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Bacterial production in the stratified karstic estuary of the Krka River

Dragica FUKS, Robert PRECALI and Massimo DEVESCOVI

"Ruđer Bošković" Institute, Center for Marine Research, Rovinj, Croatia

Spatial bacterial abundance and production in the stratified Krka River estuary were analyzed in the fall of 1990. The bacterial abundance (0.3 - 2.6 x 10^9 cells l^{-1}) within the estuary was several times higher than in the open central Adriatic waters, but was lower than in the mostly eutrophicated western part of the northern Adriatic. Meanwhile, the bacterial production (0.11 - 5.76 μg C l^{-1} h^{-1}) was of the same ranges as in the open northern Adriatic waters. A strong overall correlation between chlorophyll a content and bacterial numbers, production and growth rate, respectively, was recorded along the depth profile. The bacteria to phytoplankton biomass index lower than 1 at the brackish layer indicates that bacterial production is mostly supported by phytoplankton activity. Halved drop (35%) of bacterial number between the brackish and seawater layers compared to a 60% decrease of the flagellates number indicated a net bacterial production below the halocline which grazing was unable to match. A prevailing bacterial remineralization in the seawater layer is supposed.

INTRODUCTION

Since heterotrophic bacteria consume a large fraction of primary production (~ 50%) in both marine and freshwater pelagic ecosystems (COLE *et al.*, 1988), the understanding of the variation and control of bacterial production is essential for examination of the fate of primary production and structure of pelagic ecosystems. Although primary production is important in determining the rate of bacterial production, other factors apparently complicate this relationships. The studies of ALBRIGHT (1983), and DUCKLOW and KIRCHMAN (1983) suggested that "normal" relationships between bacteria and phytoplankton do not hold in river plumes. Recently, KIRCHMAN *et al.* (1989) ascribed the microbial loop relationships disruption in the Rhone River plume to the allochtonous carbon input.

The estuary of the Krka River on the eastern Adriatic coast is characterized by low terrigenous input due to karstic drainage area. A permanent stratification occurs between the bottom seawater which enters the estuary at the depth and the surface fresh or brackish water (ŽUTIĆ and LEGOVIĆ, 1987). Previous depth profile examinations of the Krka River estuary did not reveal a significant correlation between chlorophyll *a* and bacterial abundance and activity (FUKS *et al.*, 1991). In this paper relationships between chlorophyll *a* and bacterial production in the Krka River estuary are described and discussed.

MATERIALS AND METHODS

The Krka River estuary, which has a surface area of 21.3 km^2 and a volume of 0.3 km^3 extends 25 km into the heartland of the karstic Dalmatian region of Croatia. In September 1990 during three consecutive days sampling was conducted at five stations in the Krka River estuary and at two stations in the central Adriatic coastal waters (Fig. 1). Scuba divers collected water samples, who used horizontally NISKIN samplers at the surface (0.5 m depth), halocline layer, at 6 m depth and at 1 m above bottom.

