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DISTRIBUTION OF HETEROTROPHIC BACTERIA IN THE COASTAL AND OPEN MIDDLE ADRIATIC

RASPODJELA HETEROTROFNIH BAKTERIJA U OBALNOM I OTVORENOM MORU SREDNJEG JADRANA

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Distribution of heterotrophic bacteria and their reproductive rate were studied in the coastal and open sea waters of the middle Adriatic on a monthly basis from 1968—1978. Horizontal distribution, annual and seasonal variations were observed as affected by some abiotic factors: temperature, salinity and organic phosphate quantities. It was statistically established that organic phosphate quantities mostly affected the distribution of heterotrophic bacteria. Their quantity is usually higher in the coastal area than in the open sea, predominantly for an order of magnitude. Annual maxima as a rule coincided in time with the ingression of the eastern Mediterranean water (in 1968 and 1976—1978). Seasonal oscillations showed summer maximum. Generation time of heterotrophs ranged from 0.6—180 hrs in the Kaštela Bay and from 0.6—145 hrs in the open sea. In the majority of cases rate of heterotrophic bacteria reproduction was higher in the open sea where land effects are not felt and environmental conditions are considerably more stable (than in the coastal area).

INTRODUCTION

The role of heterotrophic bacteria is very important in marine ecosystems owing to their biochemical activities. Therefore they have become the subject of a growing number of researches carried out in the sea all over the world. Distribution of this bacterial group in the Adriatic was studied by Cviić (1955, 1956, 1963, 1964), Ristić and Letić (1972), Krstulović and Šobot (1982), Krstulović (1980) and Šobot (1981).

This paper is a further attempt to contribute to the knowledge of distribution of heterotrophic bacteria and factors they are affected by.

MATERIALS AND METHODS

Samplings were performed on monthly basis at an open sea station (Stončica) and coastal station (Kaštela Bay) (Fig. 1) in the 1968—1978 period.

Station Kaštela Bay (43°12'N 16°22'E) is in the shallow closed area characterized by great oscillations of biological and chemical-physical parameters consequent to the direct affects of the adjacent mainlad and small depths.

Station Stončica (43°12'N and 16°20'E) is southeast of the cape Stončica on the Vis Island. Depth is 107 m. It is affected by the open sea so that the oscillations of biological and chemical-physical parameters are usually smaller than at the preceding station.

Samples were collected from 0, 10, 20 and 35 at the Kaštela Bay station and from 0, 10, 20, 30, 50, 75 and 100 m at Stončica.

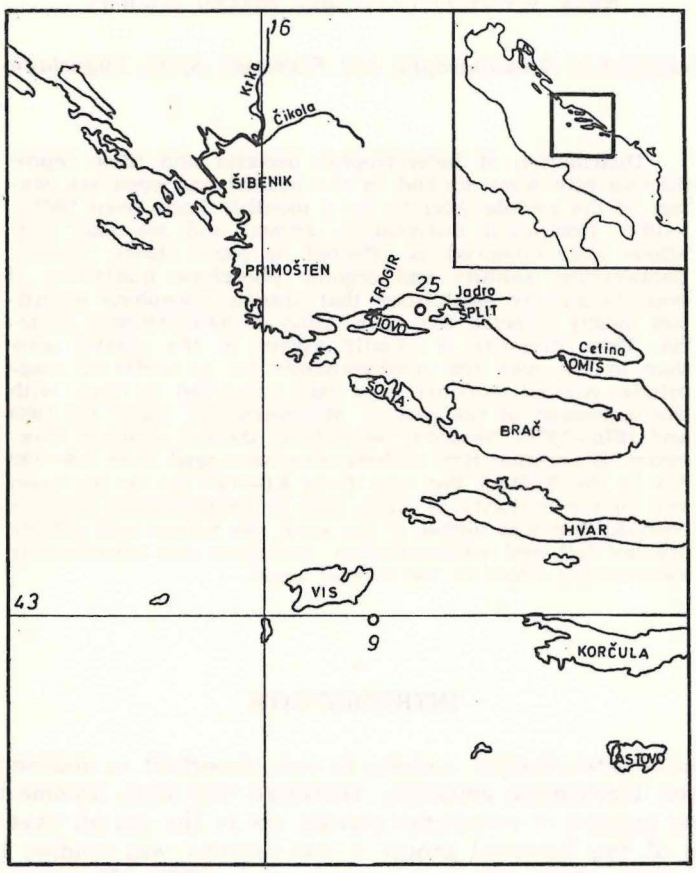

Fig. 1. Study area
Stations: No. 25 - Kaštela Bay
No. 9 - Stončica

Samples were worked out immediately on board the research vessel by the pour plate method with the standard ZoBell's medium 2216 (Z o B e l l, 1946). Reproduction rate of heterotrophic bacteria was observed »in situ« for 6 hours after the method of I v a n o v (1955).

