay .................................................................................... 2
   1.3. Harmful Algal Blooms (HABs) ............................................................................... 4
   1.4. Objective .................................................................................................................. 4

2. Material and methods ...................................................................................................... 5
   2.1. Cruises ...................................................................................................................... 5
   2.2. CTD casts .................................................................................................................. 5
   2.3. Weather conditions ................................................................................................. 5
   2.4. Sampling ................................................................................................................... 5
   2.5. Nutrients ................................................................................................................ 6
   2.6. Chlorophyll ............................................................................................................. 6
   2.7. Microphytoplankton ............................................................................................... 7
   2.8. Nanophytoplankton and cyanobacteria ............................................................... 8

3. Results ............................................................................................................................. 10
   3.1. CTD casts and weather conditions ........................................................................... 10
   3.2. Nutrients and chlorophyll ....................................................................................... 13
   3.3. Microphytoplankton ............................................................................................... 15
   3.4. Nanophytoplankton and cyanobacteria .................................................................. 19

4. Discussion ......................................................................................................................... 20

5. Conclusions ....................................................................................................................... 24

6. References .......................................................................................................................... 26
1. INTRODUCTION

1.1. Alfacs Bay

Alfacs is a shallow bay situated in the south of the Ebro delta (NW Mediterranean sea 40°40'N, 0°40'E) (Fig.1). This semi-enclosed estuary is separated from the Mediterranean by a sand barrier that leaves a 2500 m wide opening to the open sea. The total area of the bay is about 49 km$^2$ with a maximum depth of 6.5 m at the center of the bay (mean depth of 3.13 m) (Camp et al. 1992).

As other estuarine environments, it is an ecosystem with high biological productivity that provides important resources and socio-economical services: this area is considered a successful fish and shellfish aquaculture site (Colomé et al. 1997), as it is Catalunya's biggest fishing harbour. Thus, it is exposed to both natural and anthropogenic stresses. In one hand, climatic factors drive the short-term variability, while freshwater inputs of anthropogenic origin result in a yearly cycle (Llebot et al. 2011; Vidal et al. 1997).

![Map of the area of study. The x shows the Raft Station. (Llebot et al. 2011)](image_url)
Compared to the adjacent Mediterranean waters, the productivity of Alfacs bay is high, due to the freshwater input from the rice fields of the delta. This freshwater runoff contains a high concentration of inorganic nitrogen but a very low concentration of phosphorous, most likely due to its retention on the rice fields (Llebot et al. 2010). Therefore, phytoplankton growth in the bay is usually phosphorus limited (Mura et al. 1996).

The freshwater inflow creates a stratification that dominates over tidal mixing, which is lower than 0.2 m (Llebot et al. 2011), yielding a strongly stratified, two layered system. In this situation, the main flow is characterized by a classical estuarine circulation pattern (Camp 1994), where marine water enters at lower depth and freshwater flows out at the surface (Camp 1994; Dyer 1979 in Camp and Delgado 1987; Llebot et al. 2011). Solé et al. (2009) described two basic states for the bay’s hydrography: a) one transient state of a short period (2-5 days) consisting of the mixing of the water column caused by storm events or winds stronger than 4.5 m·s⁻¹; b) a steady state that maintains the estuarine typical dynamics (stratification, salt-wedge) almost all the year. This steady state presents three periods: open channel period (from April to September), semi-open period (from October to December) and closed channel period (from January to March/April). The alternation between stratification and mixing depends on three main factors: freshwater input, wind forcing and solar radiation (Camp and Delgado 1987).

1.2. Phytoplankton in Alfacs Bay

Climatologic studies performed by Solé et al. 2009 showed a seasonal cycle in the bay due to climatic forcing. Solar radiation, for instance, is the major drive of the phytoplankton annual cycle, whereas its short-term variability is given by factors related to the water column motion. In other words, variability of the physical conditions is a major factor controlling the dynamics of the phytoplankton communities. Thus, understanding the coupling between environmental and biological variables is an important research topic in this productive area, and it will be a major objective of the present study.

In this line, most studies performed in Alfacs Bay have focused on microphytoplankton, especially diatoms and dinoflagellates, which have been
recorded over almost 20 years (IRTA monitoring program) using the Utermöhl (1958) method (e.g. Delgado et al. 2004; Fernández-Tejedor et al. 2008). This is highly due to the fact that several dinoflagellates are involved in recurrent harmful events that threaten aquaculture (Garcés et al. 1999). Those two groups show different ecological strategies as it was illustrated in the Margalef’s Mandala (Margalef 1978; Annex Fig. 1). In this conceptual frame, different phytoplankton biologic types occur as an adaptation of the species to a double gradient of nutrient concentration and turbulent kinetic energy (Margalef 1980). Hence, in temperate latitudes, dinoflagellates are supposed to be abundant in summer-autumn due to high radiation and warm temperatures, and are usually the most numerous group in stratified and nutrient poor waters (K strategy). This ability is due to their capacity for mobility. In contrast, diatoms follow an R strategy, they respond fast to high nutrient concentrations. Although they have mechanisms to diminish sinking (small size, morphology), turbulence is decisive for diatoms in order to avoid their sinking (e.g. Lalli and Parsons 1997, Vila and Masó 2005). Therefore, during summer we would expect to find dinoflagellate’s blooms as a property of these coastal enriched waters, while diatoms would be associated with winter and spring, where there is no stratification and relatively high nutrient concentration (Vila and Masó 2005).

