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The Role of Bacteria in Marine Food Web

Uloga bakterija u morskom hranidbenom lancu

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Summary

Biomass and production of bacteria and heterotrophic nanoflagellates (HNF) as well as grazing on bacteria were measured seasonally during 1992/93 in Kaštela Bay (Adriatic Sea). Strong seasonality of all studied parameters was established with maximum values obtained during the warmer summer-autumn period. In that period bacteria showed a twofold higher production while HNF showed a threefold higher biomass and ninefold higher production then during colder winter-spring period. Moreover, bacterial predators showed sevenfold higher grazing on bacteria in warmer period. During the summer-autumn period bacterial carbon daily flux through predator organisms was higher than bacterial biomass pool, while in the colder period bacterial daily flux accounted only for 8 % of presented biomass pool. These results suggest that during the summer-autumn period in Kaštela Bay, bacteria could be an important link between primary production and higher trophic levels.

Sažetak

Biomasa bakterija i heterotrofnih nanoflagelata (HNF) i njihov prinos u jedinici vremena te hranidba bakterijskih predatora istraživani su sezonski u razdoblju od 1992. do 1993. u Kaštelanskom zaljevu. Utvrđena je jaka sezonska ovisnost svih određivanih parametara o maksimalnim vrijednostima tijekom toplijeg ljetno-jesenskog razdoblja.

Tada je u jedinici vremena prinos bakterija bio dva puta veći, HNF-a devet puta veći, a biomase HNF-a tri puta veći od vrijednosti utvrđenih u hladnijem zimsko-proljetnom razdoblju. Osim toga zapaženo je u toplijem razdoblju sedmerostruko povećanje hranidbe bakterijskih predatora koji se hrane bakterijama. U ljetno-jesenskom razdoblju dnevni je protok bakterijskog ugljika kroz organizme predatora bio veći od utvrđene bakterijske biomase, za razliku od zimsko-proljetnog razdoblja kada je dnevni protok bakterijskog ugljika bio svega 8 % od te biomase. Izneseni rezultati pokazuju da za toplijeg vremena u Kaštelanskom zaljevu bakterije mogu biti značajna veza između primarne proizvodnje i viših trofičkih razina.

Introduction

In the 'classical' view bacteria were considered only as decomposers of organic material in the pelagic food web. However, with the introduction of new techniques, our perception of the functioning of the pelagic food web has changed in the last 10 years. It is clear today that the biomass and production of all microorganisms is much greater than was previously assumed, and that bacteria and their microbial consumers are generally important components of energy flow in aquatic ecosystems. Moreover, it has been hypothesized *(1)* that there is a separate flow of energy through the microbial components of the food web ('microbial loop') which exist within pelagic systems. In the microbial loop planktonic bacteria are able to use dissolved organic substrates, derived from phytoplankton, incorporating them in their own biomass. In such a way heterotrophic bacteria consume more than 50 % of primary production *(2)*. Bacterial biomass is channelled through the microbial food web by protozoan grazing. Within the bacterivorous protozoa the heterotrophic nanoflagellates (HNF) and ciliates are the most important *(3-6)*. The aim of this study was to determine seasonal patterns of bacterial and HNF biomass and production, as well as grazing rate on bacteria by HNF and ciliates in a coastal marine ecosystem. Seasonal fluctuations of bacterial carbon flux through microbial loop was particularly studied.

Experimental

Samples were collected seasonally at the station located in Kaštela Bay (middle Adriatic Sea). The average surface temperatures were 13.3 °C in spring, 22.3 °C in summer, 19.8 °C in autumn and 11.9 °C in winter. Sampling was performed from 0, 10, 20 and 35 m depths, and mean values integrated for the entire water column were used as input data for the analysis. Counting of

