# IMPORTANCE OF PHAGOTROPHIC PIGMENTED FLAGELLATES (MIXOTROPHS) IN THE OLIGOTROPHIC EASTERN MEDITERRANEAN, A FIRST APPROACH

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**Abstract**

The vertical distribution and abundance of mixotrophic nanoplankton (MNAN) was examined in the oligotrophic Cretan Sea (East Mediterranean) in the framework of MAST/MTP-II MATER. Fluorescently labelled bacteria and minicells were used to identify potential algal grazers. Mixotrophic algae biomass was almost as great as the biomass of typical heterotrophs and represented 27 ± 2% of the total nanoplanktonic biomass in the 0-100 m layer. Based on minimal estimates, 12 ± 8% of total number of chlorophyll containing organisms were found to be phagotrophic.

*Key-words: Phytoplankton, bacteria, biomass, Eastern Mediterranean*

## Introduction

The ecological role of natural populations of mixotrophic flagellates (MNAN) is a new research field (1). Photosynthesis is presumably the primary energy source for these flagellates; phagotrophy is used to acquire nutrients in a particulate form when dissolved forms are scarce (2, 3, 4) and/or carbon, if photosynthesis is light limited (5, 6). The few existing data suggest a quite variable abundance and grazing activity of MNAN in aquatic environments (*e.g.* 4, 7, 8, 9). It would appear reasonable to assume that mixotrophy among phytoflagellates would be relatively more important in oligotrophic environments. However, to our knowledge, only one previous study has been conducted in an oligotrophic marine environment, the Sargasso sea (10). The present study was designed to obtain quantitative information on the nanoplanktonic mixotrophic algae (MNAN) in a pelagic oligotrophic ecosystem, the Eastern Mediterrean. For this we conducted *in situ* grazing experiments and quantified the relative contributions of apochlorotic nanoplankton (HNAN) and of chloroplast containing phototrophic (PNAN) and phagotrophic nanoplankton (MNAN) to the total nanoplanktonic flagellate population.

## Materials and methods

This study was carried out from 6 to 9 March 1997 in the oligotrophic Cretan Sea (South Eastern Mediterranean), during the first cruise of the MATER programme, on the RV *Aigaio*. During the sampling period the water column was well mixed, with very low nitrate and phosphate concentrations and T 14.2°C. Four stations were sampled (South Aegean MATER stations, MSB 1, 2, 6 and 7, depth 1300-2000 m) water samples were collected in the euphotic zone at 5, 10, 30, 50, 75 and 100 m depths. To distinguish which of the chlorophyll containing nanoflagellates are potentially phagotrophs we added fluorescent food tracer particles: FLB (Fluorescent Labelled Bacteria) or fluorescently labelled minicells. The FLB (length 1.6-2.4 $\mu$m, ESD 0.8-1.0 $\mu$m), were prepared following the protocol of (11) the fluorescent mini-cells (0.65 $\mu$m diameter) were prepared following the protocol of (12). FLB and minicells were sonicated (1 min.) on-board before every experiment to obtain monodispersed prey items. The final concentration of the prey items in the experimental bottles was approximately half of the natural bacterial density. This concentration was high (usual additions do not exceed 5-15% of natural bacteria) and of course changed the total bacterial density in the sample, but when the final minicell or FLB density is less than $10^5$ ml$^{-1}$ it is difficult to detect tracer particle uptake by flagellates.

Acid-cleaned 150 ml glass bottles were filled with seawater from each depth in duplicate. Before inoculation with the fluorescent food tracers, bottles were left undisturbed for 1 hour in a thermoregulated water bath (14.2°C). After adding the FLB and minicells, subsamples were immediately withdrawn for T0 counts, and counts of bacteria, cyanobacteria, initial densities of tracer particles, and nanoplanktonic organisms. Samples were preserved with buffered formol (1% final concentration). Subsequent subsamples of 25 ml were removed from bottles after 30 and 60 min. Samples were filtered within the same day on black Nuclepore filters (0.2 $\mu$m for picoplankton counts and 0.8 $\mu$m for nanoplankton counts), stained with DAPI (13) and stored at -20°C until counting. All populations were enumerated using epifluorescence microscopy, autofluorescence was distinguished under blue (nanoflagellates, labelled bacteria) and green (cyanobacteria) light excitation.

Among the nanoplankton in the size range 2-20 $\mu$m we differentiated three functional groups: HNAN (apochlorotic cells, mainly flagellates), PNAN (chloroplast-containing nanoplanktonic protists) and MNAN (mixotrophic nanoflagellates, chloroplast containing nanoplanktonic protists with ability to ingest particles). The organisms were classified in different size categories using an ocular micrometer. Biovolume-carbon conversion factors were 250 fg C $\mu$m$^{-3}$ for cyanobacteria (14), 220 fg C $\mu$m$^{-3}$ for HNAN and PNAN (15). Bacterial abundance data were converted to biomass using 20 fg C cell$^{-1}$ (16).

