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BIOMASS DISTRIBUTION AND PHYSIOLOGICAL CAPABILITIES OF BACTERIA IN THE WATER COLUMN ABOVE A SEAGRASS SYSTEM

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ABSTRACT - The distribution pattern of bacteria in the water column above and within a *Posidonia oceanica* bed near Ischia (off the Gulf of Naples) was investigated and correlated with DOC and POM data for autumn and winter. Data from samples of 5 stations along a transect from shallow to deep showed rather low bacterial densities (10^4. ml^{-1}) for both seasons. The number of attached bacteria was higher in late winter as compared to autumn when the leaves are shed from the stands. Except for occasional peaks DOC ranged between 1.5 and 4.5 mg C/l while POM concentrations ranged from 4 to 14 mg AFDW.l^{-1}. Hydrolytic capabilities of isolated bacterial colonies showed that more carbohydrate substrates could be fermented in winter as compared to autumn. This is correlated with an input of POM into the system due to the leaf fall of the seagrass which takes place in late autumn. The problem of structural carbohydrate breakdown is discussed.

Key words: seagrass bed, DOC, POM, fermentation, cellobiose.

RÉSUMÉ - La distribution des bactéries dans l'eau sus-jacente et dans un herbier de *Posidonia oceanica* a été étudiée à Ischia (au large du Golfe de Naples) et reliée aux concentrations de COD et MOP en automne et en hiver. Les données de 5 stations le long d'un transect montrent que la densité bactérienne est faible (x 10^4) pour les deux saisons et que le nombre de bactéries fixées est plus élevé en hiver qu'en automne. La concentration en COD varie entre 1,4 et 4,5 mgC/l et celle en MOP entre 4 et 14 mg de matière organique l^{-1}. Les caractéristiques hydrolytiques des souches purifiées indiquent qu'un plus grand nombre de substrats carbohydratés peuvent être fermentés en hiver. Cette caractéristique peut être reliée avec la chute des feuilles de l'herbier en automne, qui représente une augmentation importante de matière organique dans l'écosystème. Le problème de la dégradation des carbohydrates de structure est discuté.

Mots clés : herbier, COD, MOP, fermentation, cellobiose

INTRODUCTION

Most of the work on the ecology of mediterranean seagrass beds has concentrated on growth and production (Bay, 1978; Ott, 1980), the related daily and seasonal variations in carbohydrate and amino acid concentrations (Velimirov and Pirc, 1983; Pirc, 1984), or consumer interactions (Ott and Maurer, 1977; Traer, 1980; Velimirov, 1983). The importance of microorganisms as decomposers of macrophyte material or as a potential food source for filter and deposit feeders has been rather neglected. Although information on the role of bacteria on *Posidonia* derived macrophyte debris and on metabolically active *Posidonia* leaves is now available (Velimirov *et al.*, 1981; Novak, 1984), data on the distribution and abundance of bacteria within and above seagrass beds are still lacking.

The growth cycle of *Posidonia oceanica* is marked by the leaf fall of the seagrass in late autumn (October, November). This brings about a release of particulate organic matter

(POM) amounting to 15% of the yearly production, resulting in extensive wrack beds on the shore as well as along the edges and within the stands. During a one year study to quantify seasonal variations of bacterial density in the water around a seagrass meadow (Velimirov, in prep.), the bacteria from November samples and those from February (the begin of the main growing season) were correlated to DOC and POM values and were also tested for their fermentation capabilities. Since most of the wrack beds are progressively reduced in size until spring it is of interest to investigate whether the bacterial population exhibits different hydrolytic properties in late autumn as compared to early spring.

Material and methods

The study area was located at the north coast of the island Ischia (Lacco Ameno), off the Gulf of Naples in southern Italy. One liter water samples were taken with a PVC-syringe from 5 stations along a line transect through a *Posidonia* meadow extending from 0.5 to 33 m depth. Station 1 to station 4 covered a range from 0 to 20 m depth in 5 m intervals, station 5 was situated at 30 m depth. At all stations samples were taken from the water surface as well as within and above the meadow at the proximity of the leaf tips. At stations 2 to 5 additional samples were taken from the water column.

