

Bacterial numbers, bacterial production, and heterotrophic nanoplankton abundance in a warm core eddy in the Eastern Mediterranean

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ABSTRACT: Bacterial numbers and production, and abundance of heterotrophic nanoplankton (HNAN) were recorded over 24 h at the core and boundary of the Cyprus Eddy, a warm-core eddy in the Levantine Basin of the Eastern Mediterranean Sea. The basin is known as one of the most oligotrophic on record. Bacterial numbers in the euphotic zone were close to the lower threshold value for bacterial abundance in the oceans, with means of 2.7×10^5 bacteria ml^{-1} at the boundary and 4.9×10^5 ml^{-1} at the core. Bacterial production, measured as the rate of DNA labelling by thymidine (TdR), ranged from 0.01 to 0.70 $\text{pmol TdR l}^{-1} \text{h}^{-1}$, values which fall at the lower end of ranges reported from other freshwater and marine ecosystems. No diel cycles of bacterial numbers or rate of TdR incorporation were observed, but the TdR rate normalized to cell numbers showed marked diel variation with the highest rates usually found in early morning hours. HNAN were characteristically small (mostly $< 3 \mu\text{m}$ in diameter) and more abundant in the core (37 to 850 cells ml^{-1}) than in the boundary station (19 to 414 cells ml^{-1}). The long bacterial doubling times (ca. 50 d), and the parallel distribution of bacteria and HNAN, suggested low grazing pressure. A possible alternative food source for HNAN is prochlorophytes. Higher microbial standing crop and activity are anticipated in the core of the eddy in winter, when deep mixing occurs.

INTRODUCTION

Bacteria and heterotrophic nanoplanktonic (HNAN) grazers play major roles in the cycling of organic matter and nutrients in aquatic ecosystems. In oligotrophic oceans bacteria often consume up to half of the primary production via dissolved organic matter, and are then consumed by protozoan grazers that also feed on small algae (Azam et al. 1983). Recent studies demonstrate that in oligotrophic oceans bacterial biomass often exceeds that of phytoplankton (Fuhrman et al. 1989, Cho & Azam 1990). However, most studies have been carried out in coastal systems; those from oligotrophic offshore waters are scarce (Ducklow 1986, Fuhrman et al. 1989).

The pelagic waters of the Levantine Basin of the Eastern Mediterranean Sea have been shown to be among the most oligotrophic on record, with excep-

tionally low primary productivity (Berman et al. 1984, 1986), chlorophyll and nutrient concentrations (Salihoglu et al. 1990, Krom et al. 1991), and exceptionally deep light penetration (Berman et al. 1985, Megard & Berman 1989). To our knowledge, data on the abundance of bacteria and heterotrophic nanoplankton, and on rates of bacterial production in this oligotrophic sea have not been published.

Recent studies highlight the potential importance of mesoscale eddies as sites of episodic nutrient injections into the photic zone, leading to localized enhancement of primary productivity (Jenkins 1988, Falkowski et al. 1991). Fronts of warm core rings were shown to sustain high bacterial abundance and activity (Ducklow 1986). Recently, Brenner (1989) and Brenner et al. (1991) described a persistent warm-core eddy located south of Cyprus (the Cyprus Eddy) in the Levantine Basin. Krom et al. (1992) reported enhanced primary produc-

tivity in the core of the Cyprus Eddy in winter, as a result of deep mixing, and as was observed in the core of other warm-core eddies (Tranter et al. 1980, Bradford et al. 1982).

Our study constituted part of an effort organized by GAP (Group on Aquatic Productivity) to measure for the first time a range of microbiological parameters in the Cyprus Eddy and to compare organism abundances and rate processes between the eddy's core and its boundary. Waters in the core of the eddy were to a large extent sealed off from the surrounding water (Brenner et al. 1991, Krom et al. 1992). Measurements taken over 24 h in the core were therefore likely to follow diel patterns in the same water parcel except, perhaps, at the surface (ca 0 to 40 m) mixed layer. We report here our findings on the abundance and production of bacteria, and on the abundance of HNAN, their potential grazers, over 24 h cycles.