bacteria and HNF were made by epifluorescence microscopy using the standard AODC technique *(7)* for bacteria, and proflavine staining technique *(8)* for HNF. For biovolume estimates, length and width of cells were measured with an eyepiece graticula (New Porton G12; Graticules, Ltd, England). Bacterial volumes were calculated as 0.785 W^2 (L-$W/3$), where W is width and L is length *(9)*, and biovolume was converted to carbon biomass assuming 0.121 pg μm^{-3} *(10)*. Volumes of HNF were calculated for rounded cells as spheres and for elongated cells as rotational ellipsoids with circular cross-section given by $V = (\Pi \times L \times W^2)/6$, where W is width and L is length. HNF biovolume was converted to carbon biomass assuming 0.22 pg μm^{-3} *(9)*. Bacterial cell production was measured with the ^{3}H-thymidine incorporation technique *(11)*. (Methyl-^{3}H) thymidine was added in 10 mL samples at final concentrations of 10 nmol L^{-1} (specific activity 86 Ci $mmol^{-1}$). Triplicate samples and a formalin killed adsorption control (final volume ratio 0.5 %) were incubated for 1 h. The incubations were stopped with formalin (final volume ratio 0.5 %). The thymidine samples were extracted with ice-cold TCA *(11)*. The TCA-insoluble fraction was collected by filtering the sample through a 25 mm 0.2 μm pore size Sartorius filter. The conversion factors to estimate bacterial cell production from ^{3}H-thymidine incorporation were calculated from the < 1 μm size fraction *(12)*. Cell production of HNF was estimated using a filtration/inoculation method *(13)*. Production rate was expressed as an increase in flagellates number in grazers free samples (filtered through a 1 μm Nucleopore polycarbonate filter) after incubation at in situ temperature. Grazing on bacteria in HNF (< 8 μm), and ciliate (< 100 μm) fractions were calculated from the difference between bacterial cell production measured with the ^{3}H-thymidine technique and observed numbers of bacteria *(14)*. Grazing on HNF was estimated from the difference in HNF growth between ungrazed (< 8 μm) and grazed (< 100 μm) samples.

Results and Discussion

Bacterial carbon biomass ranged from 8.65 μg L^{-1} in autumn to 19.02 μg L^{-1} in summer showing mean value of 15.11 μg L^{-1}. The values for HNF varied from 2.21 μg L^{-1} in spring to 11.75 μg L^{-1} in autumn with mean value of 6.93 μg L^{-1}. High bacterial biomass presented during spring and summer was followed with marked decrease in autumn. Another bacterial peak was observed in winter (Fig. 1A). On the other hand, HNF biomass was significantly higher during the warmer part of the year (summer-autumn) in comparison with colder winter-spring period. High HNF biomass in summer and autumn could be a response to bacterial biomass summer peak, but there is no response to bacterial winter peak. Therefore, correlation between bacterial and HNF biomass was not established across the year, probably as a result of predator-prey oscillations, and complex trophic interactions with numerous feedbacks *(15,16)*. The ratio between bacterial and HNF abundance ranged between 0.7 in autumn and 7.1 in spring with mean value of 4.1. These values are in accordance with several field studies *(17,18)*. Higher values of ratio were observed during the colder part of the year (winter-spring). In autumn, HNF biomass maximum corresponded to bacterial biomass minimum resulting in higher HNF biomass in comparison with bacterial biomass.

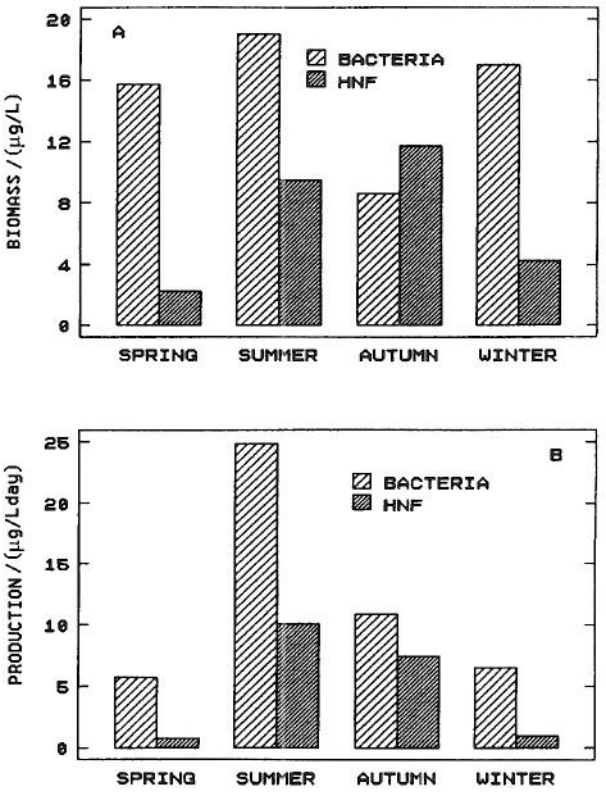

Fig. 1. Seasonal fluctuations of bacterial and HNF biomass (A) and production (B)

Slika 1. Sezonska kolebanja bakterijske i HNF biomase (A) i njihova prinosa u jedinici vremena (B)

