SEASONALITY OF PICO-PHYTOPLANKTON ABUNDANCE AND CELL DEATH IN A MEDITERRANEAN BAY (BAY OF PALMA, MAJORCA ISLAND)

P. ALONSO-LAITA¹*, N. NAVARRO², C. M. DUARTE¹, S. AGUSTÍ¹

¹Institut Mediterrani d’Estudis Avançats IMEDEA (CSIC – UIB), Miquel Marqués, 21, 07190 Esportes, Mallorca, Spain
²Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Tulipán sn 28933 Móstoles, Madrid, Spain
*corresponding author: patricia.alonso@uib.es

ABSTRACT. – A pico-phytoplankton community, dominated by Synechococcus sp., was present throughout the year in the Bay of Palma, showing high dynamics, with changes across the Bay observed at a scale of a week. The populations of pico-phytoplankton showed high seasonality. Synechococcus sp. abundance was maximal during summer, with an important bloom observed at mid August. Prochlorococcus sp. showed three moderate blooms at spring, summer, and a larger one in winter. Pico-eukaryotes abundance varied seasonally, showing the highest cell concentration by mid April. Despite these differences in cell abundance, the three pico-phytoplanktonic groups showed similar patterns in the percentage of living cells, with peaks in late winter and in early summer, corresponding to the major peaks in nutrient concentration in the Bay. Similarly, the lowest % living cells was observed at late spring, indicative of high pico-phytoplankton cell death when nutrient concentration decreased markedly. The high pico-phytoplankton cell viability of late winter, was followed by moderate increase in cell abundances indicating that factors other than nutrients were controlling pico-phytoplankton populations. Despite the reduced pico-phytoplankton cell death at early summer, only Synechococcus sp. developed a sizeable bloom, indicating that summer conditions were more favourable for Synechococcus sp. as compared to other groups. Cell viability helped to elucidate the role of losses vs. stresses in controlling the population dynamics of pico-phytoplankton communities in the Bay of Palma.

INTRODUCTION

Pico-phytoplankton, including Synechococcus sp., Prochlorococcus sp. and pico-eukaryotes, are the dominant autotrophic components of the oligotrophic ocean and are important in Mediterranean coastal areas (Vaulot et al. 1990, 1996, Agawin et al. 1998, Jacquet et al. 1998). Phytoplankton productivity and biomass shows a seasonal variability in the coastal NW Mediterra-
nean Sea characterized by an important late winter bloom (Margalef & Castelví 1967, Margalef & Ballester 1967, Estrada 1979, Duarte 1999), and the dominance of pico-phytoplankton in the summer (Mura et al. 1996, Vaulot 1990). The seasonal variability of phytoplankton in coastal Mediterranean areas is also influenced by the summer influx of tourists, which typically generates a 2- to 10-folds increase in population of the coastal towns (Duarte et al. 1999), resulting in increased inputs of nutrients associated to the water discharges to the sea.

Changes in the abundance and composition of phytoplankton communities are the combined result of varying growth and losses of the phytoplanktonic groups forming the community in response to the varying environmental conditions (e.g. Harris 1986). Analysis of the distribution and changes in pico-phytoplankton communities have been generally focused on the role of growth capacities (e.g. Partenski et al. 1996, Vaulot et al. 1996) neglecting the potential role of losses and differential sensitivity to stress as structuring processes. Yet, recent studies have identified differential cell death across taxa to be a major factor structuring phytoplankton communities in the Mediterranean littoral (Agustí & Sánchez 2002) and Atlantic Ocean (Agustí 2004). These studies showed that phytoplankton lysis rates experienced high seasonally in the Mediterranean littoral (Agustí & Duarte 2000), and that the proportion of living cells of the phytoplanktonic community varied during the summer among the taxa forming the communities, including pico-phytoplankton (Agustí & Sánchez 2002).

In this study, we examined the distribution and seasonal variability of the pico-phytoplankton community in the Bay of Palma, an oligotrophic Bay located in Majorca Island (Balearic Islands, NW Mediterranean Sea). We also quantified the proportion of living cells for the different groups forming the pico-phytoplankton community in an attempt to elucidate the importance of environmental conditions and the different cell death of the pico-sized groups in explaining the seasonal patterns of the pico-phytoplankton community observed in the Bay.

**METHODS**

Sampling was conducted in the Bay of Palma, a large (mean depth 31 m, 19.3 km across, 215.8 km²) oligotrophic bay. The bay contains an important human population, concentrated in the city of Palma de Mallorca (~360 000 inhabitants) and a seasonal maximum population of approximately 500 000 inhabitants in summer.