METHODS AND STUDY SITE

The study was conducted aboard the RV 'Shikmona' during a GAP/IOLR cruise to the Cyprus Eddy. A station located at the boundary of the eddy (34° 23' N, 33° 44' E; maximum depth 586 m) was occupied on 11–12 September 1989, and a station at its core (33° 43' N, 35° 30' E; maximum depth 2200 m) on 13–14 September 1989. Water samples were collected by means of a General Oceanics rosette system equipped with six 5 l Niskin bottles. An attached Neil-Brown STD was used for recording salinity and temperature. At each station water samples were collected at 4 h intervals over a 24 h cycle from 9, 56 and 130 m depths, corresponding approximately with the depths of 69, 10 and 0.5 % surface irradiation as estimated from Secchi disc depth (Megard & Berman 1989). Additional water samples from 500 and 1000 m were collected at the core station. All water samples were prefiltered through 300 µm mesh Nitex net to eliminate large particles and zooplankton. Subsamples for bacteria and HNAN enumeration, and for determination of rates of bacterial production were processed without delay.

Water samples for the enumeration of HNAN and bacteria were preserved with the Lugol-Formaline decoloration technique (Sherr et al. 1989) and kept refrigerated. Cells were enumerated using epifluorescence microscopy and DAPI staining (Porter & Feig 1980). We classified as 'HNAN' all nucleated, nanoplanktonic (< 20 µm), non-pigmented organisms, and distinguished them from chlorophyll-containing cells by their fluorescence characteristics. Biovolumes of HNAN were estimated from microscopically measured linear dimensions and approximated geometrical shapes.

Bacterial production was measured using the [methyl-³H]thymidine (TdR) incorporation method (Wicks & Roberts 1987). Working solutions of TdR (41 mCi mmol⁻¹; Amersham, UK) were made daily with 0.2 µm filtered, autoclaved, double-distilled water. For each sample, triplicate 25 ml subsamples were placed in sterile polystyrene scREW-top tubes immediately upon collection; 2 were live replicates, 1 an NaOH-killed (1.25 ml of 5 N NaOH) control. Labeled thymidine was added to each incubation tube to give a final concentration of 18 nM, a concentration that should fully inhibit de-novo DNA synthesis (Bell 1990). The tubes were incubated at air temperature (ca 25 °C) for 60 min. In a preliminary experiment the uptake of TdR was found to be linear over 4 h. Thymidine incorporation in the live tubes was stopped by the addition of 5 N NaOH. After 15 min labeled DNA was precipitated in ice-cold TCA, collected on 0.2 µm pore-size cellulose nitrate membrane filters, washed with phenol-chloroform (50 % w/v) and then 80 % ethanol. After removing the non-filtering area, the filters were dissolved completely in 0.75 ml of ethyl acetate and placed in 5 ml of scintillation cocktail (70 % toluene, 30 % Lumax; Lumac, Belgium). Tritium incorporation into DNA was determined with a liquid scintillation counter and counts were corrected for quench (with an external standard) and machine counting-efficiency. Replicate TdR incorporation rates were usually within 20 %. Bacterial cell production was calculated using the conversion factor of 1×10^{18} cells produced mol⁻¹ TdR incorporated (Moriarty 1988, Bell 1990).