Bacterial carbon production ranged from 5.70 μg L^{-1} day^{-1} in spring to 24.87 μg L^{-1} day^{-1} in summer, with mean value of 12.28 μg L^{-1} day^{-1}. Production of HNF varied between 0.76 μg L^{-1} day^{-1} in spring and 10.09 μg L^{-1} day^{-1} in summer with mean value of 4.80 μg L^{-1} day^{-1}. Both bacterial and HNF production were at the maximum during summer (Fig. 1B). Therefore, high correlation between bacterial and HNF production was established ($R^2 = 0.81$; $P < 0.001$; $n = 48$) suggesting a strong trophic relationship between these two groups of organisms. HNF biomass production accounted on average for 39 % of bacterial production. This value varied from 13 % in spring to 68 % in autumn. An average biomass-specific productivity, which is useful in comparing the productivity of microorganisms of different sizes, was 0.034 h^{-1} and 0.024 h^{-1} for bacteria and HNF, respectively. Population doubling time of bacteria ranged from 0.6 days in autumn to 4.1 days in spring, with mean value of 2.1 days. Doubling time of HNF population ranged from 0.8 days in summer to 3.8 days in winter, with mean value of 2.7 days. Therefore, population doubling time was very similar for both bacteria and HNF, but their seasonal distribution was somewhat different.

Total grazing on bacteria by protozoa (expressed as carbon biomass) ranged from 2.25 μg L^{-1} day^{-1} in spring to 24.18 μg L^{-1} day^{-1} in summer with mean value of 12.32 μg L^{-1} day^{-1}. Seasonal differences of grazing rates

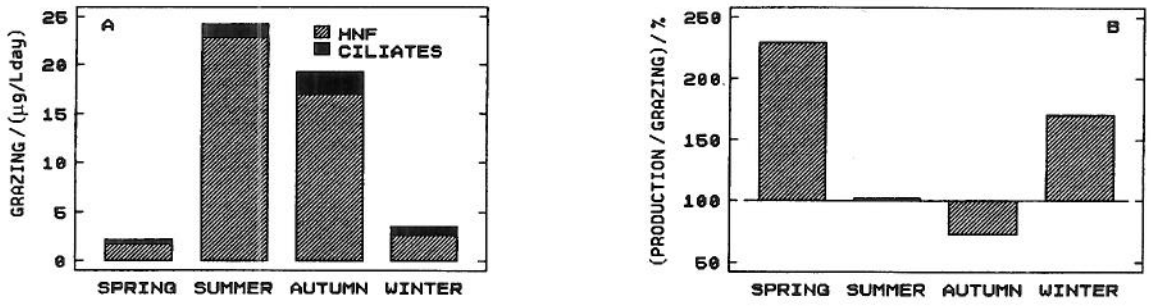

Fig. 2. Seasonal fluctuations of grazing rate on bacteria (A) and ratio between bacterial production and total grazing on bacteria (B)
Slika 2. Sezonska kolebanja brzine hranidbe bakterija (A), te omjer između prinosa bakterijske biomase i ukupne brzine hranjenja (B)

were very expressed with maximum values during the warmer part of the year (summer-autumn period). On the other hand, very low grazing was measured during the winter and spring (Fig. 2A). Small HNF (< 8 µm) were the most important bacterial grazers throughout the year accounting on average for 80 % of the total grazing on bacteria. The contribution of HNF to total grazing on bacteria ranged from 75 % in winter to 94 % in summer. Thus, in the warmer part of the year bacterial standing stock was nearly completely controlled by HNF. Similar results were observed in other marine environment *(6,19,20)*. An average HNF cell ingested 21.8 bacteria per hour, and cleared 19.5 nL of seawater per hour, which corresponded to clearance of 80.4 % of the water column per day by HNF population. These values are supported by a number of studies from different marine and freshwater environment *(21)*. Grazing activity of HNF was at the maximum during the warmer part of the year. Maximum ingestion rate of 39.4 h^{-1} was observed in summer, and minimum of 11.2 h^{-1} in winter. Water column clearance by HNF also showed strong seasonal oscillations. In the warmer part of the year HNF cleared > 100 % of the water column per day (113 % in summer, and 182 % in autumn), while in the colder part of the year < 20 % of the water column was cleared per day (9 % in spring and 16 % in winter). Grazing on bacteria showed a strong positive relationship with bacterial production ($R^2 = 0.81$; $P < 0.001$; $n = 48$). Bacterial production was significantly higher than grazing on bacteria in winter and, particularly, in spring. On the other hand, grazing on bacteria exceeded bacterial production in autumn, while in summer bacterial growth was approximately balanced by HNF plus ciliates grazing (Fig. 2B).