Sampling was conducted at Bahia station (39° 31.8’ N, 2° 36.60’ E) located at the littoral of the Bay, with an interval of 15 days to a month between the sampling events, from October 2001 to October 2002. Integrated (0 to 7 m) water samples were collected with a vertical 7 m long tube to deliver the contents into acid-washed carboys (Navarro et al. 2004). Pico-phytoplankton abundance and cell viability (cell digestion assay, Agustí & Sánchez 2002) were analyzed in fresh sub-samples of the integrated water by flow cytometry as indicated below.

Two cruises were performed during March 2002 (EUBAL-I) and June 2002 (EUBAL-II), on board the RV Tethys and RV Mytilus respectively, to examine patterns of spatial variation in pico-phytoplankton distribution and other properties of the Bay of Palma. During the two cruises, water samples were taken by using Niskin bottles attached to a CTD-Rossette system. Two surveys, involving the surface sampling at 5-11 stations, were made during EUBAL-I (surveys on 7 and 11 March 2002) and EUBAL-II (surveys on 21 and 26 June 2002). All the surveys included the sampling at Bahia station (39° 31.8’ N, 2° 36.60˚E), and at two other reference stations (Posidonia station (39° 30’ N, 2° 32.3’ E) and Cap Enderrocat station (39° 21’ N, 2° 44.4’ E), Gazeau et al. 2005). Water samples for pico-phytoplankton cell abundance distribution were fixed with glutaraldehyde (1% final concentration), frozen in liquid nitrogen and stored at –80°C until analyzed at the laboratory using flow cytometry.

For both the temporal study and the surveys, 15 ml sub samples were taken for nutrient (phosphate and nitrate+nitrite) determination, and stored frozen until colorimetric analysis by standard auto-analyzer methods (Grasshof et al. 1999).

Samples of 200 mL were filtered through Whatmann GF/F filters to estimate total chlorophyll a concentration (chl a). Chl a was measured fluorometrically (Turner Designs fluorometer) in 90% acetone extracts of filters preserved frozen following Parsons et al. (1984).

The proportion of living and dead cells in the populations of the pico-phytoplankton communities was quantified using the cell digestion assay (Agustí & Sánchez 2002). The cell digestion assay was applied to duplicate samples, by adding 100 µl of DNAse I solution (400 µg ml⁻¹ in HBSS (Hanks’ Balanced Salts)) to 0.5 ml water sample, followed by incubation for 15 min at 36°C. After this time, 100 µl of Trypsin solution (1% in HBSS) were added, followed by incubation for additional 30 min at 36°C. At the end of the incubation samples were placed in ice to stop the reaction and analysed by flow cytometry. Parallel to this analysis, fresh (or fixed with glutaraldehyde from the surveys) duplicated samples from each sampling event were analyzed by flow cytometry to quantify the total abundance of pico-phytoplankton cells.

Samples for the determination of pico-phytoplankton total cell abundance (untreated samples) and living cells (after the cell digestion assay) were counted in duplicate on a FACSCalibur flow Cytometer (Beckton Dickinson). An aliquot of a calibrated solution of 1 μm diameter fluorescent beads (Polysciences Inc.) was added to the samples as an internal standard for the quantification of cell concentration. The fluorescence scattering signals of the cells and beads were used to detect different populations and to differentiate them from the fluorescent beads (Marie et al. 1999).

The cells counted after the cell digestion assay represent the living cells in the community, whereas the cells
counted in untreated samples represented the total population (living and dying cells). The percentage of living (or viable) cells was calculated as the ratio between the concentration of cells after the enzyme digestion, and the cell concentration of untreated samples, which represent the total (dead plus living) cell population.

RESULTS

The Bay of Palma showed high variability in nutrient concentration associated with the seasonality of the Bay as indicated by the changes in water temperature (Fig. 1). Nitrate+Nitrite concentrations were higher during the wintertime, with the maximum concentration detected in February, and showing lower values during the summer when the lowest concentration was detected (0.3 µM, Fig. 1). Phosphate concentration however, showed low values throughout the year, increasing during the summer time and in fall (Fig. 1). Chlorophyll a concentration varied also seasonally, showing a peak in late winter and a second peak in summer (Fig. 1).

