APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1982, p. 1227–1237
0099-2240/82/061227-11$02.00/0
Vol. 43, No. 6

Attached and Free-Floating Bacteria in a Diverse Selection of Water Bodies

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Received 21 September 1981/Accepted 3 February 1982

The contribution of attached and free-floating bacteria to the bacterial numbers and heterotrophic uptake in 44 diverse aquatic environments was studied. A factor analysis reduced the variability of the raw data base to three major factors explaining 53.6% of total variance. These factors were (i) salinity, (ii) heterotrophic uptake, and (iii) particulate load. A cluster analysis categorized the 44 habitats into five distinct environmental types based on these three factors. There was no significant pattern in the distribution of attached versus free-floating bacteria when assessed by epifluorescent microscopy. However the contribution of attached bacteria to the uptake of an amino acids mix was reduced in marine waters. Heavy particulate loads resulted in an increased percentage uptake of amino acids and glucose from the attached bacteria. Uptake response was found to be substrate specific especially in oligotrophic freshwater. Amino acid uptake was more associated with the attached fraction, whereas glucose uptake was mediated more by the free-floating fraction.

The association of bacteria with particulate material is of considerable importance in aquatic environments. Bacteria are one of the major agents responsible for the alteration of particulate matter through processes such as decay and flocculation (16–18). They are able to convert dissolved organic matter to particulate organic matter (2), and their ultimate fate in the water will be greatly influenced by the material to which they are attached (20).

Most authorities agree that in the open ocean planktonic bacteria are unattached and free floating (6, 22, 24). In freshwater environments the degree of attachment varies considerably (4, 5, 7, 8, 14, 19). However evidence is accumulating in studies on rivers that as the freshwater flows into the sea the degree of attachment decreases with increasing salinity (9, 25).

We observed a similar decrease in numbers and activities of attached bacteria in the Fraser River Estuary as it entered the Strait of Georgia (3). To assess how universal the relationship between attachment and salinity was and also to explore other influences, we embarked on a study of 44 aquatic environments, 15 of which were coastal marine. The results of this study are reported herein.

MATERIALS AND METHODS

Collection of water samples. A total of 44 water samples were collected over the period 26 May 1980 to 25 November 1980. The majority of these samples (40) were taken from the surface water in sterile Nalgene containers. The remaining four samples were collected from the depths listed in Table 1 with a 7-liter Van Dorn bottle. All samples were processed immediately after collection.

Physicochemical determinations. Temperature was measured with a simple mercury thermometer. pH and salinity and conductivity were determined with a Fisher model 107 portable pH meter and a Yellow Springs Instruments model S-C-T 33 salinometer, respectively. The total volume and modal size of particulates were counted later in the laboratory with a Coulter Counter TA II with 280- and 100-μm-diameter apertures. This covered particles in the diameter size range of 1.26 to 80.64 μm. Seston was determined as an estimate of the organic particulates in the waters. A known volume of water was filtered through a predried and tared Whatman GFF filter which was fixed by placing in a petri plate with a pad soaked in Formalin. The filter was later ashed in a furnace at 500°C for 24 h. The weight of material burned off was reported as seston. Particulate organic carbon (POC) was defined as the organic carbon retained on a GFF filter previously baked for 24 h at 500°C. The filtrate was considered to be dissolved organic carbon (DOC). Both POC and DOC samples were fixed in the field by freezing in dry ice and were kept frozen until ready for analysis. POC was determined with a Perkin Elmer Elemental Analyser 240. DOC was analyzed on a Beckman Tocamaster model 915-B.

Information on the location and elevation of each sample site was taken from topographical maps. Two location parameters, called X coordinate and Y coordinate, were entered into the statistical analyses to detect any influences attributable to the proximity of the urban and industrial centre of Vancouver. The X coordinate measures minutes of longitude west of sample no. 32, Lost Lagoon. The Y coordinate measures minutes of latitude north of sample no. 32. Lost