Detailed depth profiles of temperature, salinity, nutrients, chlorophyll and picoplankton abundance were measured concurrently by others (Krom et al. 1992, Wood et al. unpubl.). Water temperature ranged from 27.3 °C at the surface to 16.4 °C at 130 m at the core station and from 26.2 °C to 16.3 °C at the boundary station. Temperature and salinity profiles at both stations showed a surface mixed layer of about 40 m, consisting of high salinity warm water, below which was a 10 to 20 m layer of low salinity water of Atlantic origin (Brenner et al. 1991). Beneath the Atlantic water was a homothermal and constant salinity thermostad, extending down to 400 m at the core station, and down to 150 m at the boundary station (Brenner et al. 1991), indicating that the core station was well within the eddy. At both stations the temperature, salinity and nutrient depth profiles showed minor changes over 24 h (Wood et al. unpubl.), suggesting that the same parcels of water were sampled over time.

Nitrate concentrations at 9, 56 and 130 m ranged from 0.32 to 1.35 µM at the core station and from 0.02 to 0.49 µM at the boundary, while phosphate concentrations ranged from undetectable to a maxi-

imum of 0.04 μM (Krom et al. 1992). Significantly higher nutrient concentrations were found at 500 m and 1000 m at the core station. At our sampling depths within the upper 130 m chlorophyll *a* concentrations ranged between 21 and 87 ng l^{-1} , and were highest at 130 m and lowest at 9 m. The highest chlorophyll concentration occurred at about 100 m at both stations and did not exceed 130 ng l^{-1} (Krom et al. 1992).

RESULTS AND DISCUSSION

Cho & Azam (1990) claimed that bacterial abundance in the ocean's euphotic zone has a lower threshold of ca 3×10^5 cells ml^{-1} . Bacterial numbers from the Cyprus Eddy clustered around this value, with numbers below the threshold occurring at the base of the euphotic zone at 130 m (Fig. 1). The bacteria were generally small ($<0.3 \mu\text{m}$) coccoid or rod-shaped cells. At the core station bacterial numbers ranged between 7.9×10^4 and 2.4×10^6 cells ml^{-1} , with a mean value of 4.9×10^5 cells ml^{-1} . Bacterial numbers were generally higher at 9 m ($\bar{x} = 6.6 \times 10^5$ cells ml^{-1}) and 56 m ($\bar{x} = 5.9 \times 10^5$) than at 130 m ($\bar{x} = 2.2 \times 10^5$). Bacteria numbered 2.4×10^4 cells ml^{-1} at 500 m depth and 1×10^4 at 1000 m depth. At the boundary station bacterial numbers were generally lower than at the core (Fig. 1). The mean was 2.8×10^5 cells ml^{-1} , with highest numbers at 56 m ($\bar{x} = 4.7 \times 10^5$ cells ml^{-1}), about half of that at 9 m, and about a fourth at 130 m.

Bacterial production at the core station ranged between 0.11 and 0.70 $\text{pmol TdR l}^{-1} \text{h}^{-1}$ over 24 h. The mean rate was $0.39 \text{pmol l}^{-1} \text{h}^{-1}$ with highest values usually occurring at 9 m depth (Fig. 1). At 500 m the rate was $0.09 \text{pmol TdR l}^{-1} \text{h}^{-1}$, while at 1000 m the rate was below detection level. At the boundary station thymidine incorporation rates were generally lower than at the core station, ranging between 0.13 and $0.56 \text{pmol TdR l}^{-1} \text{h}^{-1}$, with a mean rate of $0.24 \text{pmol TdR l}^{-1} \text{h}^{-1}$.

No diel pattern of bacterial numbers or the rates of TdR incorporation was found at either station. However, when bacterial production rates were expressed as specific rates per bacterial cell ($10^{-21} \text{mol TdR incorporated cell}^{-1} \text{h}^{-1}$), a distinct pattern appeared at both stations (Fig. 1). The cell-specific rates ranged between 0.10 and $7.43 \times 10^{-21} \text{mol TdR cell}^{-1} \text{h}^{-1}$ at the core station, and 0.12 and $9.62 \times 10^{-21} \text{mol TdR cell}^{-1} \text{h}^{-1}$ at the bound-

ary. The cell-specific rates were high in the early morning hours, declined during the day to a minimum in the late afternoon (core station) or at night (boundary station), and increased again in the early morning hours. At both stations this pattern was most noticeable at 130 m depth. The rates of thymidine incorporation into DNA as well as the cell-specific rates in the eddy were low, but within the range of values reported from other oligotrophic seas, such as the Celtic (Joint & Pomroy 1987) and the Sargasso (Jonas et al. 1988, Fuhrman et al. 1989).