However, there is no information in Alfacs Bay about the smaller components of the phytoplankton community, namely flagellates (or nanoflagellates: NF), that can play an important role along the succession of the different phytoplankton taxons, including the harmful species. NF population represents an important link in the microbial food web. Heterotrophic Nanoflagellates (HNF) are grazers, and they consume bacteria and picophytoplankton. This trophic relationship is an essential link in the transfer of carbon (C) to higher trophic levels. HNFs are also involved in nitrogen (N) regeneration through microheterotrophic activity. Autotrophic Nanoflagellates (ANF) carry out photosynthesis and that is why they are essential components among the primary producers (Safi and Hall 1997). We decided to include the NFs in this work because of the lack of information regarding this group in Alfacs bay so far.
1.3. Harmful Algal Blooms (HABs)

Alfacs bay, due to high nutrient loads and high water residence times, favors the development of phytoplankton communities. Sometimes, excess biomass causes ecosystem damage (water discoloration, water quality deterioration) because of the hypoxic conditions when decomposing. Some species produce potent toxins that can be bioaccumulated in shellfish, which in turn constitute a health risk to humans if consumed. These events often take place in confined waters. All in all, the exploitation of the Catalan coastline shows an increment in man-made sheltered waters resulting in more than 40 harbours along 400km of coast. Each of those harbours is a modified shallow ecosystem having reduced turbulence, high water residence times and high nutrient concentration, what means a suitable condition for the proliferation of phytoplankton, including harmful species (Vila et al. 2001). However, the dynamics of HABs is very complex as it involves interactions among physical, chemical, biological and ecological processes that operate at different spatio-temporal scales (Chang and Dickey 2008). In Alfacs several toxic species are harmful, and will lead to closure of the bay even if they are present at low concentrations. For example, *Dinophysis* spp. will cause the closure of the shellfish exploitation at only 100 cells·L$^{-1}$ and *Alexandrium minutum* at 100 cells·L$^{-1}$. Given the importance of these phenomena known as Harmful Algal Blooms (HABs) that cause both ecological and economic problems (GEOHAB 2001) it is so important investigating the general dynamics of phytoplankton, and in particular that of the harmful species.

1.4. Objective

The objective of this work was to improve our comprehension of the physico-chemical and biological conditions along the development of the phytoplankton community in Alfacs Bay in spring. In order to understand the variability and possible links among the environmental and biological processes, weekly samplings were performed from March to July within the context of the ECOALFACS project CTM2009-09581. Nevertheless, the present study focuses on the 31st May to 21st June period at a central station only. Our aim was to analyze the relatively small spatio-temporal scale
variability on the physico-chemical (temperature, salinity, inorganic nutrients) water properties and to explore whether this variability affected the vertical distribution and the temporal succession of the main phytoplankton groups. A particular attention was devoted to track the presence of harmful phytoplankton taxons, in order to understand whether the observed variability could explain their presence or absence in the studied period.

2. MATERIAL AND METHODS

2.1. Cruises

During spring 2011 four weekly cruises (May 31st, June 7th, June 14th and June 21st) were made at the Raft Station (40º 35'398 N, 000º 39'506 E), using an 8 m boat.

2.2. CTD Casts

A SAIV A/S SD204 STD (Annex Fig.2) was lowered manually from surface to the bottom (0 - 6 m approximately). The shallowest 0,2 m were not taken into account for data analysis, because the sensors may not be completely submerged. The CTD carried optional sensors for fluorescence, turbidity and oxygen. Unfortunately, the oxygen sensor did not work in the first week and the fluorescence sensor did not work in weeks 2 and 3. Data download and export was performed with Minisoft SD200W and graphs were created with Surfer 10.

2.3. Weather Conditions

For the weather description, in situ observations and meteorological data from the Servei Meteorològic de Catalunya (SMC) were combined. The SMC is stationed at the IRTA parking space, at 2 m height and gives continuous data, sampling every hour.

2.4. Sampling

Samples for the estimation of nutrients, chlorophyll concentrations and phytoplankton were collected every meter (from 0,5 to 5,5 m) with a low
pressure pump connected to a 12V battery. There was a hose for the direct transfer of the water samples to their transport containers. The bottles were immediately put into ice and in the dark to slow down their degradation. At a field laboratory, samples were further treated.

2.5. Nutrients

40 mL of sample were taken into sterile Falcon tubes and kept on ice and in the dark. Immediately upon arrival at the field station the samples were frozen at -20°C. The samples were then taken to the ICM’s Nutrient Analysis Service (Mara Abad) for the estimation of total nitrogen and phosphorous, nitrate, nitrite, phosphate, silicate and ammonium following EPA and ISO standard protocols (Grasshoff et al. 1999) using a Bran+Luebbe Autoanalyzer with Continuous Flow Analysis.