Because of the probability that not all MNAN were consuming bacteria at a given time in the samples, as well as the possibility of selection against fluorescent prey and the egestion during fixation we calculated "minimal" and "maximal" abundance of MNAN (Table 1). Minimal numbers were calculated based solely on "confirmed grazers" *i.e.*, cells with ingested food tracers; and maximal abundance were calculated based on the concentration of the mixotrophic morphotype, that is the abundances of cells with the same morphology as the "confirmed grazers". Morphotypes were distinguished by cell shape, the number and insertion of flagella, as well as chloroplast location, shape and number.

Table 1. Mean population abundances (±SD, n = 6 depths) in the 0-100 layer determined from water samples from 4 stations in the Cretan Sea (East Mediteraanean) during March 1997.

| Station date | Bacterial abundance | Chroococcoid cyanobacteria | Total phototrophic nanoplankton (PNAN)) | Total mixotrophic nanoplankton (MNAN) | Mixotrophic cells containing prey (HNAN | Heterotrophic nanoplankton |
|---|---|---|---|---|---|---|
| | $10^5$ ml$^{-1}$ | $10^5$ ml$^{-1}$ | $10^3$ ml$^{-1}$ | $10^3$ ml$^{-1}$ | $10^3$ ml$^{-1}$ | $10^3$ ml$^{-1}$ |
| 2 6 march | 4.3±0.6 | 0.21±0.01 | 0.68±0.14 | 0.23±0.10 | 0.09±0.07 | 0.30±0.04 |
| 1 7 marc | 3.6±0.4 | 0.22±0.01 | 0.54±0.13 | 0.25±0.03 | 0.08±0.04 | 0.25±0.05 |
| 7 8 march | 3.7±0.4 | 0.19±0.01 | 0.64±0.15 | 0.23±0.05 | 0.05±0.01 | 0.38±0.2 |
| 6 9 march | 5.8±4.2 | 0.21±0.07 | 0.70±0.3 | 0.30±0.16 | 0.05±0.04 | 0.43±0.09 |

## Results

The water column 0-100 m was nearly isothermal on the sampling dates (14.2°C). The vertical distribution of pico- and nanoplakton in the 4 profiles studied was almost homogenous in the 0-100 layer, only slightly decreasing under 75 m (Fig. 1). The biomass structure of the microbial community was represented by an "inverted pyramid" characteristic of oligotrophic waters, where bacterial biomass is greater

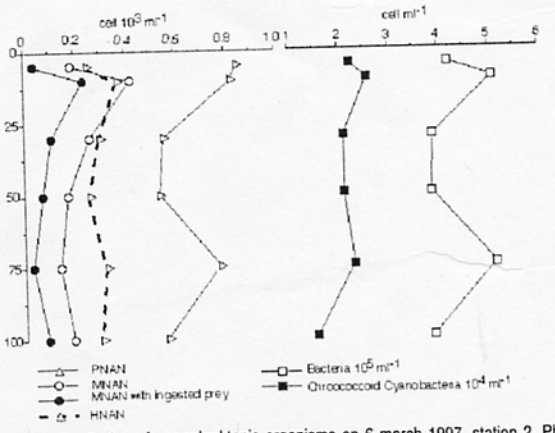


Fig. 1. Vertical distribution of nanoplanktonic organisms on 6 march 1997, station 2. Phototrophic (PNAN total number of cells containing chlorophyll), heterotrophic (HNAN), and Mixotrophic nanoplankton (MNAN), MNAN with ingested prey = confirmed grazers.

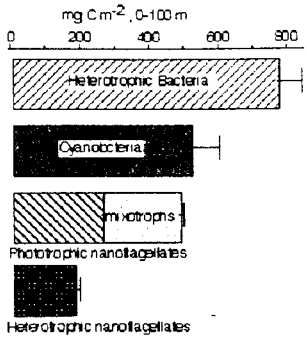


Fig. 2. Mean integrated biomass 0-100 m ($\mu$g C m2) of pico- and nanoplankton in the 4 studied stations.

than autotrophic biomass (Fig. 2). Chlorophyll-bearing cells numerically dominated the nanoplanktonic protistan assemblage (Table 1, Fig. 3). The nanoplanktonic community was composed largely of small cells : *e.g.*, dominant PNAN, MNAN, HNAN of 2-3 $\mu$m, 3-5 $\mu$m, 2-3 $\mu$m respectively. The dominant morphotype of mixotrophs were "prymnesiophyceae like" cells 2-4 $\mu$m.

