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 μm, ESD 0.8-1.0 μm), were prepared following the protocol of (11) the fluorescent mini-cells (0.65 μm 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 μm for picoplankton counts and 0.8 μm 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 μm 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 μm^{-3} for cyanobacteria (14), 220 fg C μm^{-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

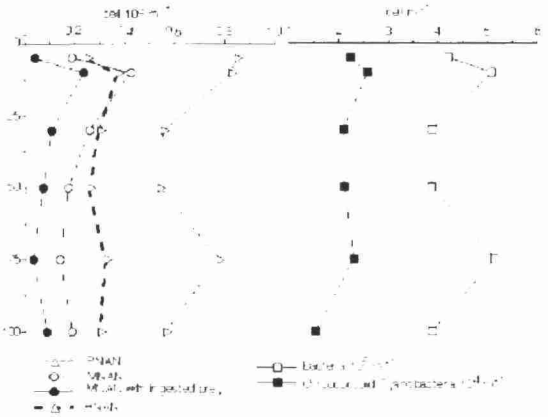

Fig. 1. Vertical distribution of nanoplanktonic organisms on 6 march 1997, station 2. Phototrophic (PNAN total number of cells containing chlorophyll), heterotrophic (HNAN), and Mixotrophic nanoplankton (MNAN). MNAN with ingested prey = confirmed grazers.