2.6. Chlorophyll

Samples of 250 mL were taken in polycarbonate bottles every meter (0.5m, 1.5 m, 2.5 m, 3.5 m, 4.5 m and 5.5 m) and kept dark and on ice for transport. Filtration was done manually through a Swinnex system (Annex Fig. 3) within 2 h after taking the sample. For the estimation of total chlorophyll a volume of 60 mL was filtered onto glass fiber filters (Whatman GF/F; nominal pore size 0.7 µm). To estimate the chlorophyll fractions smaller and bigger that 10 µm (equivalent diameter) 120 mL were filtered through polycarbonate filters with a pore size of 10 µm (PC 10 Millipore). Filters were stored in cryovials and frozen immediately in a -20°C freezer. Then, at the Barcelona laboratory they were stored at -80 °C. Chlorophyll analysis was conducted using a Turner Designs Fluorometer (Annex Fig. 4), following the protocol of Yentsch and Menzel (1963) (in Parsons et al 1984). Samples were passively extracted in 6 mL 90% acetone in the dark, in capped 10 mL polypropylene vials, at 4 °C for 24h. For each reading light intensity was regulated manually to a signal scaled in between 2 and 8 (Annex Fig. 5).
We calculated the amount of total Chlorophyll $a$ in $\mu \text{g} \cdot \text{L}^{-1}$ with the following formula:

\[
\text{Pigment [}$\mu\text{g/L}$\text{]} = \frac{\text{fluorescence reading - blank}}{\text{fluorometer scale}} \times \text{calibration factor} \times \frac{\text{acetone volume}}{\text{filtered volume}} \times \text{dilution} \times 1000
\]

The calibration factor allows the transformation of fluorescence units to concentration.

### 2.7. Microphytoplankton

We used dark glass bottles for these samples. The bottles were already cleaned and contained 2 mL of Hexamine buffered formaldehyde as fixative. The bottles were filled with 120 mL of sample, directly from the hose on board, as transport has shown to greatly influence the results of cell counts in previous studies (M.L. Artigas, pers. comm.), and kept on ice. The phytoplankton counting was carried out by the taxonomist and Alfacs bay specialist, Dr. M. Delgado. To identify and quantify phytoplankton cells the Utermöhl method was used (Utermöhl 1958). 50 mL subsamples were concentrated by settling in cylindric chambers for 24 h. Cell counts were performed in the appropriate area of the cuvette following the criteria indicated in e.g. Andersen and Throndsen 2003 using an inverted Zeiss Axiovert microscope. The minimum species abundance detected with this approach is 20 cells·L$^{-1}$.

In order to explore the degree of similarity or distance among the phytoplankton communities sampled every week, we used PERMANOVA (PERmutational Multivariate ANalysis Of VAriance), applying the software PRIMER 6, version 6.1.13 including the PERMANOVA+ add-in, version 1.0.3 (PRIMER-E Ltd) for multivariate analysis. A 2-factor PERMANOVA with 9999 permutations was carried out with weeks as fixed factor and depth as random factor. We used another PERMANOVA, applying the same settings, as post hoc test for pair-wise comparisons of the 4 different weeks. For the visualization of the data we chose MDS (Multi-Dimensional Scaling) plots into which we included the main vectors driving the distribution. Distances for the
resemblance matrix of both the PERMANOVA and the MDS plots were estimated with Canberra metric (Konrad et al. 2012). Prior to analysis we pretreated the data as such: coccolithophorids were joined into two main groups, smaller and bigger than 10 µm; all taxons that appeared in less than 20% of the samples as well as the counts for nanoflagellates were excluded; the high abundances and low resolution of nanoflagellates that are reached with Utermöhl would otherwise result in a strong bias of the PERMANOVA. We included zooplankton data in our analysis, summarized into the following taxons: ciliates, tintinnids, nauplia, copepods, bivalve larvae and gastropod larvae. We assumed each week to be independent and to consist of 6 independent samples (one per depth), disregarding the eventual temporal and spatial connectivity of the samples.

2.8. Nanophytoplankton and Cyanobacteria

Nanoflagellates and cyanobacteria enumeration was conducted using the 4',6-diamidino-2-phenylindole (DAPI) technique (Kapuscinski 1995), a DNA specific fluorochrome. Water samples were transported in polycarbonate bottles, kept dark and on ice. At the field station, 30 mL of sample were added into sterile 50 mL falcon tubes containing 3 mL of 10% glutaraldehyde for fixation. Glutaraldehyde will perforate cell membranes, thus facilitating the probe’s entrance into the cell, and will preserve chlorophyll’s autofluorescence (Crumpton 1987). Samples were stained and filtered within 24 h after fixation (in the Barcelona laboratory).