RESULTS AND DISCUSSION

Density of heterotrophic bacteria in the coastal area (Kaštela Bay) as a rule exceeded that in the open sea (Stončica). The analysis of annual means for the open sea showed their percentage proportion to vary from 7.3% to maximum 47.7% (Table 1) in relation to the coastal area.

Table 1. Annual mean values of heterotrophic bacteria (colonies/ml)

Year	Stations		
	Kaštela Bay	Stončica	K. Bay/Stončica %
1968	777.1	151.7	19.7
1969	447.3	72.1	16.1
1970	208.1	66.7	32.0
1971	300.7	143.6	47.7
1972	445.8	78.1	17.5
1973	401.4	69.4	17.3
1974	295.9	21.6	7.3
1976	336.9	99.6	29.6
1977	653.0	86.3	13.2
1978	592.9	148.6	25.1

The analysis of percentage proportion of heterotrophic bacteria in relation to the number of samples shows that heterotrophic bacteria occur in concentrations 0—9 colonies/ml in a very small number of samples from the Kaštela Bay. They occurred in greatest percentages in 100—999 colonies/ml category (Table 2).

Table 2. Density of heterotrophic bacteria in the Kaštela Bay % of colonies of heterotrophic bacteria/ml

Year	No of samples	0—9	10—99	100—999	>1000	>5000
1968	44	0.0	11.4	51.4	22.7	2.3
1969	48	4.2	27.1	56.2	12.5	0.0
1970	40	0.0	20.0	80.0	0.0	0.0
1971	40	0.0	10.0	85.0	5.0	0.0
1972	40	0.0	15.0	75.0	10.0	0.0
1973	36	0.0	16.6	77.8	5.6	0.0
1974	32	6.2	25.0	62.5	6.3	0.0
1976	44	0.0	31.8	61.4	6.8	0.0
1977	36	5.5	19.4	58.5	16.6	0.0
1978	28	14.3	17.8	50.0	17.8	0.0

Presence of heterotrophic bacteria at Stončica is given in Table 3. Their density varied within the limits of the first and second categories in the majority of cases. This means that their density in the open sea was for an order of magnitude lower than in the coastal area.

Table 3. Density of heterotrophic bacteria at Stončica

Year	No of samples	% of colonies of heterotrophic bacteria/ml 0—9	10—99	100—999	>1000
1968	77	47.6	19.1	21.4	2.4
1969	84	25.0	45.2	17.8	8.3
1970	63	25.4	46.0	28.6	0.0
1971	70	1.4	52.8	42.8	1.4
1972	63	6.3	68.2	25.4	0.0
1973	63	15.9	63.5	20.6	0.0
1974	56	32.1	67.9	0.0	0.0
1976	70	7.2	60.0	32.8	0.0
1977	63	0.0	79.4	20.6	0.0
1978	59	0.0	51.0	49.0	0.0

These great differences in density of heterotrophic bacteria are very likely due to the quantity of available nutrient matter and inflow of chemo-organic bacteria from the land which are retained in the sea for some time. Similar was established in this area by Cviić (1955), Ristić and Letić (1972), Krstulović (1980) and Krstulović and Šobot (1982).