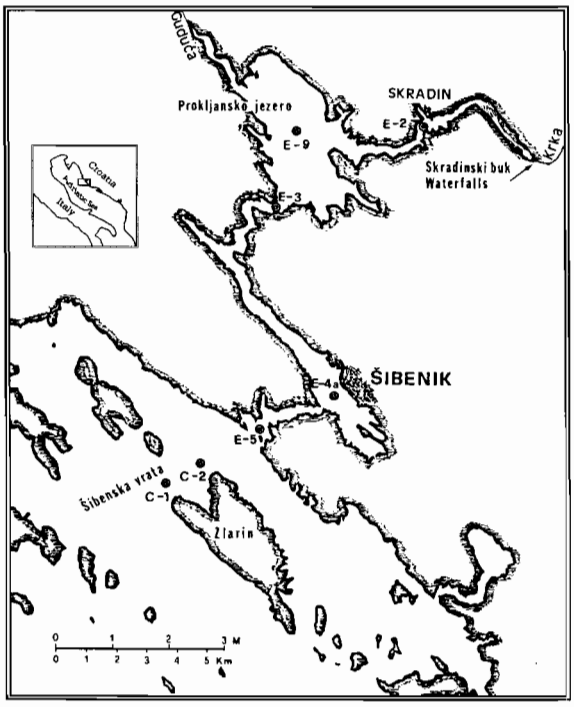

Fig. 1. The investigated area with sampling stations

Numbers of bacteria and heterotrophic nanoflagellates were determined by epifluorescence microscopy using acridine orange staining for bacteria (HOBBIE *et al.*, 1977.) and the fluorochrome primulin staining for flagellates (CARON, 1983), respectively. The bacteria cell volume was measured by the image analysis (BJØRSEN, 1986). The bacterial biomass was estimated through a conversion factor of 20 fg C cell^{-1} (LEE and FUHRMAN, 1987).

Incorporation rates of [^{3}H] - thymidine (TdR) were measured to estimate bacterial production (FUHRMAN and AZAM, 1982). Five nM (final concentration) of [^{3}H] - thymidine (85 Ci mmol^{-1}) was added to 10 ml subsamples. Three subsample replicates and formalin-killed blank were incubated at room temperature (20 °C) in dark. After 1 hour incubation, the subsamples were immersed in ice-water for 1 min and then 1.1 ml of ice-cold 50% trichloracetic acid (TCA) was added to samples to extract the soluble pool from the cells. After 5 min of extraction on ice the cold-TCA-insoluble material was collected by filtration through a 0.45 μm pore-size Milipore celluloze nitrate filter. The filters were rinsed with ice-cold 5% TCA and radioasseyed after at least 24 hours. A conversion factor of 2 x 10^{18} cells produced (mol TdR incorporated)$^{-1}$ was used (FUHRMAN and AZAM, 1982). In order to convert the bacterial number into a carbon equivalent a conversion factor of 20 fg C cell^{-1} was used (LEE and FUHRMAN, 1987). Growth rate (averaged for the entire bacterial assemblage) was estimated by dividing the production rates by the bacterial abundance.

Chlorophyll *a* (Chl *a*) concentration was measured in acetone extracts by fluorometry (STRICKLAND and PARSONS, 1972). The phytoplankton biomass was estimated from chlorophyll *a* concentration by assuming 30 μg C (μg Chl *a*)$^{-1}$.

Salinity was measured by an Autolab MK 4 bench salinometer, and temperature by reversing thermometers.

RESULTS AND DISCUSSION

Bacterial abundance (0.3 - 2.6 x 10^9 cells l^{-1}) within the Krka Estuary was of the same range as in the coastal waters (Kaštela Bay) but several times higher than the bacterial abundance in the open central Adriatic waters (KRSTULOVIĆ, 1989). Meanwhile, the bacterial abundance in the estuary was several times lower than in the mostly eutrophicated western part of the northern Adriatic (FUKS and DEVESCOVI, 1990). The highest values were registered in the surface layer at the stations under anthropogenic influence (stations E-2 and E-4a; Fig.2). The same bacterial number variations were noticed during