On the filtration ramp black filters (Whatman Grey B 110659 polycarbonate 25 mm diameter, 0.8 µm pore size) were placed (glossy side showing upwards) upon another filter (Millipore RAWPO2500, Type RA filters, white, 25 mm diameter, 1.2 µm pore size) that allowed an even distribution of the filtration pressure. For the staining, 20 mL of sample were filtered at low pressure (max. 2 mbar). Then the valve was closed and the last 10 mL of sample were added together with 3 µL of DAPI. After a 5 minutes staining, the valve was re-opened and the last 10ml of sample were filtered. The filters were then transferred to pre-cleaned and pre-labeled glass slides using a Millipore stainless steel feather forceps. Finally the samples were put into
non-transparent slide boxes and frozen at -80 ºC until analysis under the epifluorescence microscope (OLYMPUS BX40). A x100 objective lens and x10 ocular were employed with the aid of an immersion oil drop (OLYMPUS immersion oil Type-F) above the cover slide. We used different light filters, that is to say, different wavelengths, corresponding to ultraviolet and blue light. DAPI has an absorption maximum at a wavelength of 358 nm (ultraviolet) and its emission maximum is at 461nm (blue).

For counting, the main criteria were the distinction between HNF and ANF, separated into two different size classes: bigger and smaller than 5 µm. If possible, the ANF were further distinguished into dinoflagellates, *Phaeocystis*-like organisms, *Micromonas*-like organisms, and two further prominent organism groups labeled as “suns” and “big sperm-like” organisms, because its taxonomic identification was not possible (Fig. 2). Centric and pennate diatoms were also counted and separated into two size classes: centric diatoms bigger and smaller than 5 µm, pennate diatoms bigger and smaller than 10 µm. Cyanobacteria (mostly *Synechococcus*) were also identified. However, these countings will not be considered here. Parallel samples were taken to be estimated by flow cytometry, a more appropriate method, and will be part of future of further studies. For each sample two transects of one millimeter were counted. If the amount of ANFs per sample was lower than 50 cells, another millimeter was counted. The following formula was used for obtaining concentration values (cells·L⁻¹):

\[
\text{Cell·L}^{-1} = \left(\frac{33}{30}\right) \times \frac{\text{cells} \times F}{\text{filtered vol.} \times \text{num. of transects}} \times 1000
\]

\[
F = \frac{\pi R^2}{\text{transect surface}}
\]

where \((33/30)\) refers to the dilution factor due to the fixative and \(F\) applies the factor relating the filtered surface \((\pi R^2, \text{where } R \text{ is the radius of the filter})\) and the counted transect's surface.
Fig. 2. Epifluorescence microscope (OLYMPUS BX61) images of DAPI in ultraviolet (A₁ and B₁) and blue light (A₂ and B₂) illuminating the chloroplasts. A (from left to right): two “Micromonas-like”, one heterotrophic nanoflagellate (HNF) and a “Phaeocystis-like organism. B (from the top): a “Phaeocystis-like”, a HNF and a Chaetoceros sp.

3. RESULTS

3.1. CTD casts and weather conditions

CTD casts revealed that the water column was initially well mixed, subsequently stratified during the 2nd and 3rd weeks, and mixed again in the last sampling. Indeed, the temperature, salinity and turbidity profiles showed a well-mixed water column in weeks 1 and 4 (Fig. 3), though salinity showed a slight increase with depth (a difference of 1 - 1.5 ‰ from surface to bottom). Both weeks showed similar salinity and turbidity ranges, 35 - 37 ‰ and 0 - 10 FTU (Formazin Turbidity Units), respectively. However, temperature increased between week 1 (21.5 to 22 °C) and week 4 (25 to 25.5 °C). In weeks 2 and 3, the water column was stratified, with the thermo- and
halocline located at 4 m depth. The cast showed a depth-dependent increase of salinity and turbidity, and a temperature decrease. The variability of the values of the different parameters from the surface to the bottom ranged from 25.5 to 22.5 °C for temperature, from 34.5 to 37 ‰ for salinity and from 8 to 50 FTU for turbidity (Annex, Fig. 6).

Both the observed and recorded weather conditions showed high variation over the four weeks (Annex Table 1). The night before the cast of the first week, it was cold and stormy, as the Mestral (NW wind) with a force around 5.2 m·s⁻¹ blew. Weeks 2 and 3 presented calm and sunny weather, barring a 4.9 m·s⁻¹ force Mestral, two hours before the cast of week 2. In the fourth week the weather was cloudy and cold, with a breeze around 5 m·s⁻¹ and a strong water current (data not shown).

It is also important to note that in weeks 1 and 4 it was noticed the presence of a bloom of the ctenophora Mnemiopsis leidyi.
3.2. Nutrients and Chlorophyll

Phosphate was the nutrient with the lowest concentrations over the four weeks, usually with values below or around 0.1 µmol·L⁻¹, distributed homogeneously in the water column. The values for silicate were higher, with a marked increase from week 1 to week 4. Its distribution was relatively homogeneous over depth in weeks 1 (between 2.5 - 3.0 µmol·L⁻¹) and 4 (6 - 9 µmol·L⁻¹), while in weeks 2 and 3 it presented a maximum at 3.5 m and 4.5 m, respectively. Ammonium concentrations declined from maximum values of 2 µmol·L⁻¹ in the first two weeks to reach lower concentrations (almost 0 µmol·L⁻¹ in week 3 and around 0.7 µmol·L⁻¹ in week 4) during the rest of the sampling period. Nitrate and nitrite behave similarly to ammonium as well: higher concentrations in the first two weeks (with the highest values on the surface layer around 3-3.5 µmol·L⁻¹), reaching almost undetectable values in the last two weeks (Fig. 4).