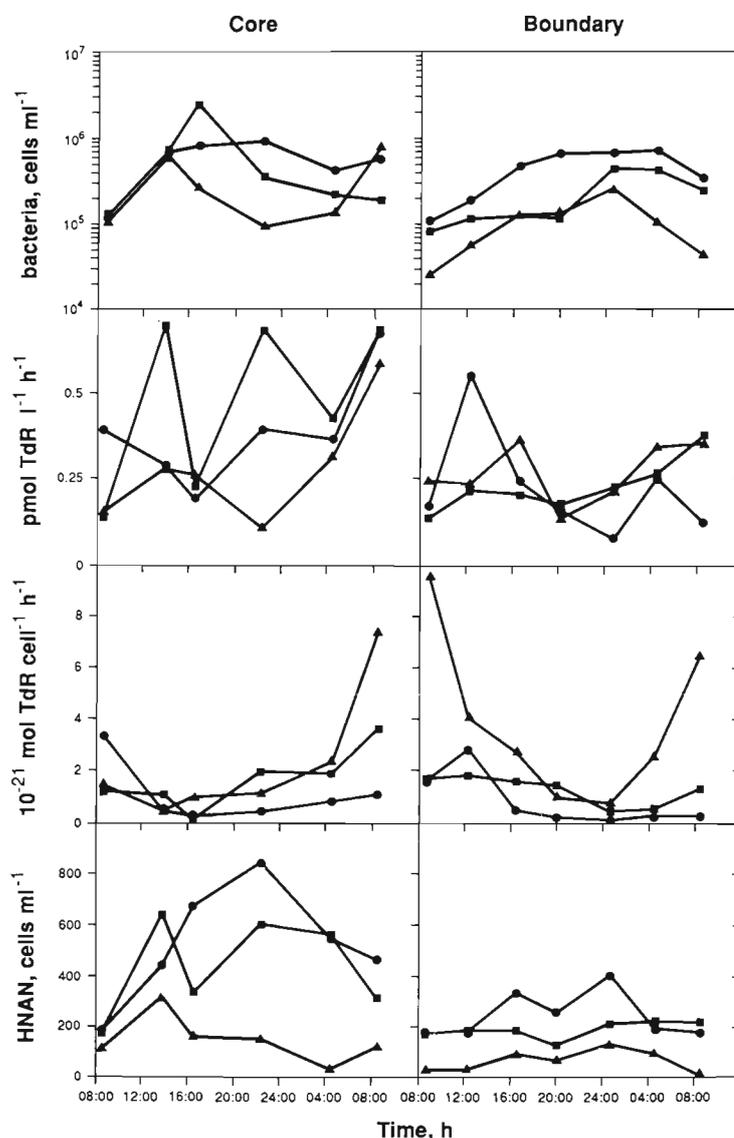


Fig. 1. Diel patterns of bacterial abundance (cells ml^{-1}), rates of thymidine incorporation into DNA ($\text{pmol TdR l}^{-1} \text{h}^{-1}$), specific rates of thymidine incorporation per bacterial cell ($10^{-21} \text{mol TdR cell}^{-1} \text{h}^{-1}$), and of HNAN abundance (cells ml^{-1}) at 9 m (■), 56 m (●) and 130 m (▲) at the core and boundary of the Cyprus Eddy, Eastern Mediterranean Sea, September 1989

Table 1 Bacterial production in a warm-core eddy in the Eastern Mediterranean Sea, September 1989. Values are averages of 7 measurements taken over 24 h, except for the data from 500 m which was a single measurement