TABLE 1. Site number and location

Site no.	Location	Longitude	Latitude
1	Porlier Pass	123°35'W	49°01'N
2	Active Pass	123°19'W	48°52'N
3	Nanaimo River Estuary	123°54'W	48°08'N
4	Howe Sound (250 m)	123°15'W	49°05'N
7	Howe Sound (8 m)	123°15'W	49°05'N
8	Indian River Estuary	122°53'W	49°29'N
10	Indian Arm (200 m)	122°52'W	49°23'N
11	S.F.U. Reflecting Pool	122°55'W	49°17'N
12	Fraser River	122°55'W	49°12'N
13	Burnaby Lake	122°57'W	49°15'N
14	Buntzen Lake	122°52'W	49°20'N
15	Chadsey Lake	122°09'W	49° 7'N
16	Marion Lake	123°10'W	49°33'N
17	Cheakamus Lake	122°56'W	50°01'N
18	Lindeman Lake	121°27'W	49°07'N
19	Levette Lake	123°11'W	49°50'N
20	Pacific Ocean	125°40'W	49°43'N
21	Pacific Ocean (150 m)	125°40'W	49°43'N
22	Alberni Inlet	124°48'W	49°09'N
23	Trevor Channel	125°09'W	48°51'N
24	Goldie Lake	122°56'W	49°22'N
25	Henriette Lake	123°20'W	49°41'N
26	First Lake	123°11'W	49°23'N
27	Wedgemount Lake	122°49'W	50°10'N
28	Lafarge Lake	122°46'W	49°17'N
29	Killarney Lake	123°21'W	49°24'N
30	Greendrop Lake	121°26'W	49°09'N
31	Mill Lake	122°19'W	49°03'N
32	Lost Lagoon	123°08'W	49°18'N
33	Garibaldi Lake	123°01'W	49°55'N
34	Hicks Lake	121°42'W	49°20'N
35	Pitt Lake	122°35'W	49°21'N
36	Serpentine River	122°45'W	49°08'N
37	Squamish River	123°16'W	49°53'N
38	Chilliwack River	121°58'W	49°06'N
39	Bute Inlet	124°50'W	50°53'N
41	Homathko River	124°52'W	50°57'N
42	Calm Channel	125°06'W	50°21'N
43	Robert Burnaby Park Creek	122°56'W	49°14'N
44	Post Creek	121°29'W	49°06'N
45	Steveston Drainage Ditch	123°05'W	49°08'N
46	Fraser River Estuary	123°08'W	49°06'N
47	Georgia Strait	123°30'W	49°09'N
50	Sewage Effluent (Annacis Island)	122°58'W	49°10'N

Lagoon was chosen as a convenient reference point as it is situated in downtown Vancouver.

Biomass determinations. Chlorophyll *a* was determined spectrophotometrically after acetone extraction with Millipore 0.8-μm pore size filters (23). The filters were removed from the field wrapped in foil and analyzed within a few days. Bacterial numbers were estimated by the acridine orange direct count (AODC) method of Hobbie et al. (10). Bacteria attached to particles, which included bacterial aggregates and bacteria attached to plankton, were distinguished from free-floating bacteria after removal of the bulk of the particles by gently filtering 10 ml of water sample through Nuclepore 1.0-μm pore size filters. This procedure has been tested extensively in the varying conditions of the Fraser River Estuary (3). Between 60 and 97% of all particulates in the estuary were retained by this filter, yet in samples which had a predominance of free-floating bacteria, 98% of the bacteria passed through the filter. Samples were fixed in the field with 2% (vol/vol) Formalin.

Heterotrophic uptake. The turnover time of glucose and an amino acid mix was calculated by the method of Azam and Holm-Hansen (1). D-[6-^{3}H]glucose (specific activity, 30 Ci/mmol; New England Nuclear Corp.) and a mixture of 15 tritiated L-amino acids (New England Nuclear Corp.; code no. NET-250) were diluted to a working concentration of 10 μCi/ml. The relative uptake of each amino acid within the mixture could not easily be determined, so calculations to specific concentrations of amino acids and glucose were not attempted. The water sample was