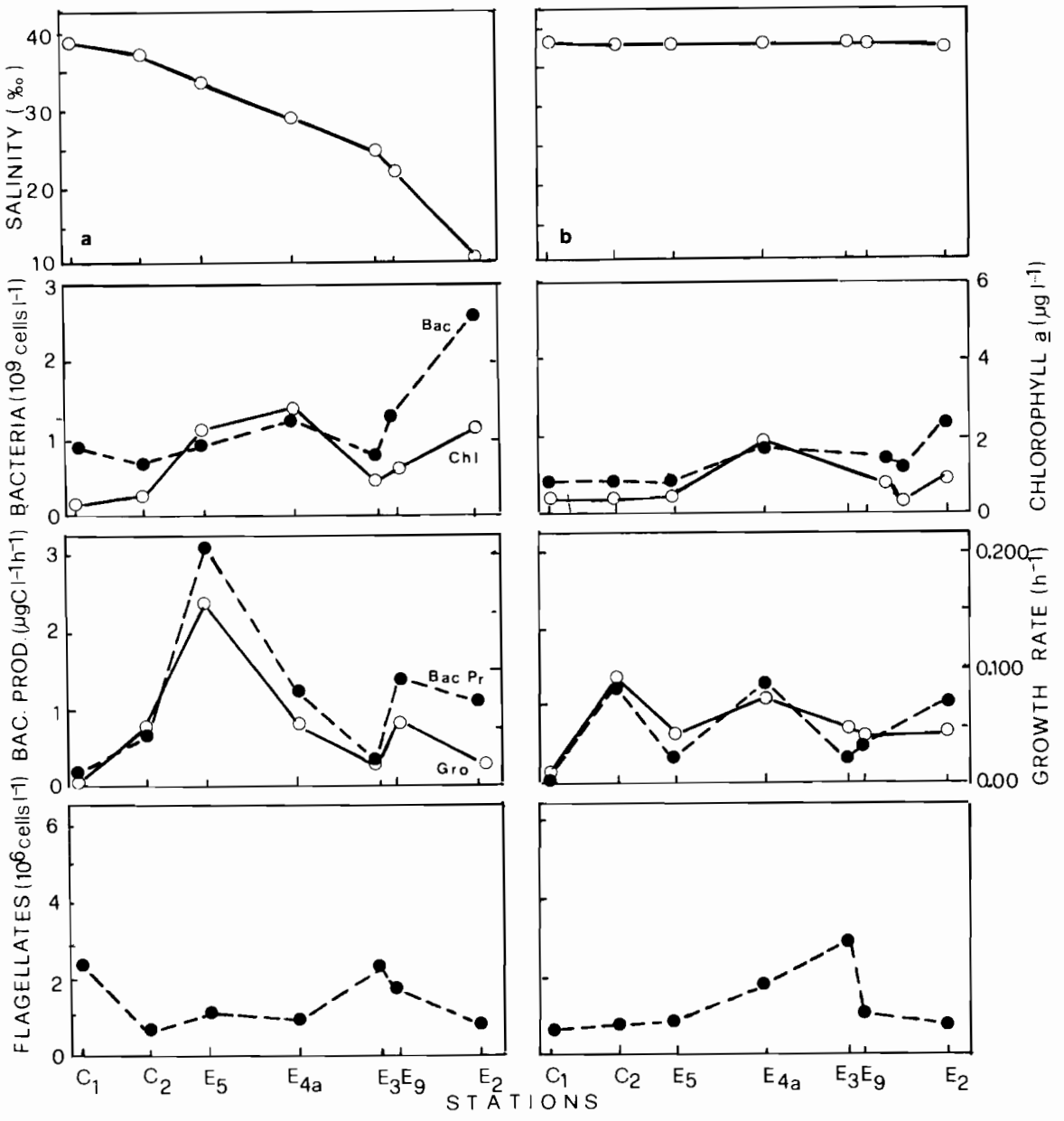

Fig. 2. Transects of salinity and microbial parameters in the surface layer (a) and below halocline (6 - m depth; b) on September 28, 1990.

the springtime sampling over the 1987 - 1989 period (FUKS *et al.*, 1991), as well as during the consecutive days sampling in the fall of 1990.

Results for bacterial abundance within the estuary depth profile obtained on dial time scales during springtime (FUKS *et al.*, 1991). revealed a several fold higher bacterial abundance in a boundary than in a riverine or coastal seawater environment. During the fall an identical pattern was obtained only at station E-2 (Fig. 3). Namely, due to the lowest river flows occurring in late summer and fall, a strong estuary stratification was recorded only at station E-2 (Fig. 2). Also, cell volume measurement revealed free-living smaller bacteria prevalence at the halocline layer of the depth profile (station E-2; Fig. 3). When different salinity layers are concerned the same bacterial volume varia-