The pico-phytoplankton in the Bay of Palma was composed of populations of *Synechococcus* sp., *Prochlorococcus* sp. and pico-eukaryotes, with a year averaged abundance of $6.59 \pm 2.20 \times 10^4$, $1.66 \pm 0.30 \times 10^4$, and $6.51 \times 1.34 \times 10^3$ cells ml$^{-1}$ (mean ± SE) for *Synechococcus* sp., *Prochlorococcus* sp. and pico-eukaryotes respectively, indicating, that *Synechococcus* sp. tended to be more abundant than the other pico-phytoplanktonic groups.

The distribution of pico-phytoplankton at the surface of the Bay of Palma was not homogeneous and showed changes in the distribution across the Bay that could be observed at a scale of a week (Fig. 2, 3). Besides these changes, there were differences according to the time of the year. During March the highest concentration of the three pico-phytoplankton groups were found in the external area of the Bay, away from the littoral (Fig. 2) and in June the highest concentrations of pico-phytoplankton were located at the littoral area (Fig. 3).

![Fig. 1. – Temporal variation of water temperature (grey discontinuous line), nutrient concentration (Nitrate+Nitrite and Phosphate respectively) and chlorophyll a (solid lines, full circles) in the Bay of Palma (Bahia station). Solid lines: fitted smoothed line for nutrients and chlorophyll a variability.](image-url)
There were also differences in the cell concentration of pico-phytoplankton between the periods of the surveys, associated with the seasonal variability (Fig. 4A). *Synechococcus* sp. abundance was maximal in summer (Fig. 4A) with an important bloom observed at mid August reaching a maximum abundance of $4.62 \times 10^5$ cells ml$^{-1}$. The abundance of living cells during the annual cycle of *Synechococcus* sp. paralleled that for the total population abundance and reached a maximum in the summer with $1.84 \times 10^5$ cells ml$^{-1}$. The pattern of temporal abundance of *Prochlorococcus* sp. (Fig. 4A) differed from that observed for *Synechococcus* sp., with the maximum abundance reached by mid December with $6.5 \times 10^4$ cells ml$^{-1}$. Three moderate blooms of *Prochlorococcus* sp. were detected, with the largest one observed in winter (December), followed by periods of increased abundance in April and during the summer time, from July to September (Fig. 4A). *Prochlorococcus* sp. living cells followed the same pattern than the total cell abundance with an increase in the concentration by mid December, and secondary maxima to mid April and July.

Pico-eukaryotes abundance was also highly seasonal, with a bloom found in April and May showing the highest cell concentration by mid April ($2.48 \times 10^4$ cells ml$^{-1}$), although the cell concentration of Pico-eukaryotes was highly variable during this period (Fig. 4A). Pico-eukaryotes abundance increased again during the summer time showing low cell concentration during fall and winter (Fig. 4A). The low concentration of the pico-eukaryotic community detected in some periods precluded the regular quantification of living cells that was difficult when total cell concentration decreased below $4.5 \times 10^3$ cells ml$^{-1}$.

The percentage of living cells found ranged from 16 to 94% for *Synechococcus* sp., from 26 to 100% for *Prochlorococcus* sp and from 20 to 94% for pico-eukaryotes. Two periods of increased viability were observed for both *Synechococcus* sp. and *Prochlorococcus* sp. during the period studied (Fig. 4B). *Prochlorococcus* sp. maximum living cells was found at late winter, with the highest percentage of living cells found from mid January to March, preceding the April bloom of *Prochlorococcus*, and a second period of high via-

---

**Fig. 2.** Distribution of *Synechococcus* sp., *Prochlorococcus* sp., and pico-eukaryotes cell abundance (cells ml$^{-1}\times 10^4$) at the surface waters of the Bay of Palma, during two sampling surveys in March 2002. Full circles: sampling stations.
bility observed at the early summer. The fall and the beginning of winter represented a period of higher cell death for *Prochlorococcus* sp. with maximum cell death (minimum % living cells) observed in late spring, concurrent with the decay of the April bloom. *Synechococcus* sp. showed the highest % living cells also at late winter (February and March, Fig. 4B) and a second period of high viability observed from the beginning to mid summer preceding the summer bloom (Fig. 4B). As observed for *Prochlorococcus* sp., the % living cells of *Synechococcus* sp. declined greatly in spring (Fig. 4B) indicating high cell death.

Despite the limited data on viability on pico-eukaryotes, the temporal distribution of the viability values obtained indicated that their cell death varied in agreement with the patterns of viability found for the pico-cyanobacteria, with increasing values of % living cells at late winter and at the beginning of the summer, and showing higher cell death, indicated by a minimum in the percentage of living cells, by May (Fig. 4B).