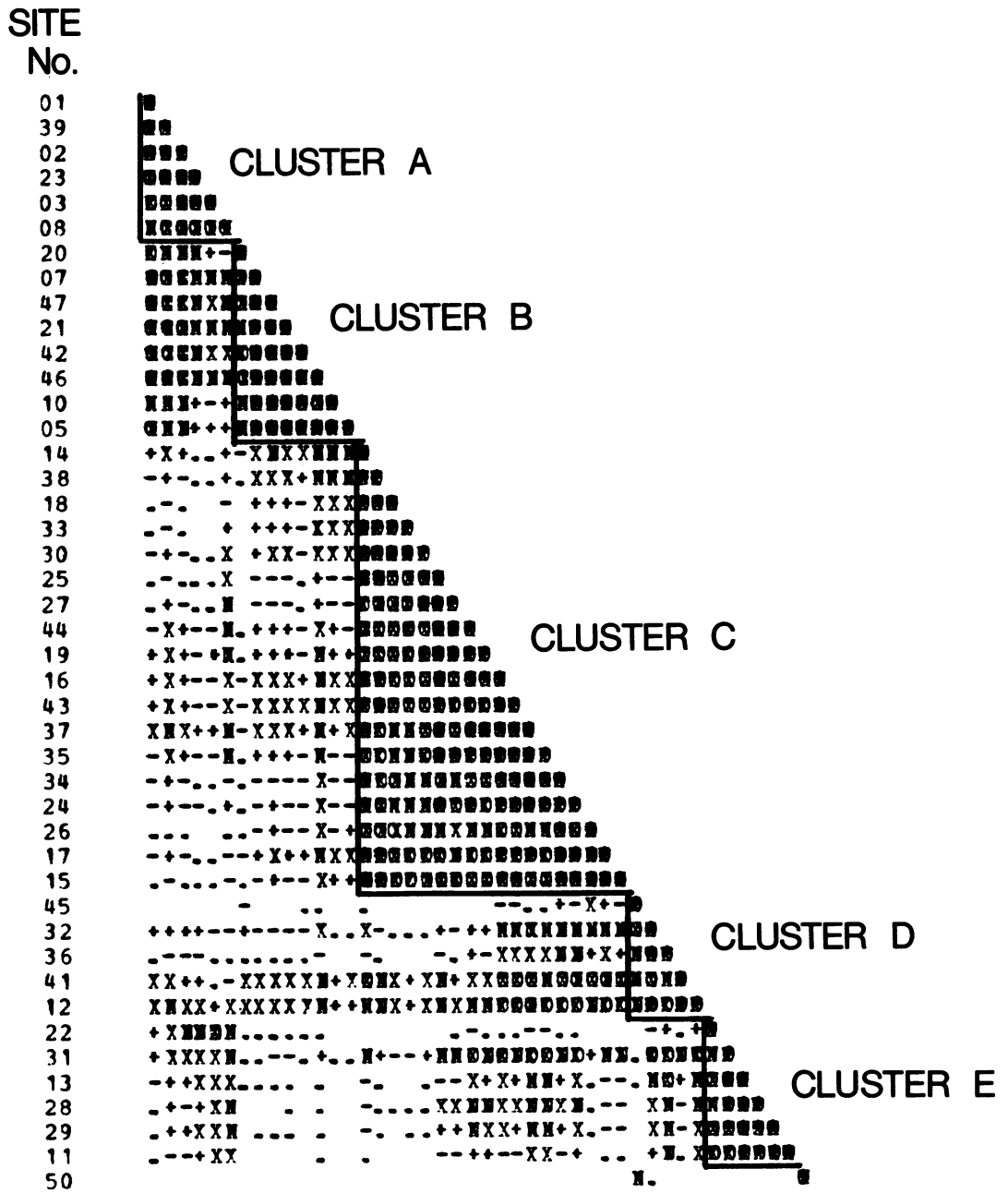

FIG. 1. Distance matrix of sites arranged in clusters. The distances are represented in shaded form according to the following scheme: ●, <0.953; ○, 0.953 to 1.487; ■, 1.487 to 2.002; ×, 2.002 to 2.268; +, 2.268 to 2.535; −, 2.535 to 2.802; -, 2.802 to 3.279; open space, >3.279.