Along the studied period, most chlorophyll was contributed by phototrophic organisms smaller than 10 µm (Fig. 4) and showed a similar vertical distribution: chlorophyll concentration increased from surface to 4.5 m depth, where the chlorophyll maximum was situated, except for week 1, which showed the maximum at 5.5 m. Unfortunately, some chlorophyll samples were lost on week 3 due to technical problems (Fig. 4).
Fig. 4. Chlorophyll a concentrations (total, smaller and bigger than 10 µm) versus nutrient concentrations (nitrate+nitrite, ammonium, silicate and phosphate) week per week over depth.
3.3. Microphytoplankton

Regarding the general composition and trends of the phytoplankton community, NFs were markedly the group with the higher abundances both over time and depth (Fig. 5). Note that in the first week it was not possible to count the sample from 5.5 m depth due to floculation. Dinoflagellates and diatoms decreased from week 1 to week 4, barring the diatom’s increase in the second week. However, NFs followed an increasing trend during weeks 1, 2 and 3. Then their peak, which moved deeper over time, decreased in week 4.

Looking to each phytoplankton group more specifically (Fig. 6), it can be seen that dinoflagellates were specially abundant (values between $3.08 \times 10^4$ and $1.23 \times 10^5$ cells·L$^{-1}$) in the upper layer (2.5-3.5 m) in the first week, decaying during weeks 2 and 3 and slightly increasing again in week 4 at deeper layers (around 4.5 m). Diatoms, compared to the other three groups, presented an homogeneous distribution over depth. For this group, the highest values ($9.03 \times 10^5$ cells·L$^{-1}$) occurred in week 2 around 3.5 m depth, decreasing from then on, reaching pretty low abundances (a minimum of $8.05 \times 10^4$ cells·L$^{-1}$) in the last week at the deeper layers. Coccolithophorids were mainly found in the upper layers and they were the group with the lower abundances. The first two weeks they presented values always below $1.1 \times 10^4$ cells·L$^{-1}$ and were homogeneously distributed throughout the water column. Then, they increased in the 3rd sampled week, reaching their maximum concentration of $5.28 \times 10^4$ cells·L$^{-1}$ at 1.5 m, and decreased thereafter. Finally, nanoflagellates, always with the highest concentrations both over time and depth, presented the highest abundances below the picnocline (around 4 m). The lower concentrations were found in the upper layers, with a minimum value of $3.01 \times 10^6$ cells·L$^{-1}$ at 0.5 m in the second week. The peak was observed at 4.5 m in week 3, $1.97 \times 10^7$ cells·L$^{-1}$. 
Fig. 5. Abundances of the four main phytoplankton groups over time and depth. Dinoflagellates, diatoms and coccolithophorids counted with the Utermöhl method; and nanoflagellates counted using the DAPI technique. A, B, C and D refer to week 1, 2, 3 and 4, respectively.
Fig. 6. Variability of the abundance and vertical distribution of the four main groups of phytoplankton.
The PERMANOVA analysis showed that the variability of the phytoplankton distribution was highly influenced by time (i.e. the sampled week) and depth (p<0.0001 and p=0.0092, respectively). When exploring the particular influence of the sampled depths, as the 6 categories for depth resulted in a loss of 5 degrees of freedom further analysis did not give additional information due to lack of statistical power. However, the MDS graph (Fig. 7) revealed that samples from 4.5 and 5.5 m were grouped somehow apart (towards the center) from the rest of the samples (towards the periphery). The pair-wise test revealed that each week significantly differed from the others (Table 2). The main vectors for week 1 were Protoperidinium diabolus and P. oblongum, Pseudo-nitzschia spp. (potentially Amnesic Shellfish Poisoning – ASP-producer) and small dinoflagellate cysts; for week 2, small, unidentified dinoflagellates and for week 3, heterotrophic Gyrodinium spp. and Gymnodinium elongatum. Week 4 was characterised by coccolithophorids smaller than 10 µm and Pseudo-nitzschia sp.1. Using Utermöhl, it was not possible to identify Pseudo-nitzschia to species level, yet this species could clearly be distinguished from the whole Pseudo-nitzschia spp. group.

Fig. 7. MDS plot showing the Canberra metric dissimilarities between samples; labels refer to depth of sample. Vector’s represent variables with most influence on sample distribution. Vector length represents influence of taxons to dissimilarity.
Regarding the potentially harmful phytoplankton species, the following taxa were seen along our studied period. The dinoflagellates *Alexandrium minutum*, several species of *Dinophysis*, *Gonyaulax polygramma*, *Gonyaulax spp.*, *Gymnodinium sanguineum*, *Karlodinium spp.*, *Lingulodinium polyedrum* and *Protoceratium reticulatum*. And the diatom *Pseudo-nitzschia spp*. Barring *Karlodinium spp.* and *Pseudo-nitzschia spp.*, all of the observed harmful species presented low concentration values (between 20 and 600 cells·L\(^{-1}\)). *Pseudo-nitzschia spp.* decreased from more than 50000 cells·L\(^{-1}\) in the 2\(^{nd}\) week to a mean value of 300 cells·L\(^{-1}\) in the last week. And finally *Karlodinium spp.* presented a maximum concentration of 77000 cells·L\(^{-1}\) in week 1, but this specie is not toxic below 100000 cells·L\(^{-1}\). Thus, regarding these results, we did not take into account anymore for this study.