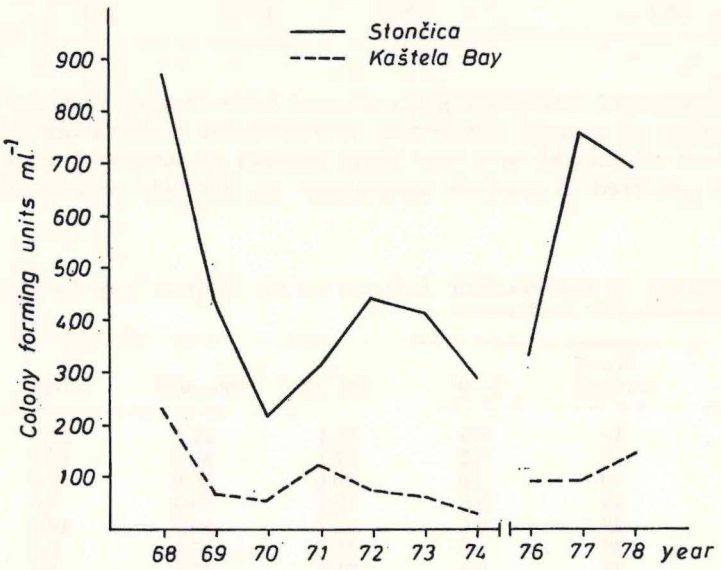

Fig. 2. Annual variations of heterotrophic bacteria

Annual variations in density of heterotrophic bacteria were also observed in the 1968—1978 period (Fig. 2). These variations mainly coincided at both stations what pointed to the possibility that both the coastal and open sea areas are affected by the same factors. Their maximum density was recorded in 1968 that is during the period of strong ingression of the water from the eastern Mediterranean (Buljan and Zore-Armanda, 1979) as well as during the three last years of our researches (1976—1978) once again due to the ingression that occurred in 1976. It should be pointed out that density increase is more marked in the Kaštela Bay. This, in addition to the ingression, may be due to the land effects.

Monthly variations in distribution of heterotrophic bacteria were analyzed from the monthly means over a ten-year period of researches.

Graphs of monthly means show two density maxima of heterotrophic bacteria in the Kaštela Bay; winter-spring and summer ones (Fig. 3). Since the standard error of these means is fairly great, that is monthly oscillations for individual months were very marked, the significance of differences between minima and maxima were tested by T-test. It was established that the winter-spring maximum is a seeming maximum since $P<0.05$, in fact there is no significant difference at the significance level of 5%.

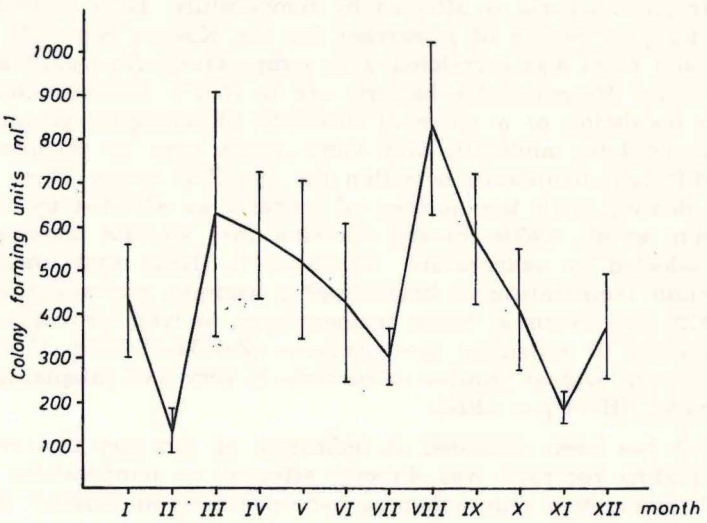

Fig. 3. Monthly variations of heterotrophic bacteria in Kaštela Bay

Summer maximum was as well tested and the difference was established at 5% significance level. In general the variability of this parameter at the Kaštela Bay station was established to be considerably in excess of that at Stončica. This is also very likley due to the land effects and increased pollution of this area.

Monthly oscillations were less marked at Stončica with the exception of heterotrophic bacteria is affected by temperature. Each individual datum significance level of 5% in relation to all the other moths (Fig. 4).

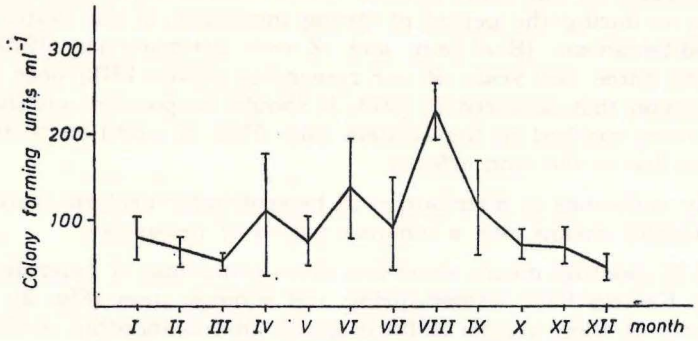

Fig. 4. Monthly variations of heterotrophic bacteria at Stončica

Mentioned summer maxima may be indicative of the fact that distribution of heterotrophic bacteria is affected by temperature. Each individual datum from the ten-year period of researches for the Kaštela Bay (366 pairs) and Stončica (602) pairs was correlated with temperature. No significant correlation was found. Heterotrophic bacteria are in fact a heterogeneous bacterial population consisting of a series of different physiological groups of which proteolytic, lipolytic, amiolytic and some others may be predominant with peaks at different temperatures within the 18—28°C range. There is a series of papers dealing with the number of bacteria as affected by temperature (S c h e w a n *et al.*, 1960a, b) and showing that specific taxonomic groups were not selected by temperature. S i e b u r t h (1967) established that common optimum temperature of heterotrophic bacteria was about 18°C. Maximum density may occur at lower temperatures, as well, provided that sufficient quantities of nutrients are available (Z o B e l l and C o n n, 1940). Furthermore, the largest number of bacteria is very well adaptable to temperature changes (H o p p e, 1978).