Total bacterial counts and cell volumes were determined using the method of Hobbie *et al.* (1977). Additionally, subsamples of 150 ml were filtered through 0.2 μm nuclepore polycarbonate filters for SEM observation of suspended particles and associated microbes. For the isolation of bacterial strains, two water samples were taken from 5 and 15 m depth including suspended particulate matter. The samples were pooled in a sterile glass bottle and kept dark at 6°C during transport. Plate cultures were prepared on ZoBell Agar at 20°C and incubated for 72 hours. Only the colonies appearing within this time were isolated and used for the inoculation of the specific media to perform routine morphological and physiological tests. Working from the perimeter of the plate in a spiral to the center, approximately 15% of the colonies were isolated and restreaked to purity. Oxidative utilization of carbohydrates was indicated by acid formation under aerobic conditions using a carbohydrate nutrient broth (modified after Hallmann and Burkhardt, 1974), containing phenol red as indicator.

To estimate the POM load of the same water samples 800 ml were filtered through preashed (480°C) Watman GF/F filters (nominal pore size 0.7 μm) and then trough 0.2 μm Sartorius filters, dried to constant weight at 70°C and ashed at 480°C for 6 hours. 15 ml of the 0.2 μm filtered water were acidified with 2% HCl, sparged with synthetic CO_2 free air for 10 to 15 minutes and injected into a total organic carbon analyser (Beckman Tocamaster, Model 915-B) fitted with IR detector and calibrated with anhydrous potassium biphtalate in carbon free double distilled water. Dissolved free carbohydrates in the same samples were determined according to Dawson and Liebezeit (1983).

Results

The distribution pattern of DOC, POM and bacteria in November are shown in Figure 1. Only slight variations occur in DOC, ranging from 1.5 to 3.2 mg/l, exept for station 4 where a peak of 31.3 mg/l was recorded in the water column at 15 m. The samples taken just above and within the meadow show that the water body between sediment bottom and the leaf tip niveau is homogenous. A similar distribution pattern is noticed for POM, which varies from 8 to 14 mg AFDW/l over the entire depth range. A different situation is evident in the case of the bacterial distribution. Densities vary from 0.5×10^4 to 4×10^4

cells. ml^{-1} with peaks in the water column at 5 m (station 2) and 15 m (station 4). The amount of attached bacteria is highest in the surface water at station 3 with 11.4 %, rods being the dominant morphological type (Tab. 1).

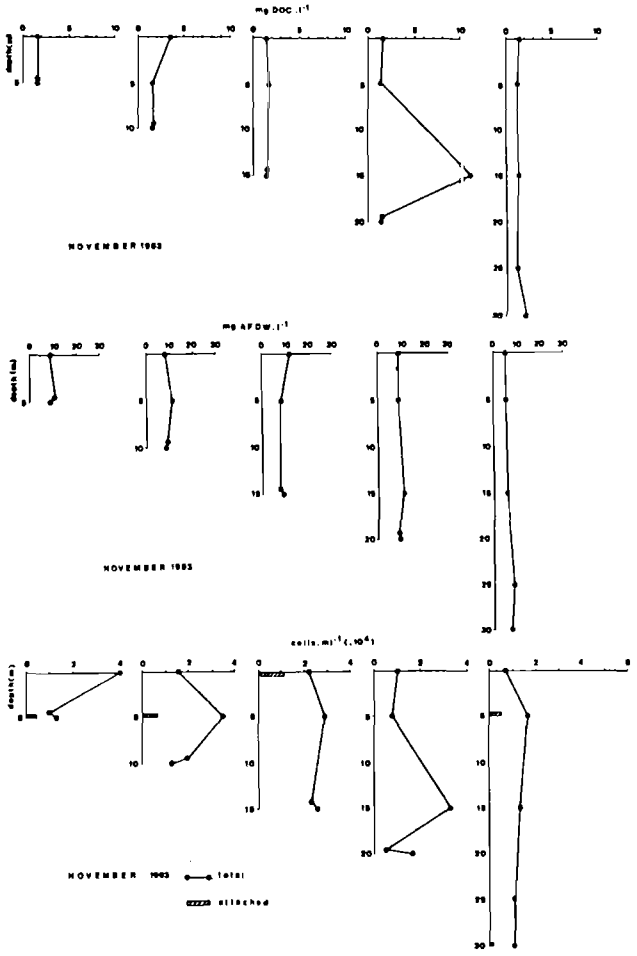

Figure 1 : Variations in bacterial density, POM and DOC concentrations in November