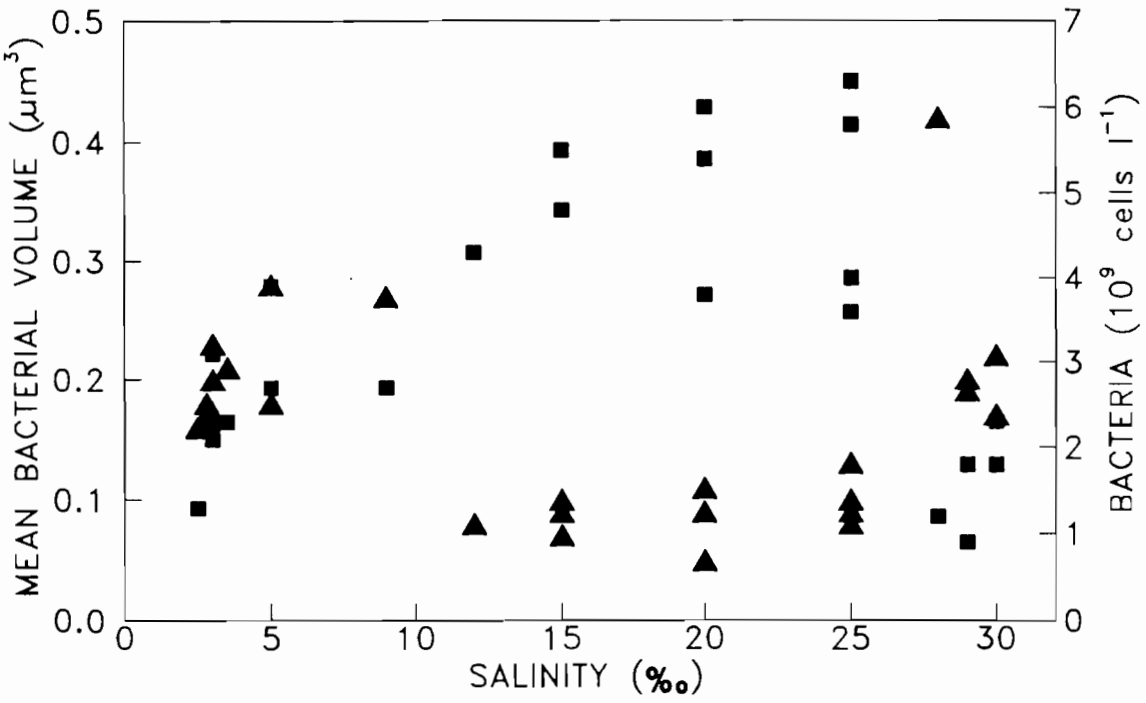

Fig. 3. Variations of bacterial abundance (■) and mean bacterial volume (▲) in salinity profile at station E-2.

tions were recorded in halocline (Sal.: 12-25‰; cell volume: 0.05-0.13 μm^3) and seawater (Sal.: 34-38‰; cell volume: 0.07-0.11 μm^3), respectively. Larger bacteria prevailed in river water (Sal.:2.5-9‰; cell volume: 0.16-0.28 μm^3) and in the lower part of halocline (Sal.: 28-30‰; cell volume: 0.17-0.42 μm^3). When all data were separated into two categories based on salinity values (Table 1), bacterial abundance means of particular layer differed significantly (ANOVA, $F_{1,28} = 7.22$, $p < 0.01$), but a correlation between salinity and bacterial abundance was not recorded when all results were lumped (Table 2).

The pattern of bacterial abundance in an estuary is a result of the bacterial substrate sources in that specific estuary. The substrate supply is a function of physical, chemical and morphological characteristics of each estuary. For example, the highest bacterial numbers could result from the riverine input (ALBRIGHT, 1983), from tidal flushing of a surrounding salt marsh (WRIGHT and COFFIN, 1983), and in the stratification period from higher phytoplankton production (SHARP *et al.*, 1986), respectively. Most of the time, the Krka River is a clear organic-poor medium (ŽUTIĆ and LEGOVIĆ, 1987). Dissolved organic carbon (DOC) in the river water is of the same order as in the seawater (1 - 2 mg l^{-1}). The DOC, which mainly originated from phytoplankton (excretion and autolysis) slightly increased in the halocline (CAUWET, 1991). Although in the fall a lower planktonic activity was noticed in the central estuary (E-4a, max Chl *a* = 4.5 $\mu g\ l^{-1}$) compared to the springtime examination (E-4a, max Chl *a* = 15.2 $\mu g\ l^{-1}$; FUKS *et al.*, 1991), chlorophyll *a* concentration in the upper brackish layer of the entire estuary significantly differed from the seawater layer (Table 2; ANOVA, $F_{1,22} = 8.85$, $p < 0.01$). Contrary to the springtime (FUKS *et al.*, 1991), a high correlation between Chl *a* content and bacterial number appeared during the fall examination (Table 2; $r = 0.70$, $p < 0.001$). Ratios of bacterial to phytoplankton biomass were below 1 in the brackish layer and differed significantly ($p < 0.05$) from those in the seawater layer where the index varied from 0.3 - 1.8 (Table 1). A biomass index far below 1 in the brackish layer indicated that bacterial production and numbers were mostly supported by phytoplankton activity.