All that has been discussed is indicative of the fact that none of the summer maxima recorded was directly affected by temperature. It is very likely that temperature only enhances some processes in summer which affect the number of bacteria.

The analysis of correlation between heterotrophic bacteria and salinity showed no significance for the data at Stončica. Namely, salinity variations are very small in this area and therefore no significant influence on heterotrophic bacteria is to be expected since they are adapted to the coditions of the environment.

However, in the Kaštela Bay where salinity variations are more considerable the coefficient of correlation between salinity and number of hetrotrophic bacteria is higher. If the total number of pairs (366) is taken into

account the correlation coefficient is —0.2. However, if annual correlation coefficients are considered they are significant in some cases (Table 4). This is also likely due to a certain number of bacteria which enter the sea from the land and are not adapted to higher salinity. Similar was established in the paper by Palumbo and Ferguson, (1978) for the estary of the Newport River, North Carolina.

Table 4. Coefficient of correlation between heterotrophic bacteria and salinity in the Kaštela Bay

Year	No of pairs	Correlation coefficient
1968	44	—0.03
1969	40	—0.38
1970	40	—0.49
1971	40	—0.42
1972	32	—0.70
1973	32	—0.02
1974	31	—0.51
1976	43	—0.20
1977	36	—0.25
1978	30	—0.05
Total no of pairs	366	—0.20

In addition it was attempted to bring the distribution of heterotrophic bacteria into connexion with the phosphate quantities in the sea. Therefore monthly means of the number of heterotrophic bacteria were correlated with the monthly organic phosphate means. Correlation coefficient was 0.30 for the Kaštela Bay and 0.36 for Stončica. Studying the relationship between bacteria and different abiotic factors Starzecka (1979) obtained that the coefficient of correlation between total heterotrophic bacteria and organic phosphates was highest.

Generation time of heterotrophic bacteria varied from 0.6 to 145 hours (485 analyzed samples) at Stončica and from 0.6 to 180 hours (297 analyzed samples) in the Kaštela Bay.

As shown by monthly means (Table 5) reproduction rate at Stonč.ca exceeded that in the Kaštela Bay in the majority of cases. This may, at first sight, appear contradictory to the fact that the number of chemoorganotrophs is greater in the coastal area. However, quantities of bacteria in a defined area is not determined only by their rate of reproduction but as well by elimination due to unfavourable environmental conditions. Namely, the coefficient of correlation between the number of heterotrophic bacteria and their generation time was 0.25 in the Kaštela Bay and 0.30 at Stonč'ca. The rate of reproduction is not therefore the single factor affecting the density of heterotrophic bacteria.

Table 5. Monthly mean value of generation time (h)

Stat./Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
K.B.	32.2	22.0	33.3	49.6	28.3	18.0	14.4	11.1	17.7	17.4	7.8	16.3
St.	11.5	10.7	16.7	15.2	23.3	43.7	13.3	21.1	8.9	12.7	11.9	9.0

The Kaštela Bay is a coastal area exposed to particularly strong pollution effects and very variable environmental conditions (temperature, salinity), Therefore, it should be assumed that these factors as well affect the reproduction of bacteria. In addition it was also observed that the proportions of dominant groups of heterotrophic bacteria varied a lot (K r s t u l o v i ć, unpublished data).

In addition, considerable differences in reproduction rate were also recorded between different layers. Mean generation time of all the samples was recorded in different layers (10 and 20 m) and lowest in the surface layer (Table 6). Similar was recorded from the open sea (Table 7). This may be due to the phytoplakton quantity in the surface layer and effects of UV radiation.