		November			February		
Station	depth	free	attached	total	free	attached	total
1	5	68.9	7.5	76.4	45.5	21.9	67.4
2	10	78.3	4.9	83.2	57.8	-	57.8
3	15	80.5	11.4	91.9	13.6	63.0	76.6
4	20	87.4	-	87.4	39.9	8.0	47.9
5	30	68.9	8.1	77.0	14.7	18.5	33.2

Table 1. Free, attached and total rod shaped bacteria expressed as percentage of total cells per ml. in stations 1 to 5 (depth in meter)

In February none ot the water samples reveal DOC concentrations over 5 mg/l (Fig. 2) while the organic particle load of the water reaches 20 mg AFDW/ l at station 5, which is the lower border of the seagrass bed. At all other stations POM ranges between 4.2 and 14 mg AFDW/ l. A higher percentage of attached bacteria is noticed for this month, being again most abundant in the water surface. At 15 m depth (station 3) a higher percentage of attached (63 %) versus free floating rods is recorded (Tab. 1). The volume of rods and cocci was the same for both seasons with means 0.21 μm^3 and 0.041 μm^3, respectively (100 measurements for the two types per season).

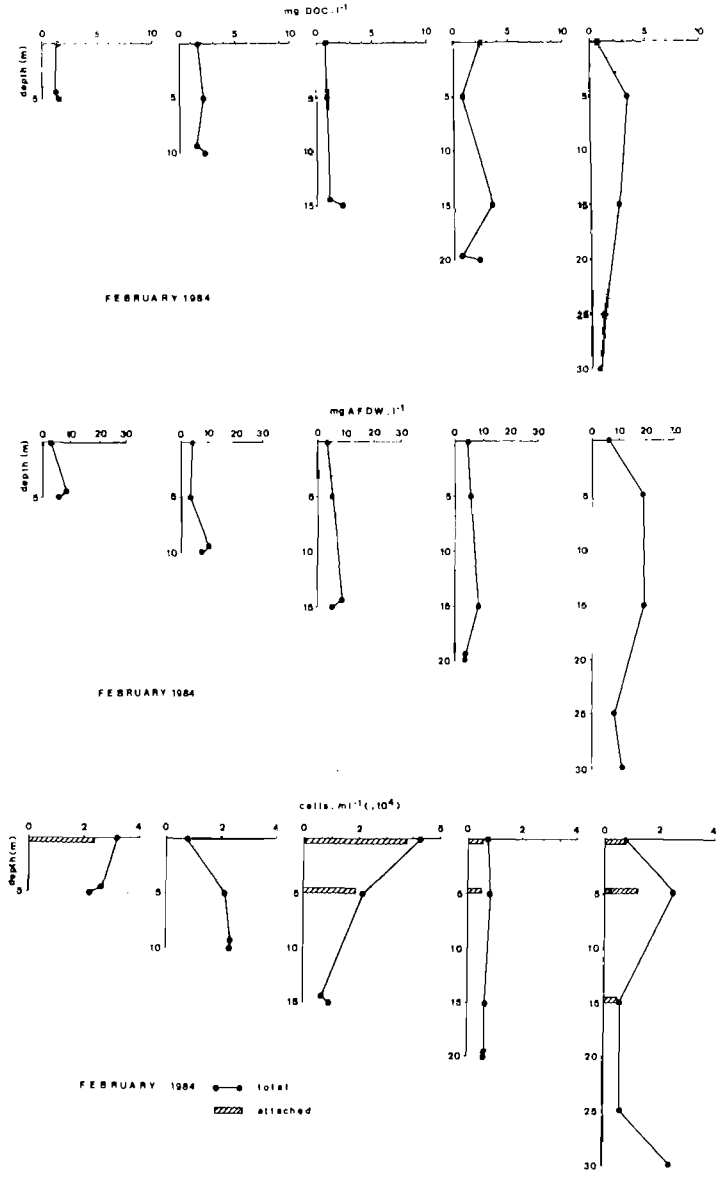

Figure 2 : Variations in bacterial density, POM and DOC concentrations in February