Table 1. Summary of selected parameters (± SE) for the Krka River estuary. Stations: E-2, E-3, E-4a, E-5, E-9

Parameter	Brackish water	Sea water
No. of samples	14	16
Salinity (‰)	21.11 (± 8.12)	37.94 (± 0.34)
Temperature (°C)	19.2 (± 0.7)	20.7 (± 1.3)
Chl *a* (μg l^{-1})	1.98 (± 1.35)	0.75 (± 0.47)
Bacterial abundance (10^9 cells l^{-1})	1.16 (± 0.58)	0.73 (± 0.25)
Bacteria / plankton biomass	0.33 (± 0.18)	0.88 (± 0.31)
Bacterial production (μg C l^{-1} h^{-1})	1.84 (± 1.91)	0.72 (± 0.40)
Bacterial growth rate (h^{-1})	0.070 (± 0.064)	0.048 (± 0.017)
Heterotrophic flagellates (10^6 cells l^{-1})	3.12 (± 2.01)	1.18 (± 0.76)

Table 2. Spearman rank correlation matrix for relationships between variables in the water column. S = salinity; Chl *a* = chlorophyll *a*; Bac = bacterial abundance; Prod = bacterial production; Gr = bacterial growth rate; HFl = heterotrophic flagellates abundance. Stations E-2, E-3, E-4a, E-5, E-9.

	S	Chl *a*	Bac	Prod	GR	HFl
S	1.00					
Chl a	-0.53 *	1.00				
Bac	-0.27	0.70 ***	1.00			
Prod	-0.19	0.75 ***	0.86 ***	1.00		
GR	-0.05	0.67 ***	0.62 **	0.90 ***	1.00	
HFl	-0.79 ***	0.48 *	0.09	0.12	0.03	1.00

*, **, *** significant at 0.05, 0.01, 0.001 levels, respectively. The total number of samples was 22.

Within the estuary the bacterial production varied in range from 0.11 - 5.76 μg C l^{-1} h^{-1}, while in the adjacent coastal waters (stations C-1 and C-2) the production ranged from 0.02 - 0.87 μg C l^{-1} h^{-1}. Bacterial production of the Krka River estuary ranges were also registered in polluted coastal waters of the central Adriatic (KRSTULOVIĆ, 1989), as well as in the open waters of the mostly eutrophicated western part of the northern Adriatic (FUKS, unpublished data). The highest bacterial production registered in the surface layer at station E-5 (Fig. 2) was probably provoked by stimulation with oxidized nutrients (ALBRIGHT, 1983) available from mixed seawater and surface layer at this station. The bacterial activity was significantly higher in the brackish layer compared to the seawater layer (Table 1; ANOVA, $F_{1,25} = 4.91$, $p < 0.05$).

A strong overall correlation between bacterial production and Chl *a* content was recorded (Table 2, $p < 0.001$). Although the lumping of results generated a strong correlation between bacterial growth rates and Chl *a* content (Table 2, $p < 0.001$) no significant difference ($p > 0.20$) of growth rates between brackish water and seawater was recorded.

Phytoplankton activity (Chl *a*), bacterial production and heterotrophic flagellates, respectively, dropped for 60% in average between the brackish and seawater layer (Table 1). On the contrary, below the halocline, bacterial abundance decreased for 35% compared to upper layer. Coexistent decrease of bacterial abundance for 35% and bacterial production for 60% between brackish and seawater layer indicated bacterial net production in seawater layer. Although flagellates grazing on bacteria was not measured the net bacterial cells production in seawater layer, compare to upper layer, is only possible for grazing (by flagellates or other bacterivores) was unable to match a 60% decreased bacterial production. Further examination is needed to elucidate whether net bacterial production is result of low grazing pressure in seawater layer or the lag between bacterial production and bacterivores response. It is supposed that more energy is used for cell maintenance than for transfer to a higher trophic level, and the remineralization of DOM under the halocline is a prevailing role of the bacterial standing stock. The intensive use of small organic molecules as energy sources for degradation of higher molecular weight organic compounds was also recorded within the estuary below the halocline during springtime (FUKS *et al.*, 1991).