**DISCUSSION**

The pico-phytoplankton community observed in the Bay of Palma was composed by *Synechococcus* sp. *Prochlorococcus* sp. and pico-eukaryotes and showed abundances comparable to those found in other Mediterranean areas (Jacquet *et al.* 1998, Ferrier-Pages & Rassoulzadegan 1994, Vaulot *et al.* 1990, Agawin *et al.* 1998). *Synechococcus* sp. was the major component of the pico-phytoplanktonic community during all the seasons. The three pico-phytoplankton groups present in the Bay of Palma showed important seasonality and differed in the period of peak abundance, which were observed in winter, summer and spring for *Prochlorococcus* sp., *Synechococcus* sp. and pico-eukaryotes, respectively. Despite the differences found in the timing of the occurrence of peak abundance, *Prochlorococcus* sp. and pico-eukaryote abundances were positively correlated to that of *Synechococcus* sp. ($r = 0.53$, $P<0.0001$, $r = 0.52$, $P<0.0001$ for *Prochlorococcus* sp. and pico-
eukaryotes respectively), as observed for Mediterranean communities elsewhere (Vaulot et al. 1990, Ribes et al. 1999). This suggests that, despite differences described for growth requirements (Scanlan & West 2002, Vaulot et al. 1999), the populations were affected by similar controls in the Bay of Palma (e.g. water temperature, nutrients availability, grazing, etc.).

The development of a late winter phytoplankton bloom is one of the most coherent seasonal features of the NW Mediterranean sea (Margalef & Castellvi 1967, Margalef & Ballester 1967, Mura et al. 1996, Duarte et al. 1999, Duarte et al. 2004). This bloom was also described for the Bay of Palma, which showed the maximum chlorophyll a concentration in February-March 2002 (Navarro et al. 2004). The temporal distribution observed for the pico-phytoplankton community indicated their minor contribution to the late winter phytoplankton bloom, suggesting that the factors influencing the dynamics of pico-phytoplankton should differ from that of diatoms and large flagellates (e.g. Alonso-Laita & Agustí, submitted) which comprised the late winter bloom. The development of a summer phytoplankton bloom, described for the Bay of Palma and the Mediterranean coast elsewhere (Navarro et al. 2004, Mura et al. 1996, Duarte et al. 1999) has been related to the increased input of nutrients, especially phosphate associated with the sewage from the tourist peak in the Mediterranean coast (Duarte et al. 1999). The large summer bloom of Synechococcus sp. observed in the Bay of Palma also may be related to the increased phosphate concentration in the Bay. Indeed phosphorus has been identified as the main limiting nutrient in the Mediterranean Sea (Thingstad et al. 1998, Krom et al. 1991) and the division rate and growth of Synechococcus sp. has been described to respond to low increases in phosphorus concentration in the Mediterranean summer (Vaulot et al. 1996).

Fig. 4. – A, Seasonal variation in the abundance (solid line, full circles ± standard deviation) for Synechococcus sp., Prochlorococcus sp. and pico-eukaryotes) from October 2001 to October 2002 in the Bay of Palma (Bahia station). B, Temporal variation of the percentages of living cells (% living cells, solid line, full circles ± standard deviation) of Synechococcus sp., Prochlorococcus sp., and pico-eukaryotes, from October 2001 to October 2002 in the Bay of Palma (Bahia station). Broken line: temperature.
The spatial distribution of pico-phytoplankton in the Bay of Palma during the surveys suggested a relationship between the dynamics of this community and the water circulation in the Bay (Gazeau et al. 2005). During the March surveys, the dynamics of water in the Bay was fast with a resident time of 2.5 ± 0.2 d (Gazeau et al. 2005) which explain changes in the pico-phytoplankton distribution observed at the short scale of a week. During the beginning of the first cruise (March 2002) the water entered the bay along its Western side, while the water outflow was dominantly through the Eastern side. The circulation changed to the opposite direction toward the end of the cruise (12-March-2002, Gazeau et al. 2005) influencing the distribution of pico-phytoplankton in the Bay. The residence time of the water in the Bay of Palma increased by summer (10 days, Gazeau et al. 2005), with consequences for the renovation of the nutrients, increasing the persistence of land inputs in the bay and decreasing the exchange with the external waters (Gazeau et al. 2005). During the summer surveys, the highest concentration of pico-phytoplankton was detected at the more littoral stations, indicating a local response to the increased nutrients from land inputs in the littoral zone.