Table 2 presents a comparison between total bacterial cells and its colony forming fraction. Although total counts between November and February are rather similar, the autumn samples contained less colony forming bacteria than the winter samples; for both months the counts of colony forming bacteria are less than 1.5% of the total population. The properties of the purified strains are shown, in Table 3. A lower percentage of

	November	February
Total cell count (cells. ml^{-1})	1.4 x 10^4	1.65 x 10^4
Colony forming bacteria. ml^{-1}	0.9 x 10^2	2.40 x 10^2
% of total colony forming bact.	0.64	1.45

Table 2. Total bacteria count in a pooled sample (5 and 15 m depth) taken above the meadow compared with numbers of colony forming bacteria for two seasons.

Properties	November	February
Rod shaped	90	68
Spore forming	20	25
Gram negative	70	43
Motility	70	37
Facultative anaerob	20	18
Catalase	90	87
Oxidase	50	21

Table 3. Characteristics of bacterial colonies isolated at 20°C. All values are expressed as % of total isolated colonies (n = 20 for November, n = 32 for February).

rodshaped bacteria as well as a relatively low number of gram negative bacteria is confirmed for February as compared to November. Motile strains occurred with a higher frequency in November and the higher percentage of oxidase positive bacteria indicates more strictly aerobic colonies. The tests of the fermentation spectra (Tab. 4) were chosen

Fermentation	November	February
Xylose D	40	18.7
Xylose L	0	0
Ribose	20	37.5
Arabinose D	0	6.2
Arabinose L	10	50.0
Fucose D	40	6.2
Fucose L	0	37.5
Rhamnose	0	43.7
Glucose D	50	59.4
Galactose	40	50
Mannose D	50	56.2
Fructose D	40	50
Inositol	0	3.1
Saccharose	20	46.9
Cellobiose	10	15.6
Starch	7	46.9
Cellubose	0	0

Table 4. Enzymatic characteristics of bacterial isolates for two seasons. All values expressed as % of total colony forming bacteria (n = 20 for November, n = 32 for February.

according to the easily metabolizable carbohydrate compounds which characterize the water column (Fig. 3) and those within the *Posidonia* leaves, namely fructose, glucose, inositol and saccharose (Velimirov *et al.*, 1981). Additionally, fermentation tests for the storage and structural carbohydrates starch, cellulose and cellobiose were performed. Differences were mainly reflected in a higher proportion of rhamnose, saccharose, arabinose and starch fermenting colonies in February. There were more xylose and D-fucose fermenters in November while no L-fucose fermentation could be detected (compare with February). All tests for cellulose fermentation were negative, but moderate cellobiose digestion in both seasons indicates the presence of a β-glucosidase like enzyme.

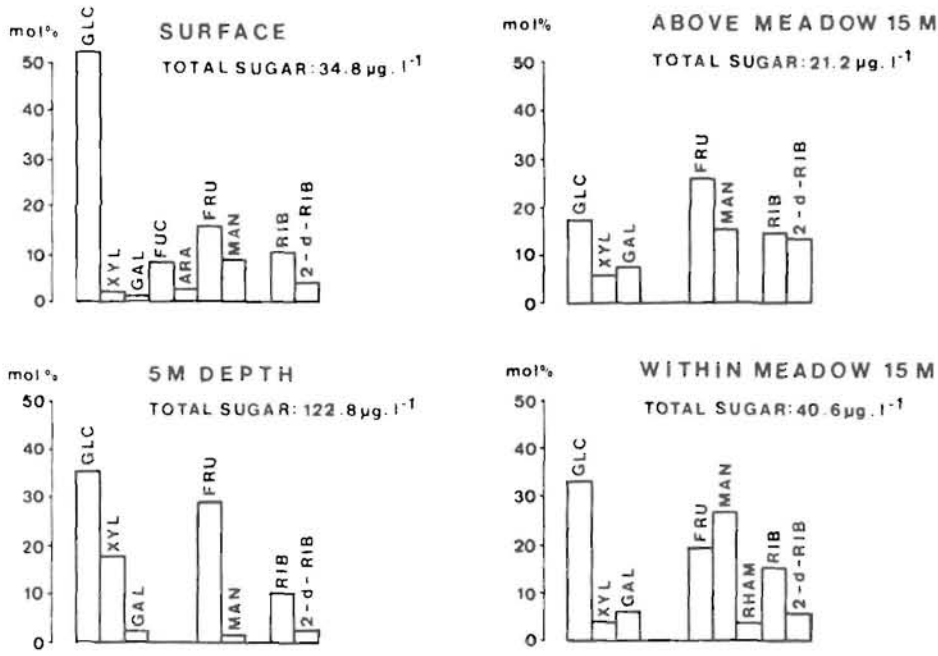

Figure 3 : Distribution of free dissolved sugar compounds and total free sugar concentrations in Station 3 (November)