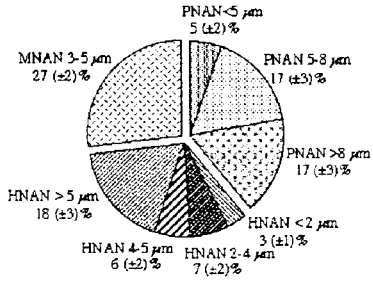


Fig. 3. Average biomass percentages of nanoplankton size classes. Mean±sd of integrated biomass data ($\mu$g C m2) in the 4 sampled stations, 0-100 m layer. (PNAN) phototrophic nanoflagellates (MNAN) mixotrophic nanoflagellates (HNAN) heterotrophic nanoflagellates.

The mean biomass of chloroplast-containing flagellates was $4.9 \pm 2.8\ \mu g\ l^{-1}$, of this biomass $1.8 \pm 0.7\ \mu g\ l^{-1}$ were MNAN morphotype, and $0.53 \pm 0.4\ \mu g\ l^{-1}$ were "confirmed grazers". The HNAN mean biomass was $2.2 \pm 1.2\ \mu g\ l^{-1}$. Thus, the mixotrophic algae biomass was almost as great as the biomass of typical heterotrophs (Figs. 1, 2, 3).

Among both heterotrophic and mixotrophic flagellates, FLB were ingested at rates lower than those estimated for minicells. HNAN did not ingest FLB at all, while only 7 ± 5% of the MNAN were found to contain FLB after 1 hour of incubation. Minicells were ingested at higher rates, by the MNAN "morphotype" than by HNAN, *i.e.*, 28 ± 14 % and 14 ± 8% respectively contained at least 1 minicell after 1 hour of incubation. Considering only "confirmed grazers", 12 ± 8 % of total number of chlorophyll containing organisms were found to be phagotrophic.

"Minimal" rates of bacterivory for the MNAN assemblages were roughly calculated assuming that all MNAN (based on morphotype) were phagotrophic. Based on this reasoning, MNAN consumed about 25 bacteria $cell^{-1}\ d^{-1}$, in terms of carbon such ingestion corresponds at only around 3% of their carbon content. The same calculation considering "confirmed grazers" only results to a maximum estimate of around 10% of their carbon content. Thus, the mixotrophic morphotype of flagellate was relatively abundant but not all cells were phagotrophically active. However, the % of HNAN ingesting minicells was even lower. As HNAN are known to be redoubtable bacterial consumers, such low ingestion suggest a selectivity against the prey analogs used.

**Discussion**

Our results underline the potential importance of MNAN within the microbial food web in the oligotrophic Eastern Mediterranean. We found that approximately 12% of chloroplast-containing nanoflagellates consumed at least one bacterial tracer per hour. The mixotrophic morphotype represented about 40% of total chloroplast-containing flagellate cell numbers. The ingestion rates we estimated and the relative contribution of mixotrophs to total chloroplast-containng flagellates are quite similar to figures concerning Sargasso Sea populations recently reported (10). With the current method used (ingestion of labelled inactive bacteria) we can not be sure of the real *in situ* ingestion rates; true ingestion rates are probably higher if flourescently-labeled prey are discriminated against, which is not unlikely. Thus, our estimates, along with the other literature values, should be considered as conservative. Clearly, many questions remain concerning mixotrophy among phytoflagellates. While mixotrophic flagellates are abundant in a variety of aquatic habitats, few generalities have emerged. Some mixotrophic species appear closer to a photoautotrophic mode while others appear to be mostly phagotrophic. Mixotrophs closer to the "phagotrophic extreme" have been encountered in environments where neither light nor mineral nutrients are limiting photosynthesis. In contrast, mixotrophs closer to the "photoautotrophic extreme" may never play an important role as grazers but use phagotrophy to aquire nutrients (see review 3). The importance of the photoautotrophic and phagotrophic modes of nutrition can vary in a given species as a function of environmental parameters such as prey particle density, light (5), inorganic nutrient concentration (2), PH (5), and perhaps dissolved organic carbon (5, 17). Our rough estimates of carbon acquisition by MNAN via bacterivory in our experiments (3-10% of cell carbon per day) indicates that in the nutrient-poor Eastern Mediterranean algae probably ingest particulate prey primarily for nutrients and/or for organic compounds that they"re unable to synthesize.

In conclusion, we have shown that MNAN are abundant in the oligotrophic Eastern Mediterrean and consequently, phagotrophy by the primary producers in this region needs to be considered in the assessments of the flows of material between compartments of the planktonic food web.

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