TABLE 2. Variation in the data from the 44 water bodies

Determination	Salinity (‰)	Conductivity (μS/cm × 10^{-2})	pH	Temp (°C)	Elevation (m)	X coordinate	Y coordinate	Particle vol (μm^3/ml × 10^{-5})	Particle size mode (μm)	Seston (mg/liter)	POC (mg/liter)	DOC (mg/liter)	Chlorophyll *a* (mg/m^3)
Mean	6.8	87.9	7.1	14.1	262	4.9	8.4	344	19.4	22.2	2.3	6.5	2.8
Standard deviation	10.6	137.8	0.6	4.4	443	61.3	29.9	1,003	9.1	9.7	3.2	5.9	6.2
Smallest value	0.0	0.1	5.5	8.0	0	−102.8	−69.7	5	3.2	3.9	0.6	1.1	0.1
Largest value	30.0	415.0	8.1	25.0	1,859	151.7	98.8	5,100	40.3	69.3	21.3	37.9	39.7
Range	30.0	414.9	2.6	17.0	1,859	254.0	168.5	5,095	37.1	55.4	20.7	36.8	39.6

AODC (per ml × 10^{-5})			NAB (per ml × 10^{-4})						Turnover time (days)				
			Amino acids			Glucose			Amino acids			Glucose	
Free	Attached	Total	Free	Attached	Total	Free	Attached	Total	Free	Attached	Total	Free	Attached	Total
3.6	3.5	7.1	9.7	15.6	25.3	6.1	9.2	15.3	171	3,988	80	334	8,605	240
6.7	7.1	11.6	14.1	28.3	37.5	10.0	15.4	19.7	152	12,056	86	419	16,092	363
0.2	0	0.2	0.03	0	0.1	0.03	0.03	0.06	8	3	3	3	5	2
33.0	32.0	65.0	58.0	130.0	188.0	34.0	80.0	92.0	642	41,667	362	2,158	41,667	2,158
32.8	32.0	64.8	58.0	130.0	187.9	34.0	80.0	91.9	634	41,664	259	2,155	41,552	2,156

prepared by pouring four 100-ml volumes, previously filtered through a 300 μm Nitex mesh, into four beakers supported in the water body with a small styrofoam raft to keep the samples at in situ temperature. Two beakers were treated as controls by adding 2% (vol/vol) Formalin. Glucose solution (200 μl) was then added to a test and a control beaker, similarly with the amino acid solution. The samples were then incubated in the dark for up to 2.5 h. Samples (10 ml) were removed every 30 min and filtered by a procedure previously described (3) to distinguish uptake mediated by attached bacteria from that mediated by free-floating bacteria. Briefly, the samples were first filtered through 1.0-μm pore size Nuclepore filters, and then the filtrate was passed through 0.22-μm pore size Millipore filters. The radioactivity on both filters was later counted on a Beckman LS 8000 scintillation counter, correcting for quench with the external standards ratio method.

Microautoradiography. Numbers of actively metabolizing bacteria (NAB) were determined by the technique of Hoppe (13) with the following modifications. Samples of water (1 ml) were filtered through 300-μm Nitex mesh and then placed in four test tubes and held at in situ temperature in the raft. Two tubes were treated with 2% (vol/vol) Formalin as controls. Tritiated glucose and amino acid solutions (100 μCi/ml) were then added, 50 μl to a tube, and incubated in the dark for 3 h. At the end of this period, the two test samples were fixed with 2% (vol/vol) Formalin, and then all four tubes were brought to a final volume of 10 ml with sterile isotonic water. Samples were filtered through 1.0-μm pore size Nuclepore filters and washed five times with 10 ml of isotonic water. The filtrate was saved and then filtered through 0.2-μm pore size Nuclepore filters and washed five times. After air drying, the filters were fixed to microscope slides with double-sided sticky tape and returned to the laboratory. The slides were dipped in Kodak NTB-2 emulsion and stored at 5°C for 7 days in black plastic boxes. The microautoradiograms were developed in Kodak D-19 for 5 min and then hardened in Anti-Scratch Hardener (Edwal Scientific Products Corp., Chicago, Ill.) for 1 min before fixing for 10 min in Edwal Quick Fix. The slides were finally washed for 1 h in tap water. Silver grains were counted by using a Zeiss Standard WL microscope under bright field illumination at 1,250× magnification.