Discussion

Defining the study area as a surface of 600 m (extension of the meadow from 0 to 33 m depth) by 320 m (from Punta Vico to Pietro del Lacco) we obtain a water wedge 33 m in height with a volume of $31.69 \times 10^5 m^3$. If we consider both the surface water and the water in the immediate vicinity of the meadow as distinct water bodies of 1 m thickness, we obtain a surface layer and a bottom layer of nearly equal volume and a large midwater body in between. In order to estimate bacterial density, DOC and POM per m^3 water above the idealized *Posidonia* bed, the average data per water body were weighted according to the water volume to obtain an overall mean per unit (Table. 5). It is evident that bacterial carbon represents only a minute fraction of DOC standing stock and that the bacterial contribution to the overall POM, assuming a carbon content of 40% per gram ash free dry weight, is insignificant. These findings are in agreement with data from pilot studies in autumn and winter of the previous year (Velimirov, 1984; Velimirov in prep.). Total number of bacteria per unit within and above the seagrass stands obtained

by direct counts is rather small when compared to other macrophyte systems such as kelp beds (Linley and Field, 1982; Davis *et al.,* 1983) where the amount of structural carbohydrates per plant is lower and cell numbers range from 10^5 to 10^8 ml^{-1}. At the present stage of reseach no explanation can be offered to account for the low densities of suspended and attached bacteria. However, it should be pointed out that the concentration of monomeric carbohydrates in the water (Fig. 3) is low, compared to the total DOC, and that dissolved free amino acids are 100 to 200 times lower than the dissolved sugar compounds (Velimirov, in prep.); also most of the DOC in the water may be present in highly polymerized from which is more resistent to degradation. Further information was obtained by SEM observations of suspended particles, showing that only 1 % of the latter.

	November	February
Bacteria (mg $C.m^{-3}$)	0.248 (0.043)	0.223 (0.102)
DOC ($gC.m^{-3}$)	4.82 (8.21)	2.15 (1.072)
POM (g $AFDW.m^{-3}$	8.27 (2.42)	10.54 (5.28)

Table 5 : Standing stock of bacteria, DOC and POM per m^3 *Posidonia* water, weighed and averaged over all stations for the two seasons.

could be identified as being seagrass derived. The main bulk of POM were flocs or inorganic aggregates with organic coating (Velimirov, in prep.).In contrast to AODC counts, only 2% of flocs and aggregates were colonized by bacteria while none of the *Posidonia* particles was colonized. Size of particles suspended in the water, as determined during AODC counts, for the 2 months ranged from 2 μm to 800 μm. Of the total (free suspended and attached) bacteria in the water column only 10 and 15% in November and February respectively, have the capability to break down cellobiose. Although the enzyme β- glucosidase which ferments cellobiose to glucose, is the last step in cellulose digestion, no indication for the presence of a β-1,4 glucanase could be found in suspended bacteria. Although we are aware of the problems in detecting a glucanase in the presence of cellobiose we assume that the breakdown of the *Posidonia* particles is insignificant or absent in the water column. We suspect that a part of the leaf is degraded by microheterotrophs on the leaf while it is still connected to the shoot (Velimirov *et al.,* 1981, Novak , 1984), the rest being broken down in the sediment after the leaf fall.

The differences in hydrolytic properties of the bacteria in the autumn and winter may well reflect the adaptive power of a specific seagrass population which synthesize enzymes as substrates change. Due to the leaf fall and the erosive effect of winter storms, which tear out whole shoots along with the rhizome system, more particulate starch is exposed to bacterial breakdown within the wrack beds than during the rest of the year. However, the high percentage of observed Gram-positive cocci in February indicates also the interference of sediment bacteria which become partially resuspended from shallow bottom by winter storms.

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