Table 6. Mean generation time in the Kaštela Bay (h)

Month/Depth	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
0 m	38.9	15.3	45.5	41.8	83.5	6.2	12.2	11.6	36.0	17.7	15.5	28.5
10 m	38.6	16.9	22.1	63.6	15.3	12.3	15.1	8.9	13.5	19.2	6.5	10.3
20 m	22.1	10.6	10.5	56.2	9.9	12.0	20.0	17.3	14.8	14.1	7.0	24.3
35.m	26.5	46.8	37.9	28.9	32.3	18.5	10.1	10.6	24.5	13.4	3.9	10.4

Table 7. Mean generation time at Stončica (h)

Month/Depth	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
0 m	38.9	15.3	45.5	41.8	83.5	6.2	12.2	11.6	36.0	17.7	15.5	28.5
10 m	8.4	16.5	35.1	16.4	49.0	11.5	6.1	25.0	12.1	9.6	6.9	9.3
20 m	15.7	12.2	22.6	20.9	9.2	25.3	2.6	24.0	9.9	5.7	4.6	6.2
30 m	24.5	10.1	19.4	7.5	5.7	19.1	10.2	8.0	7.1	5.8	8.6	4.8
50 m	6.7	4.3	11.1	12.9	12.3	34.2	6.3	24.7	7,5	7.4	7.2	4.6
75 m	13.3	13.0	10.4	19.7	14.8	40.2	6.5	17.9	7.9	3.2	8.5	8.4
100 m	25.3	16.8	9.8	11.2	23.5	48.9	6.9	29.0	4.0	17.7	5.5	14.2

It may of interest to mention that negative generation time was recorded from 19% of the samples from Stončica and 18% of the samples from the Kaštela Bay. This means that the rate of elimination exceeded the rate of reproduction. However, this was recorded from a single layer while in the layer above or below the generation time recorded was very short.

To give as good explanation as possible of the phenomena just described, the reproduction of heterotrophic bacteria as affected by basic abiotic factors, temperature and salinity, was also observed. Coefficient of correlation with temperature was —0.18 and with salinity —0.20 in the Kaštela Bay. Coefficient of correlation with temperature was 0.14 and with salinity —0.03 at Stončica. These data are another evidence that temperature is not the principal factor affecting the development of bacterial population what is in agreement with the data from the literature.

However, it should be emphasized that in the future researches much more attention should be given to the relationship between plankton communities and bacterial population since it is likely that plankton communities affect bacterial populations more than abiotic factors do.

CONCLUSIONS

Density of heterotrophic bacteria in the coastal area exceeded that in the open sea, for an order of magnitude in the majority of cases.

Annual density variations show maximum values during the periods of ingressions of Mediterranean water into the Adriatic (1968, 1976—77).

Summer maximum of heterotrophic bacteria number was established at both stations under consideration.

The analysis of density of heterotrophic bacteria as affected by salinity, temperature and organic phosphates showed that organic phosphate effects were most significant.

Monthly mean values of generation time showed that the rate of reproduction of bacteria in the open sea exceeded that in the coastal sea in the majority of cases. Environmental conditions are more stable in the open sea where land influence is not felt.

Considerable differences in reproduction rates were observed between the layers. The rate of reproduction was highest in the intermediate layers and lowest mainly in the surface layers.

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RASPODJELA HETROTROFNIH BAKTERIJA U OBALNOM I OTVORENOM MORU SREDNJEG JADRANA

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KRATKI SADRŽAJ

U periodu od 1968—78. godine vršena su mjesečna istraživanja distribucije hetrotrofnih bakterija u obalnom (Kaštelanski zaljev) i otvorenom moru (Stončica) srednjeg Jadrana.

Horizontalna raspodjela, godišnje i sezonske oscilacije promatrane su u ovisnosti o nekim abiotskim faktorima: temperaturi, salinitetu i količini organskih fosfata. Statistički je utvrđeno da količina organskih fosfata ima najviše utjecaja na gustoću heterotrofnih bakterija.

Gustoća heterotrofnih bakterija u priobalnom području redovito je veća nego u otvorenom moru i to u najvećem broju slučajeva za red veličine.

Analiza godišnjih srednjaka pokazala je da su maksimumi njihove gustoće upravo u vrijeme ingresije vode iz istočnog Mediterana (1968. god. i period od 1976—78. god.).

Signifikantnosti minimalnih i maksimalnih vrijednosti gustoće heterotrofnih bakterija testirane su T-testom na osnovu kojeg je utvrđen ljetni maksimum gustoće heterotrofnih bakterija na obadvije ispitivane postaje.

Istraživana je i brzina razmnožavanja heterotrofnih bakterija te ovisnost generacijskog vremena o različitim faktorima sredine.

Njihovo generacijsko vrijeme se kretalo u Kaštelanskom zaljevu od 0,6—180 sati, na Stončici od 0,6—145 sati. U većini slučajeva razmnožavanje ispitivanih bakterija je bilo brže na Stončici gdje se ne osjeća utjecaj kopna i gdje su uvjeti sredine znatno stabilniji nego u Kaštelanskom zaljevu.