Station	Depth (m)	Thymidine incorp. ($\text{pmol l}^{-1} \text{h}^{-1}$)	No. of cells ($\times 10^8 \text{l}^{-1}$)	Cell production ($\times 10^6 \text{l}^{-1} \text{h}^{-1}$)	Specific growth (d^{-1})	Doubling time (d)
Core	9	0.48	6.60	0.48	0.0175	57.3
	56	0.39	5.90	0.39	0.0159	63.3
	130	0.29	2.15	0.29	0.0318	31.4
	500	0.09	0.24	0.09	0.0038	266.7
Boundary	9	0.23	2.27	0.23	0.0243	41.1
	56	0.23	4.69	0.23	0.0118	84.9
	130	0.26	1.21	0.26	0.0516	19.4

At the core station average diel bacterial cell production rates ranged from 0.29 to 0.48×10^6 cells $\text{l}^{-1} \text{h}^{-1}$, giving doubling times of 31.4 to 57.3 d. At the boundary station the range was 0.23 to 0.26×10^6 cells $\text{l}^{-1} \text{h}^{-1}$, with doubling times of 19.4 to 84.9 d. The single measurement from 500 m gave a doubling time of 267 d. These doubling-time estimates are longer than reported elsewhere for bacteria in the euphotic zone (e.g. Fuhrman et al. 1989). A similar value of 65 d was reported by Cho & Azam (1988) for bacteria in aphotic water of an oligotrophic north Pacific gyre.

Estimates of bacterial doubling times depend on the conversion factor employed for calculating bacterial cell production from TdR incorporation rates, the choice of which remains controversial. While Cho & Azam (1988) used a factor of 1.18×10^{18} , similar to the 1.0×10^{18} employed here, Ducklow (1986) used a value 4-fold higher for calculating bacterial production in a Gulf Stream ring. Recently Bell (1990) argued that conversion factors that were considerably greater than the theoretical 1×10^{18} were often obtained in experiments in which the amount of TdR added was insufficient to completely inhibit de-novo DNA synthesis.

Numbers of HNAN were higher at the core station compared with the boundary station, a pattern similar to that of bacteria (Fig. 1). At the core station the HNAN numbers ranged between 36.5 and 851 cells ml^{-1} while at the boundary station the range was 18.5 to 413.7 cells ml^{-1} . At both stations highest numbers were recorded at 56 m depth and lowest at 130 m depth.

The majority of the HNAN cells were small, usually 1 to 3 μm in diameter. In all samples 93 to 99 % of the cells had a diameter of 5 μm or smaller. However, these small cells contributed only 25 to 44 % of the overall HNAN biomass, while the rare large (>5 μm) cells contributed the bulk of it.

The ratio of bacterial cells to HNAN cells was about 1000:1500, a ratio typical of aquatic systems (Azam et al. 1983). Considering the exceptionally low cell-specific thymidine incorporation rates and the long

bacterial doubling times (Table 1), we would have expected a smaller standing crop of HNAN relative to bacterial numbers. Alternatively, the HNAN population could have been utilizing other food sources, e.g. prochlorophytes, thus exerting a low grazing pressure on bacteria. Wood et al. (unpubl.) found small-sized (<0.5 μm) prochlorophytes to be abundant in the Cyprus eddy. Our hypothesis requires experimental confirmation.

The low values for bacterial abundance and production at both stations are in agreement with the exceptionally low nutrient and chlorophyll concentrations, and demonstrate again the ultra-oligotrophic nature of the Eastern Mediterranean Sea. The study did not reveal major differences between the core and boundary locations (Fig. 1). Higher bacterial standing crops and production, and greater differences in their vertical distribution between the eddy's core and boundary are anticipated to occur in winter, when deep mixing at the core (to ca 400 m) brings nutrients into the euphotic zone, supporting a short (days to a few weeks) spike of new production (Krom et al. 1992).

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