### 3.4. Nanophytoplankton and Cyanobacteria

Among the nanophytoplankton, NFs showed the highest abundances. ANF smaller than 5 µm were the most abundant group during the four weeks in all 6 depths. They followed a similar pattern over depth in all weeks, with the minimum concentration at surface and the maximum at or below the chlorophyll maximum. These concentration maxima for each week were, respectively: \(1.1 \times 10^7\) cells·L\(^{-1}\) at 5.5 m, \(6.9 \times 10^6\) cells·L\(^{-1}\) at 3.5 m, \(1.6 \times 10^7\) cells·L\(^{-1}\) at 4.5 m and \(9.6 \times 10^6\) cells·L\(^{-1}\) at 4.5 m. The NFs bigger than 5 µm, both auto- and heterotrophic, appeared in lower abundances: HNF with concentrations between \(4.2 \times 10^4\) cells·L\(^{-1}\) - \(2.5 \times 10^5\) cells·L\(^{-1}\) and ANF

---

**Table 2.** p-Values for pair-wise PERMANOVA.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 2</td>
<td>0.0243</td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>0.0081</td>
</tr>
<tr>
<td>1 vs. 4</td>
<td>0.0073</td>
</tr>
<tr>
<td>2 vs. 3</td>
<td>0.0089</td>
</tr>
<tr>
<td>2 vs. 4</td>
<td>0.0037</td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>0.0116</td>
</tr>
</tbody>
</table>
between $1.2 \times 10^5$ cells·L$^{-1}$ and $1.1 \times 10^6$ cells·L$^{-1}$. They did not follow a clear pattern over depth.

Fig. 8. Abundance and distribution of nanoflagellates (ANF and HNF bigger and smaller than 5µm) along the water column. A, B, C and D refer to week 1, 2, 3 and 4 respectively.

4. DISCUSSION

Our results showed a variable physical dynamic. The mixed water column in weeks 1 and 4 was probably caused by strong wind events. In weeks 1 and 4, strong Mestral and Garbí, wind events, respectively, probably caused the mixing of the water column. As there was no significant rain event in any of those weeks, the salinity differences were mainly due to freshwater inputs from the northern edge caused by rice cultivation. These findings are in accordance with the findings of e.g. Camp (1994) and Solé et al (2009). We expected that turbidity could match somehow the observed mixing-
stratification patterns. Turbidity is caused by the different particles suspended in the water column, including phytoplankton and particles from fresh-water run-off of the rice fields, which, close to the shore, drain directly into the bay. We would have expected turbidity to be higher in the weeks with a mixed water column than in the weeks with stratification, because the shallow water column should favor mixing with the muddy bottom of the bay. However, the maximum turbidity values for weeks 1 and 4 were, approximately, the minimum ones for weeks 2 and 3. It might be that there was more “clear” Mediterranean water coming into the bay and/or that biological filtration was enhanced. The main filter feeders in the bay are the cultivated mussels, but in those weeks a *Mnemiopsis leidyi* bloom was observed (Neszi, personal communication), an invasive ctenophora that has supposedly established its’ whole live cycle in the bay (Fuentes, Angel et al. 2010).

Nutrient concentrations were low and consumed during the studied period, except for silicate, which increased. Somehow, the values were even lower than expected. This could be the result of the consumption by phytoplankton in the previous weeks of our samplings. This hypothesis will be considered within the ECOALFACS project, as it covered a more extensive sampling period (March to July). Regarding the particular elements, previous investigations (Krom et al. 1991; Estrada et al. 1993) point to phosphorous as the main limiting nutrient in the NW Mediterranean, demonstrating that the decline of the late winter bloom is caused by the depletion of dissolved inorganic nutrient, particularly phosphorous (Duarte unpublished in Mura et al. 1996). Indeed, the Redfield ratio (Redfield 1934) in our samples N:P>>16 (not shown) corroborates this limiting role of phosphorous in Alfacs bay as also suggested by previous studies (Vidal 1994). P seems to be present also in low concentrations in the fresh water of the discharge channels (Artigas et al. submitted), indicating that it can also be retained on the rice fields (Llebot et al. 2010).

Interestingly, silicate accumulated from week 1 to 4. This trend could be related to the lack of silicate consumption by diatoms that decayed concurrently during this period (Annex Fig. 7). The decay of diatoms can be in part due to an overcompetition by nanoflagellates, as they are in high
abundances. Those organisms could uptake ammonium, a component that indicates nutrient regeneration within both, the planktonic and the benthonic microbial communities (Vidal et al. 1992).