In the most part of the year bacterial production satisfied carbon requirements of HNF. Bacterial carbon production accounted an average for 178 % of the HNF carbon requirement with maximum values of 289 % recorded in spring. HNF carbon requirements exceeded bacterial production only in autumn, when bacterial production satisfied 70 % of the HNF carbon requirement. This suggests that in autumn HNF satisfied a part of their carbon requirement from sources other than bacteria *(22)*. However, in Kaštela Bay bacteria were capable to support completely HNF growth during the most part of the year. Moreover, bacteria satisfied on average 32 % of ciliates carbon requirements with maximum value of 42 % in winter.

Incorporation of bacterial biomass into biomass of predators showed marked seasonal oscillations (Fig. 3). Thus, according to ecological importance of microbial loop, two different periods of the year were distinguished. During the warmer part of the year (summer-autumn), bacterial carbon flux through microbial loop was high, and significant part of bacterial carbon was channelled to higher trophic levels. In this period 99–143 % of bacterial production or 122–197 % of bacterial standing stock per day was removed by grazers suggesting that grazing could be a major factor in controlling bacterial standing stock. HNF incorporated in its own biomass 7–9 µg L^{-1} day^{-1} or 38–50 % of bacterial production. Ciliates incorpo-

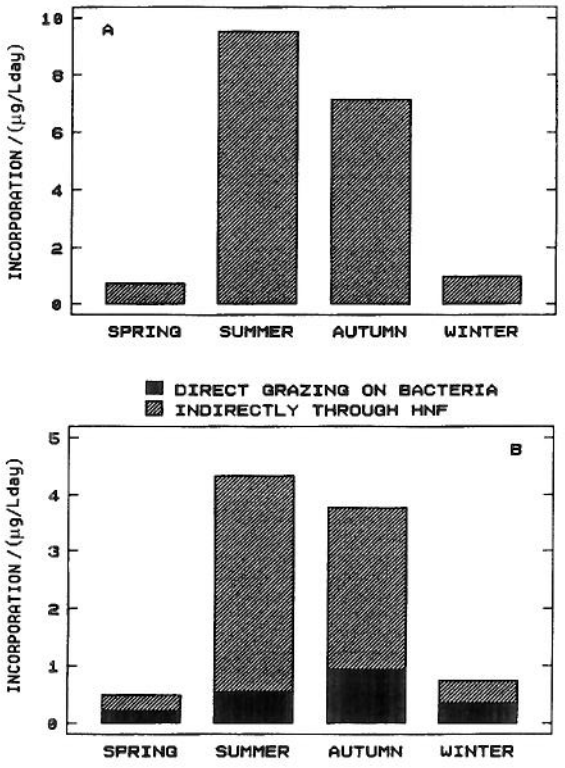

Fig. 3. Seasonal fluctuations of incorporation of bacterial biomass into HNF (A) and ciliate (B) biomass
Slika 3. Sezonska kolebanja ugradnje bakterijske biomase u biomase HNF-a (A) i ciliata (B)

rated about 4 μg L^{-1} day^{-1} of bacterial biomass or 18–29 % of bacterial production. About 80 % of bacterial biomass incorporated in ciliate component previously passed through HNF component, whereas only 20 % was the result of direct grazing on bacteria. In this period turnover time of bacterial biomass was about 1 day. Thus, during the warmer part of the year, microbial loop could be an important link between primary production and higher trophic levels. On the other hand, during the colder part of the year (winter-spring) bacterial carbon flux through microbial loop was very low. Only 12–22 % of bacterial standing stock per day (55–70 % of bacterial production) was removed by grazers. Less than 1 μg L^{-1} day^{-1} or less than 20 % of bacterial production was incorporated in both HNF and ciliate biomass. Almost 50 % of bacterial biomass incorporated in ciliate component was the result of direct grazing on bacteria. Turnover time of bacterial biomass was considerably longer than in the warmer period, and ranged between 3.8 and 5.7 days. Although the grazing pressure on bacteria was reduced, bacteria were not capable of rapid increase of production. This suggests that in this period a major factor in controlling bacterial standing stock was not grazing but environmental factors such as temperature and/or substrate supply. Therefore, during the colder part of the year microbial loop acted as a mineralization system rather than as a link to higher trophic levels.

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