CONCLUSIONS

Bacterial abundance within the Krka Estuary was higher than in the open waters of the central Adriatic, but several times lower than in the mostly eutrophicated western part of the northern Adriatic.

During the fall bacterial secondary production in the estuary was of same ranges as the production of the central Adriatic coastal waters, as well as of the open northern Adriatic waters.

Chlorophyll *a* content and heterotrophic flagellates abundance decreased significantly with the salinity increase along the depth profile. Although an overall correlation between salinity and bacterial number and production, respectively, did not appear, bacterial abundance and production in the brackish layer significantly differed from those in the seawater layer.

A strong overall correlation between bacterial abundance and chlorophyll *a* content appeared in the estuary during fall of 1990 contrary to the correlation absence in the springtime of the 1987 - 1989 period. Also, a signifficant correlation between Chl *a* and bacterial production, bacterial growth rate as well as flagellates number were recorded.

Bacterial to phytoplankton biomass index far below 1 in the brackish layer indicated that bacterial production and number were mostly supported by phytoplankton activity. On the average a 60% decrease of flagellates between brackish and seawater layers accompanied by a 35% drop of bacterial abundance indicated a bacterial net production below the halocline which grazing was unable to match. A prevailing bacterial remineralization in the seawater layer is supposed.

Further examinations are needed to elucidate whether net bacterial production in the seawater layer is the result of low grazing pressure or the lag between bacterial production and bacterivores response.

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Bakterijska proizvodnja u raslojenom krškom estuariju rijeke Krke

Dragica FUKS, Robert PRECALI i Massimo DEVESCOVI

Institut "Ruđer Bošković", Centar za istraživanje mora, Rovinj, Hrvatska

KRATKI SADRŽAJ

Broj i proizvodnja bakterioplanktona, te broj heterotrofnih nanoflagelata i koncentracija klorofila *a* istraživani su u vodama estuarija rijeke Krke (5 postaja) i u obližnjem obalnom moru (2 postaje). Broj bakterija (0.3 - 2.6 x 10^9 stanica l^{-1}) bio je viši nego u otvorenim vodama srednjeg Jadrana, ali i nekoliko puta niži od broja bakterija prisutnih u vodama eutrofnog zapadnog dijela sjevernog Jadrana. Bakterijska proizvodnja (0.11 - 5.76 μg C l^{-1} h^{-1}) u estuariju varirala je u granicama zabilježenim u obalnim vodama srednjeg Jadrana (Kaštelanski zaljev) i otvorenim vodama sjevernog Jadrana.

Broj heterotrofnih flagelata i koncentracija klorofila *a* značajno su opadali s porastom saliniteta. Iako nije zabilježena značajna korelacija između saliniteta i bakterijske proizvodnje i abundancije, vrijednosti za oba parametra značajno su se razlikovale u slojevima bočate i morske vode.

U jesen, za razliku od proljeća, zabilježena je značajna korelacija između broja i proizvodnje bakterioplanktona sa koncentracijom klorofila *a*. Indeks bakterijske prema fitoplanktonskoj biomasi znatno manji od 1 u bočatom sloju ukazuje da je bakterijska proizvodnja uglavnom vezana za fitoplanktonsku aktivnost.

Smanjenje broja flagelata za 60% u morskom sloju prema brakičnom sloju uz istovremeno polovično smanjenje (35%) broja bakterija ukazuje na prisustvo bakterijske proizvodnje koja ne može biti uklonjena iz sloja ispod halokline potrošnjom od strane flagelata. Za pretpostaviti je stoga da je remineralizacija preovladavajuća bakterijska aktivnost u morskom sloju estuarija.