The lack of increase in the chlorophyll concentration over time is coherent with the low nutrient concentrations discussed above. The fact that most part of the chlorophyll was contributed by phototrophic organisms smaller than 10 µm is consistent with a likely utilization of recycled nutrients as well. It can be also noted that the partitioning of chlorophyll agrees well with the phytoplankton counts obtained by the two used methods, i.e. Utermöhl (1958) and DAPI (Kapuscinski 1995) methods (Fig. 5).

The observed succession of the phytoplankton community (Fig. 6) could be the following. Our period of study would start with a diverse community, in which coccolithophorids are at almost undetectable limits, but dinoflagellates, diatoms and nanoflagellates coexist. In the second week, while dinoflagellates start to decrease, it can be seen a peak on the diatom's community. We could speculate that this increase was driven by the observed peak of silicate in that week at the same depth (Fig. 4). Towards week 3, diatoms decreased notably suggesting the end of what could have been their spring bloom, which is consistent with the observed increase in silicate's concentration (Fig. 4), as it is not being consumed. Contemporaneously, coccolithophorids proliferated, agreeing with Margalef's Mandala (Annex, fig.1), in which the succession for these groups would be: diatoms, coccolithophorids and dinoflagellates, as this last group proliferated again in the last week. The high concentration of NFs in the 3rd week at 4.5 m coincided with a little peak of ammonium, suggesting that they could be proliferating from recycled nutrients. It could be hypothesized that the growth of dinoflagellates in the last week could be given by the high abundance of NFs the week before, although we could not discard that they could be different water bodies. Taking all of this into account, the succession of the future will depend on the diatom's competitive capacity versus the NFs, since if these ones uptake the P, the diatoms will not be able to proliferate even with high concentration of silicate. So they would have to wait until NFs' predator outcompete them (such as dinoflagellates or high abundances of HNFs)
Our MDS plot corroborates the different succession of groups along the 4 weeks. The plot suggests cysts of dinoflagellates, *Protoperidinium oblongum*, *Protoperidinium diabolus*, *Pseudo-nitzschia* spp., *Pseudo-nitzschia* sp1 and cocolithophorids smaller than 10 µm to be the main drivers for the differences in between the weeks. This is probably related to the dynamics of the water column: the weeks more influenced by the presence of the diatom *Pseudo-nitzschia* are the first and the fourth ones, agreeing with a mixed water column. According to the conceptual model of Margalef's Mandala (1978) (Annex, Fig. 1), high turbulence favors diatoms' proliferation as this motion avoids their sinking. Our analysis suggests that week 1 was also influenced by the presence of the dinoflagellate *Protoperidinium*. Since we don't have data for what happened before, we assume that this was due to the transition between a stratified to a mixed water column. Dinoflagellates are known to be favoured in a stratified water column, as the presence of flagella gives them the capacity of movement and hence the power to follow light and nutrient gradients relevant to their growth (Estrada and Berdalet 1997).

Significant differences in between depth could be observed when used as a random factor in the overall PERMANOVA. Nevertheless, it is far fetched to state that differences in depth are truly significant, due to the lack of statistical power of our analysis. Regardless, our MDS plot indicates that the samples of 4.5 m and 5.5 m are separated from the samples above, especially in the stratified weeks. It seems logical that the weeks with the mixed water column did not show those differences as clearly as the stratified weeks did. The bottom layers were characterized by the presence of benthic microalgae, mainly diatoms, from the resuspended sediments. In weeks 2 and 3 the pycnocline was located around 4 m depth, as revealed in previous studies (e.g. Camp & Delgado 1987; Artigas et al. submitted). As expected, the organisms that marked that distribution along depth were the dinoflagellates, most likely favored under calm water conditions because their ability to swim. It is important to note however that, Alfac's bay, even during mixing periods the turbulent kinetic energy dissipation rates were low and compatible with the swimming speeds of dinoflagellates (Artigas and Berdalet, pers. comm.). Due to the low number of replicates, and since our data are not normally
distributed, a Principal Component Analysis (PCA) approach, which is often used to evaluate phytoplankton data (e. g. Llebot et al. 2011), was impossible. We are aware that our four weeks of study are not independent samples, as they are continuous in time; neither are our depths independent from each other in space. Yet, our analysis provides an important insight to the phytoplankton dynamics in the bay: high short-term temporal and spatial variability of the phytoplankton community in the bay, as also noted elsewhere (e.g. Artigas et al. submitted).

A really interesting trend could be observed in our graphical analysis: a clear trophic relationship between ANFs and HNFs (Fig. 8). It can be perfectly seen the trend they both follow: HNFs, always in lower abundances, show the same variability pattern over depth as the ANFs, they preys. This is the first study documenting the spatio-temporal changes in the abundances of these organisms. New questions can now be formulated regarding their importance in the microbial web structure and dynamics in Alfacs Bay. This is already an undergoing research in our group.

5. CONCLUSIONS

- The water column presented a high short-term variability given by physical drivers (such as wind events, radiation, freshwater discharges). It started well-mixed the first week, stratified in weeks 2 and 3 and mixed again in week 4.

- Really low chlorophyll a amounts were observed, suggesting a limitation driven by nutrients. This was supported by the low and decreasing nutrients’ concentrations from week 1 to week 4, except for silicate, which increased towards the last week due to the lack of consumption by diatoms.