Pico-phytoplankton showed an important seasonal variability in cell death, as indicated by the changes observed in the percentage of living cells. The variability in percentage of living cells, contrary to that found for the occurrence of blooms, showed a similar seasonal pattern for all the pico-phytoplanktonic groups. The viability of Synechococcus sp., Prochlorococcus sp. and pico-eukaryotes showed two peaks, indicative of low cell death, in late winter and in early summer. The two peaks in pico-phytoplankton viability corresponding to the two major peaks in nutrient concentration in the Bay of Palma. The two peaks of nutrients coincided with similar peaks in dissolved organic nitrogen, that could explain the positive response of Prochlorococcus sp. to inorganic nutrients despite the inability of this genera to use nitrate (Moore et al. 2002). Similarly, the lowest % living cells, indicative of high pico-phytoplankton cell death, was observed at the period when nutrient concentration decreased markedly and the water temperature began to increase, indicating summer stratification of the water column. A similar period of high phytoplankton cell death preceding the summer was described for the Spanish Mediterranean coast, where both pico-sized and larger phytoplankton cells showed the lowest viability, as determined by high phytoplankton lysis rates (Agustí & Sánchez 2002).

The high % living cells of pico-phytoplankton observed in late winter preceded the spring blooms of Synechococcus sp. and Prochlorococcus sp., this high cell viability, associated to the increased nutrient concentration in the Bay, resulted however in moderate cell abundances, indicating that factors other than nutrients were also important in controlling these populations. The low water temperature at this period may, by slowing growth rate, preclude the development of larger blooms, due to the described temperature-dependent growth of Synechococcus sp. and Prochlorococcus sp. (Moore et al. 1995, Agawin et al. 1998, Partensky et al. 1999). Grazers are reported to exert a strong control over pico-phytoplankton populations in the Mediterranean Sea (Agawin & Agustí 1997, Agawin et al. 2000, Agawin et al. 2004) and may be responsible for the control of the magnitude of the blooms observed, in addition to the losses imposed by cell death. During the early summer, the increase in cell viability observed, indicative of reduced pico-phytoplankton cell death, preceded the summer blooms. The increased viability of pico-phytoplankton at the early summer is associated with the increased phosphate concentration in the Bay of Palma. The summer increase in pico-phytoplankton viability, could be also facilitated by the higher water temperature. For the Subtropical Atlantic Ocean, Alonso-Laita & Agustí (submitted) found that water temperature strongly influenced pico-cyanobacteria cell viability, with Synechococcus sp. and Prochlorococcus sp. viability increasing at temperatures above 21°C (Alonso-Laita & Agustí submitted).

Despite the reduced cell death of all the pico-phytoplanktonic groups at the early summer, only Synechococcus sp. developed a sizeable bloom in the Bay of Palma. This indicates that the conditions for growth during summer are more favourable for Synechococcus sp. than for the other groups. Synechococcus sp. cell division strongly increases with increased water temperature (e.g. Agawin et al. 1998) and phosphorus inputs (Vaulot 1996). Also, the differences recently described in the sensitivity to PAR and UVR for the different pico-phytoplankton groups, indicated that Synechococcus sp. is more resistant to high solar radiation levels than Prochlorococcus sp. and pico-eukaryotes are (Agustí 2004, Llabrés & Agustí 2006 in press), which could also be an advantage for Synechococcus sp. during the summer time.

In summary, an important pico-phytoplankton community was present throughout the year in the Bay of Palma, with Synechococcus sp. population dominating the pico-phytoplanktonic community. The different groups showed different seasonal patterns in abundance, but a similar response in % of viable cells, which increased preceding the late winter bloom and in early summer, indicating these periods of relatively high nutrient availability as the most favourable for pico-phytoplankton in the Bay of Palma. The results demonstrate the usefulness of assessing viability patterns along with abundance, to elucidate the relative roles of losses vs. stresses in controlling the population dynamics of pico-phytoplankton communities.
ACKNOWLEDGEMENTS. – This is a contribution of project EUROTROPH (Nutrients Cycling and the Trophic Status of Coastal Ecosystems) funded by F5 of the European Union. We thank the crew of RV Tethys and RV Mytilus for the assistance, R Santiago & R Martinez for sampling assistance and JC Alonso for nutrients analyses. P A-L. was supported by a fellowship from the Spanish Ministry of Science and Technology.

BIBLIOGRAPHY


Reçu le 5 août 2005, received August 5, 2005
Accepté le 21 octobre 2005; accepted October 21, 2005