INTERACTIONS BETWEEN BACTERIA AND THEIR PROTOZOAN PREDATORS ON THE DIEL SCALE

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Abstract.
Diel variations in bacterial and heterotrophic nanoflagellates (HNF) abundance, bacterial production as well as grazing on bacteria were studied in Kastela Bay (Adriatic Sea). Pronounced diel patterns of all studied parameters were established. Bacterial abundance and growth rate were higher during the day than during the night. Maxima were measured during afternoon and early evening, and minima during the second half of the night and early morning. On the other hand, abundance of HNF was higher at night than during the day. Grazing on bacteria peaked in the evening, but grazing exceeded bacterial production during dark period, therefore, bacterial biomass produced during the day was removed by grazing during the night.

Key-words: bacteria, predation, Adriatic Sea

Introduction

In contrast to seasonal changes in microbial activity, which would be expected to be strongly affected by physical parameters most notably temperature, diel changes are primarily a manifestation of the diel sunlight cycle. It is obvious that primary production should have a pronounced diel cycle with carbon fixation occurring only during daylight hours. Due to strong coupling between phytoplankton and bacteria, significant diel variations of bacterial activity have also been observed (1, 2). Therefore, diel variations of bacterivorous protozoa could also be expected due to diel variations of bacteria as their main source of food. Moreover, bacterivorous protozoa might be able to regulate their feeding rates as a result of changes in light intensity (3).

However, in comparison to a number of studies reporting diel variations of phytoplankton and bacteria, there are still few data which explain interactions between bacteria and their protozoan grazers on the diel scale.

Methods

Samples were collected at a station located in the enclosed, shallow basin Kastela Bay, mid Adriatic Sea (43°31'N, 16°22'E). Samples were collected using sterile, acid washed microbiological samplers, from 1 m depth, and were processed in the laboratory within 24 h after collecting. Samplings were performed at 3 h intervals from 12 to 17 September 1994. Enumeration of bacteria and heterotrophic nanoflagellates (HNF) were made by epifluorescence microscopy using the standard AODC technique (4) for bacteria, and proflavine staining technique (5) for HNF.

Bacterial cell production was measured with the ^{3}H-thymidine incorporation technique (6). (Methyl- ^{3}H) thymidine was added in 10 ml samples at a final concentrations of 10 nM (specific activity 86 Ci mmol^{-1}; Amersham Ltd, UK). Triplicate samples and a formalin killed adsorption control (final conc. 0.5%) were incubated at *in situ* temperature in the dark for 1 h. The incubations were stopped with formalin (final conc. 0.5%). To each 10 ml sample and control an equal volume of ice-cold 10% (wt/vol) TCA was added and mixtures were kept on ice for 15 min. The TCA-insoluble fraction was collected by filtering the sample through a 25 mm 0.2 μm pore size cellulose nitrate filter. The filters were rinsed five times with 1 ml of ice-cold 5% (wt/vol) TCA. The filters were dried, placed in scintillation vials, dissolved in 10 ml Filter-count™ (Packard scintillation cocktail) and counted after 24 h storage in a scintillation counter (Packard Tricarb 2500 TR).

Grazing on bacteria was estimated by the size fractionation technique (7, 8) using diffusion chambers with and without predators, as a difference in bacterial growth between ungrazed and grazed samples.

Results and discussion

Diel fluctuations of bacterial and HNF counts are shown in Figure 1. Bacterial abundance increased during afternoon and early evening, reaching maximal number at 18 h, and declined during night, reaching minimal number at midnight. Bacterial abundance at 18 h was nearly twice that at 24 h. On the contrary, abundance of HNF peaked during night and early morning, whereas low values were measured during daylight. Maximal values of HNF abundance were recorded between 3 h and 6 h in the morning, whereas minimal abundance was found at 12 h.

Diel variations of thymidine incorporation rate (TI) and specific thymidine incorporation rate (STI) or thymidine incorporation per bacterial cell are shown in Figure 2. TI peaked in the afternoon to early evening (between 15 h and 21 h), whereas minimal values were measured at night and early morning. STI showed nearly the same diel rhythm as TI suggesting that increase in bacterial production during daylight was not only because there were more bacteria, but, on average, each cell grew faster.

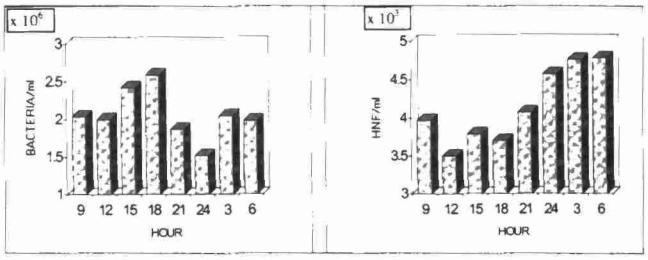

Fig. 1. Diel fluctuations of bacterial and HNF abundances.

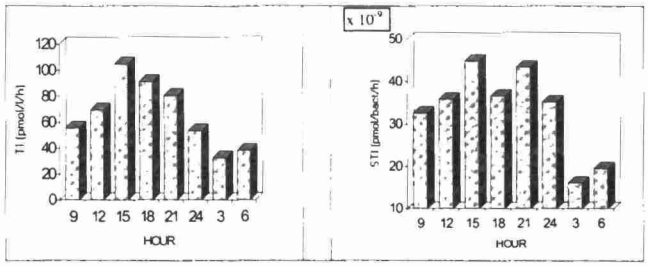

Fig. 2. Diel fluctuations of thymidine incorporation (TI) and specific thymidine incorporation (STI) rates.

The same diel pattern in bacterial production with maximal values during afternoon and early evening and minimal values during night and early morning have been reported for other marine environments (9, 10, 11). Higher bacterial production and abundance during the day than at night was also reported for the north Adriatic Sea (12), western Mediterranean Sea (13), southern California Bight (2) and Bothnian Sea (14). Diel variations in oxygen consumption and bacterial production indicated that bacteria were substrate limited during the night and early morning (11). This suggests that bacterial production may be supported by a small labile fraction of the total DOC pool which may be turned over remarkably fast (15, 16). However, other studies reported inconsistent or no diel variation in bacterial activities (1, 17, 18). The reason whether diel pattern in bacterial activities are observed or not could lie in the source of organic matter that supports bacterial growth. Thus, if phytoplankton are the predominant substrate source, diel variations in bacterial activities are expected. The higher abundance of HNF during the night, particularly during the second half of the night and early morning were in accordance with several studies in which HNF abundance increase occurred during the night (12, 19), during the night and early morning (13) and during the morning (2).

Grazing on bacteria also showed diel rhythm with maximal values between 18 h and 21 h, and minimal between 9 h and 12 h (Fig. 3). However, the average specific grazing rate on bacteria was higher during the dark period than during daylight (Tab. 1).

Production exceeded grazing during daylight, whereas grazing exceeded production during dark period (Fig. 3). Thus, positive net growth rates were recorded during daylight and negative during night (Table 1). Bacterial net production was near zero during the period from 15 h to 21 h, suggesting equilibrium between growth and grazing