- The observed phytoplankton succession was given by turbulence and nutrient availability (agreeing with Margalef’s Mandala) and also by competition between groups. The whole four weeks were notably dominated by nanoflagellates. These small organisms coexisted with diatoms and dinoflagellates the first half of the studied period, but as nutrient went
decreasing and the flagellates went increasing, they outcompeted the other organisms.

- There was an evident trophic relationship between heterotrophic (predator) and autotrophic (prey) nanoflagellates.

- Several potentially harmful species were detected, but since they were at really low concentrations, they were not taken into account.
6. REFERENCES


ANNEX
This section includes images and some data that complement the study presented here.

Fig. A. Modified Margalef’s mandala (Margalef, 1978). Graphic representation of the major phytoplankton life forms in an ecological space defined by nutrient concentration and turbulence.

Fig. B. SAIV A/S SD204 STD, used in ECOALFACS project’s cruises. Photo: Laia Viure.
Fig. C. Swinnex system used for filtering the samples for chlorophyll analysis.

Fig. D. Turner Designs Fluorometer used for the estimation of chlorophyll $a$.

Fig. E. Turner Designs Fluorometer’s scale.
Fig. F. Vertical profiles for temperature, salinity and turbidity for each week. Data given by the CTD casts.
Table 1. Weather conditions from the Servei Meteorològic de Catalunya and the in situ observations influencing the CTD cast.

<table>
<thead>
<tr>
<th>Week</th>
<th>Time</th>
<th>Wind vel. [m/s]</th>
<th>Wind dir. [°]</th>
<th>Temp. [°C]</th>
<th>Rel. humidity [%]</th>
<th>Precipitation [mm]</th>
<th>Radiation [W/m²]</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12h before</td>
<td>21:00</td>
<td>2.8</td>
<td>340</td>
<td>18.8</td>
<td>48</td>
<td>0</td>
<td>Night before sampling: cold and stormy. Presence of breeze (Mestral).</td>
</tr>
<tr>
<td></td>
<td>6h before</td>
<td>3:00</td>
<td>5.2</td>
<td>334</td>
<td>14.7</td>
<td>49</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2h before</td>
<td>7:00</td>
<td>4.1</td>
<td>341</td>
<td>16.8</td>
<td>43</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>downcast</td>
<td>9:40</td>
<td>3.9</td>
<td>359</td>
<td>18.6</td>
<td>40</td>
<td>0</td>
<td>767</td>
</tr>
<tr>
<td>2</td>
<td>12h before</td>
<td>23:00</td>
<td>3.3</td>
<td>330</td>
<td>17.9</td>
<td>52</td>
<td>0</td>
<td>Calm weather with a little rain.</td>
</tr>
<tr>
<td></td>
<td>6h before</td>
<td>5:00</td>
<td>2.5</td>
<td>335</td>
<td>15.8</td>
<td>51</td>
<td>0</td>
<td>At midnight there was little Mestral, but not too strong.</td>
</tr>
<tr>
<td></td>
<td>2h before</td>
<td>9:00</td>
<td>4.9</td>
<td>343</td>
<td>20.8</td>
<td>43</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>downcast</td>
<td>11:26</td>
<td>1.1</td>
<td>181</td>
<td>22.0</td>
<td>51</td>
<td>0</td>
<td>935</td>
</tr>
<tr>
<td>3</td>
<td>12h before</td>
<td>22:00</td>
<td>1.2</td>
<td>187</td>
<td>22.3</td>
<td>72</td>
<td>0</td>
<td>Sunny, hot and calm.</td>
</tr>
<tr>
<td></td>
<td>6h before</td>
<td>4:00</td>
<td>1.6</td>
<td>340</td>
<td>19.3</td>
<td>91</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2h before</td>
<td>8:00</td>
<td>0.6</td>
<td>56</td>
<td>23.6</td>
<td>70</td>
<td>0</td>
<td>644</td>
</tr>
<tr>
<td></td>
<td>downcast</td>
<td>10:42</td>
<td>2.5</td>
<td>125</td>
<td>24.4</td>
<td>70</td>
<td>0</td>
<td>871</td>
</tr>
<tr>
<td>4</td>
<td>12h before</td>
<td>21:00</td>
<td>5.0</td>
<td>216</td>
<td>22.7</td>
<td>33</td>
<td>0</td>
<td>Cloudy, cold and a little breeze.</td>
</tr>
<tr>
<td></td>
<td>6h before</td>
<td>3:00</td>
<td>2.4</td>
<td>194</td>
<td>22.0</td>
<td>83</td>
<td>0</td>
<td>Really strong current both the day before sampling and the day of sampling.</td>
</tr>
<tr>
<td></td>
<td>2h before</td>
<td>7:00</td>
<td>1.1</td>
<td>159</td>
<td>23.5</td>
<td>78</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>downcast</td>
<td>8:57</td>
<td>1.6</td>
<td>152</td>
<td>24.3</td>
<td>75</td>
<td>0</td>
<td>688</td>
</tr>
</tbody>
</table>

G. Vertical variability of diatoms’ abundance and distribution along the studied period.