REGULATION OF BACTERIAL ABUNDANCE ALONG THE TROPHIC GRADIENT IN THE CENTRAL ADRIATIC

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Abstract

Bacterial and heterotrophic nanoflagellates (HNF) abundance, as well as bacterial production and chlorophyll *a* levels, were measured at five sites extending from the coastal zone toward the open Adriatic in the period from March to October 1995. The investigated areas were grouped into trophic categories according to concentrations of chlorophyll *a*. All investigated parameters increased along the trophic gradient, leading to eutrophy, but they did not increase at the same rate. Different increasing rates of individual parameters caused decreasing B/chl *a* and increasing B/HNF ratios with increasing trophy which might reflect the different structures of microbial food web. In the oligotrophic system bacterial abundance was more closely related to bacterial production and chl *a* than in the eutrophic system, suggesting stronger control of bacterial abundance by substrate supply. On the other hand, the coupling between bacteria and HNF and uncoupling between bacterial abundance and production in the eutrophic system, showed that the importance of bacteriovory increased in richer system.

Key-words: bacteria, biomass, Adriatic Sea

Introduction

Many authors have shown that bacterial abundance changes with trophic state in freshwater and marine systems (1, 2, 3). Within these systems, the trophic relationships are undoubtedly very complex and characterized by numerous feedbacks. There is a considerable empirical evidence of resource control of bacteria (bottom-up control) and of grazing control of bacteria (top-down control) (4). Large scale comparative studies demonstrate strong correlations between bacterial abundance and bacterial productivity and between bacteria and chlorophyll, suggesting significant resource regulation of bacteria (1, 5, 6). On the other hand, comparisons of the abundances of heterotrofic nanoflagellates (HNF) and bacteria imply that in some cases predatory control (top-down regulation) of bacteria may be of major importance in eutrophic environments (7, 8).

One way to determine whether regulation of the bacteria is by top-down or bottom-up control is to consider how bacterial abundance and growth rates change along a resource gradient. For this purpose, we evaluated the regulation of bacterial abundance by comparing the relationship between bacterial abundance and production, between bacteria and chl *a* and between bacteria and HNF along a range of trophic gradient in the Adriatic sea.

Material and methods

Samples for bacterial and HNF counts, chl *a*, and bacterial production were collected on monthly basis from March to October 1995 at a 5 stations (A - E) located from coastal zone toward the open Adriatic. Samples for counting were poured into sterile, acid washed, glass bottles, fixed with formalin (final conc. 2%), and processed in the laboratory within two days after collecting.

Chlorophyll *a* content was measured on a Turner 112 fluorometer after acetone extraction (9).

Enumeration of bacteria and heterotrophic nanoflagellates (HNF) were made by epifluorescence microscopy using the standard acridine orange direct counting technique (10) for bacteria, and proflavine staining technique, which enable distinguishing of heterotrophic from autotrophic cells, for HNF (11). For biovolume estimates, length and width of bacterial and HNF cells were measured with an eyepiece graticule (New Porton G12; Graticules, Ltd, UK). Biovolume was converted to carbon biomass assuming 0.220 pg C μm^3 for bacteria (12) and HNF (13).

Bacterial cell production was measured with the ^{3}H-thymidine incorporation technique (14). (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).

Results and discussion

The investigated areas were grouped into trophic categories according to concentrations of chl *a* and were arranged by increasing chl *a* concentrations (Fig. 1).

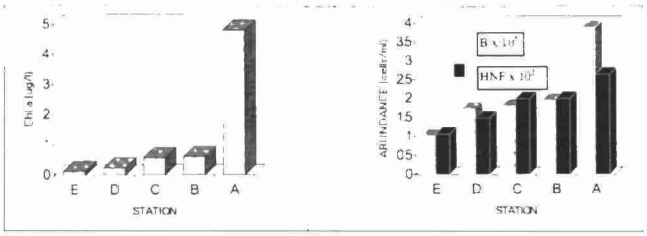

Fig. 1. Average concentrations of chl *a* and abundance of bacteria (B) and HNF at the investigated sites.

Chl *a* concentrations in the oligotrophic area (E site) ranged from 0.04 to 0.57 mg m^{-3} with a mean value of 0.126 ± 0.015 mg m^{-3} (SE), in the eutrophic area (A site) from 0.16 to 18.36 mg m-3 with a mean value of 4.8 ± 1.14 mg m^{-3}. Ratio of the average chl *a* concentrations in the oligotrophic system to that in eutrophic area was 1 : 34. In the mesotrophic area (B, C, D sites) chl *a* concentrations averaged 0.239 ± 0.04 mg m^{-3}, 0.572 ± 0.071 mg m^{-3} and 0.613 ± 0.51 mg m^{-3} at sites B, C and D respectively (Fig. 1).

Abundances of bacteria and HNF showed similar patterns along the trophic gradient as shown by chl *a* concentrations (Fig. 1), but the ratios of bacterial and HNF abundance in the oligotrophic system to those in the eutrophic systems was considerably lower than of chl *a*. Thus, a 34-fold increase in the chl *a* level was accompanied by only 4-fold increase in bacterial abundance and 2.5-fold increase in HNF abundance.

The coefficient of determination, which measure the degree of association between bacteria and phytoplankton (chl *a*) along a trophic gradient was $R^2 = 0.36$ ($p < 0.001$; $n = 35$). That is, 36% of the variability in bacterial abundance can be explained by concentration of chl *a*. However, in the oligotrophic system bacterial abundance was much more closely related to phytoplankton ($R^2 = 0.94$; $p < = 0.001$; n=7), than in the eutrophic system where R^2 was not statistically significant.

BB/chl *a* ratio also changed with trophic status. The ratio was extremely high in the oligotrophic system (10.5 ± 0.94) and decreased gradually toward the eutrophic site where BB/chl *a* ratio was only 0.8 ± 0.21 (Fig. 2). A striking result from this study is that in oligotrophic waters bacterial biomass exceeded phytoplankton biomass. We do not know what mechanisms are responsible for maintaining bacterial abundance at relatively high levels even in highly oligotrophic waters. This may be due to changes in the way algae and bacteria compete for nutrients in waters of differing trophic status. Curie (6) proposed that in very oligotrophic systems both bacterial and algal growth is P limited. Because bacteria are better competitors when orthophosphate is very scarce, bacteria obtain a larger share of the P, and increase in abundance relatively more rapidly than do algae. In richer systems, bacterial growth becomes simultaneously P and C limited. Algae then obtain greater portions of P, and algal abundance begins to increase