## SIMULTANEOUS EFFECT OF DIFFERENT VARIABLES ON BACTERIAL AND HNF ABUNDANCES IN KASTELA BAY (ADRIATIC SEA)

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# Abstract

Simultaneous effects of temperature, concentration of chl a and bacterial production on bacterial abundance, as well as of temperature, bacterial abundance and bacterial production on heterotrophic nanoflagellate (HNF) abundance were studied monthly from January 1997 to December 1998 in the coastal middle Adriatic Sea. The results showed that bacterial abundance was not limited by substrate supply. The effect of temperature on bacterial abundance was very high, and temperature obscured the effect of bacterial production, suggesting that bacterial growth itself is highly temperature dependent. About 60% of the variability in HNF abundance can be explained with bacterial abundance, bacterial production and temperature.

Key words: Bacteria, heterotrophic nanoflagellates, Adriatic Sea

#### Introduction

Distribution and dynamics of microbial organisms are result of the complex interactions among environmental variables and interspecies relationships. Heterotrophic nanoflagellates (HNF) have been identified as a major source of bacterial mortality in aquatic ecosystems, but their predation pressure is found to be dependent on temperature and trophic state of the studied area (1, 2). The effects of these factors are not always clear as they can act simultaneously, changing their relative importance spatially and temporally. The aim of this study was to gain a better understanding of seasonal patterns of bacterial and HNF abundances according to simultaneous effect of different factors.

#### **Material and Methods**

Sampling was conducted monthly, from January 1997 to December 1998 at a coastal station located in an enclosed shallow basin Kaštela Bay. Phytoplankton biomass was estimated from chl *a* concentrations using fluorimetric methods (3). Enumeration of bacteria and HNF were made by epifluorescence microscopy using the standard AODC technique (4) for bacteria, and proflavine staining (5) for HNF. Bacterial cell production was measured from DNA synthesis based on incorporation rates of 3H-thymidine (6).

## **Results and discussion**

Bacterial abundance was positively correlated with temperature (r = 0.55; P < 0.001), while correlation with bacterial production was statistically significant but relative low (r = 0.289; P < 0.05) (Table 1). On the other hand, bacterial abundance was not related to chl a concentration, suggesting that input from land is more important source for substrate supply than phytoplankton. However, since the Kaštela Bay receives large quantities of nutrients throughout the year, this location shows conditions in which substrate concentrations are almost always above saturating level. Therefore, it seems that temperature was the main factor that determined whether bacteria attain maximal growth. Analysis of simultaneous effect of temperature and bacterial production on bacterial abundance showed that effect of temperature masked effect of bacterial production. That is, the effect of bacterial production on bacterial abundance failed to occur when temperature stayed constant, suggesting that in Kaštela Bay, bacterial growth itself is highly temperature dependent seasonal phenomenon (7).

Table 1. Simultaneous effect of different variables on bacterial and HNF abundances.

| Dependent variable | Independent variables | r      | r <sub>p</sub> | β     | R     | R <sup>2</sup> (%) |
|--------------------|-----------------------|--------|----------------|-------|-------|--------------------|
| Bacteria           | Temperature           | 0.549  | 0.589          | 0.615 | 0.591 | 35                 |
|                    | Chl a                 | 0.055. | 0.254          | 0.230 |       |                    |
| Bacteria           | Temperature           | 0.549  | 0.523          | 0.519 | 0.565 | 32                 |
|                    | Bacterial production  | 0.249  | 0.160          | 0.137 |       |                    |
| Bacteria           | Chl a                 | 0.055  | 0.041          | 0.040 | 0.304 | 6                  |
|                    | Bacterial production  | 0.289  | 0.286          | 0.247 |       |                    |
| Bacteria           | Temperature           | 0.549  | 0.564          | 0.587 |       |                    |
|                    | Chl a                 | 0.055  | 0.249          | 0.216 | 0.601 | 36                 |
|                    | Bacterial production  | 0.249  | 0.132          | 0.110 |       |                    |
| HNF                | Bacterial abundance   | 0.754  | 0.665"         | 0.693 | 0.760 | 58                 |
|                    | Temperature           | 0.493  | 0.141          | 0.111 |       |                    |
| HNF                | Bacterial abundance   | 0.754  | 0.745          | 0.722 | 0.773 | 60                 |
|                    | Bacterial production  | 0.306  | 0.255          | 0.170 |       |                    |
| HNF                | Temperature           | 0.493  | 0.454          | 0.445 | 0.530 | 28                 |
|                    | Bacterial production  | 0.306  | 0.224          | 0.201 |       |                    |
| HNF                | Bacterial abundance   | 0.754  | 0.668          | 0.680 |       |                    |
|                    | Temperature           | 0.493  | 0.105          | 0.080 | 0.775 | 60                 |
|                    | Bacterial production  | 0.306  | 0.237          | 0.159 |       |                    |

r - coefficient of correlation - rp - coefficient of partial correlation - b (beta coefficient) - regression coefficients (b) stated in terms of their standard deviations - R - coefficient of multiple regression - R2 (%) - coefficient of multiple determination; measure of the proportion (percentage) of variance explained

\*\* P < 0.01; \* P < 0.05.

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HNF abundance was positively correlated with temperature, bacterial abundance and bacterial production (Table 1). The strongest correlation (r = 0.754; P< 0.001) was found with bacterial abundance. The coefficient of multiple determination (R<sup>2</sup>), which measures the overall degree of association between HNF abundance and independent variables, varied from 0.58 to 0.60. That means that about 60% of the variability in HNF abundance can be explained with bacterial abundance, bacterial production and temperature. The highest relative importance of bacterial abundance in controlling HNF abundance is shown by the coefficients of partial correlation (r<sub>p</sub>) and beta coefficients ( $\beta$ ) (Table 1). Bacterial abundance obscured the effects of both other factors, particularly the effects of temperature. This result suggests that bacterial abundance itself was highly temperature dependent, since temperature influences variation in HNF abundance indirectly through the changes in bacterial abundance.

As we stated before, inconsistent relationship between bacterial abundance and productivity could be result of conditions in which substrate supply is above saturation level, but it could also suggests conditions in which mortality factors such as bacterivory and viral lysis are very strong. It seems that in Kaštela Bay temperature controlled not only bacterial abundance but also the abundance of bacteriovorous protozoa, which in turn determined bacterial abundance by high grazing pressure. In these conditions, grazing was a main control of bacterial abundance, particulary during summer. The weak relationship between bacteria and HNF during colder months could be a result of the weak grazing pressure on bacteria by HNF, as well as of high grazing pressure on HNF by ciliates (1, 2, 7). This is supported by the study that found high ciliate abundances during winter, and low abundance and grazing pressure on HNF during summer in Kaštela Bay (8).

In conclusion, this study suggests that relative importance of various factors in regulating bacterial and HNF abundances might change over the seasonal scale. In the coastal area, bacteria were not limited by substrate supply, but were rather controlled by HNF grazing and temperature. Moreover, the fluctuation of bacterial abundance explained significant part of variance in HNF abundance.

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