Statistical analyses. All analyses of the data were performed with the computer packages BMDP Biomedical Computer Program P-series 1979 or Statistical Package for the Social Sciences, 2nd ed. The normality of all variables was checked with BMDP5D. It was necessary to perform a log transformation on the following variables to enhance normality and homogeneity of variance: conductivity, salinity, particle volume, elevation, seston, POC, DOC, chlorophyll *a*, AODC, NAB, and turnover time estimates. The factor analysis was performed on all independent variables by using BMDP4M. The correlation matrix was factored by principal component analysis with varimax rotation of factors. Factor scores were then computed for all 44 sites, and the scores on the first three factors were used to construct the pin diagram in Fig. 1. The cluster analysis of sites was obtained by using BMDP2M with factors 1, 2, and 3 as criteria. Clusters

were formed by average linkage based on the Euclidean distance between sites (i.e., the square root of the sum of squares of the differences between the values of variables for each two sites). The arrangement of data by cluster (see Table 6) was performed by BMDP7D with a one-way analysis of variance. The three-way analysis of variance was executed by Statistical Package for the Social Sciences program ANOVA with classic partitioning of variance. The significance of the differences between data sets was assessed by using these two statistical programs.

RESULTS

Description of sites. The 44 water bodies sampled comprised a random selection of marine, estuarine, lake, river, and creek environments in the southwest corner of British Columbia as described in Table 1. Table 2 indicates the diversity of the sites with the more obvious microbiological parameters showing an extensive range of values, e.g., temperature, 8 to 25°C; salinity, 0 to 30%; POC and DOC, 2.0 to 59.2 mg/liter; chlorophyll *a*, 0.1 to 39.7 mg/m^3; and total bacterial counts, 2.0×10^4 to 6.5×10^6 per ml. The total population investigated therefore represented a broad selection of the niches of heterotrophic bacteria.

Attached and free-floating bacterial activity. The aspect of these environments of interest in this study was the contribution of free-floating and attached bacteria to biomass and heterotrophic uptake. Table 3 shows the data averaged for some pertinent variables which indicated that, over the whole data base, the heterotrophic uptake (turnover time) of glucose and amino acids was greater for the free-floating portion than for the attached portion. The percentage of uptake attributed to the attached flora for amino acids (46%) was also significantly greater than that for glucose (31%). However, the greater activity of the free-floating fraction was not paralleled by an increase in the number of free-floating bacteria as estimated by AODC; the numbers in both fractions were comparable. Data on the number of active bacteria and turnover time per active cell demonstrated a different response depending on the radioactive substrate. The bacterial population actively metabolizing amino acids had significantly greater counts in the free-floating fraction compared with the attached fraction. When glucose was used as the substrate, the NAB were similar, whereas the turnover time per active cell was significantly lower in the free-floating fraction.

Factor analysis. The breakdown of the data in Table 3 into counts of sites where the free-floating fraction was greater than the attached fraction of a given variable only showed a marked difference in the turnover time of glucose, where 34 sites showed higher activity in the free floaters. To explore the data base more

TABLE 3. Distribution of bacterial numbers and activities[a]

Determination	AODC (per ml)	Turnover time (days)		NAB per ml		Turnover time per active cell (h)	
		Amino acids	Glucose	Amino acids	Glucose	Amino acids	Glucose
Free-floating fraction	$3.6 \times 10^5 \pm 6.7 \times 10^5$	170.6 ± 152.3	334.4 ± 419.2	$1.6 \times 10^5 \pm 2.8 \times 10^5$	$9.2 \times 10^4 \pm 1.5 \times 10^5$	5.5 ± 9.3	11.5 ± 22.7
Attached fraction	$3.5 \times 10^5 \pm 7.1 \times 10^5$	3,988.1 ± 12,056.1	8,605.1 ± 16,091.8	$9.7 \times 10^4 \pm 1.4 \times 10^5$	$6.1 \times 10^4 \pm 1.0 \times 10^5$	86.1 ± 501.5	319.7 ± 964.7
Significance of difference	0.886	0.041	0.001	0.116	0.223	0.293	0.040
Percent attached[b]	44 ± 31 (F22, A22)[c]	46 ± 28 (F22, A22)	31 ± 28 (F10, A34)	53 ± 34 (F25, A19)	57 ± 37 (F23, A21)	N/A[d] (F24, A20)	N/A (F20, A24)

[a] Means are given ± 1 standard deviation.
[b] Percent attached was calculated as [attached/(attached + free floating)] × 100. The reciprocal of turnover time was used in these calculations.
[c] (F22, A22) denotes 22 sites where the free-floating fraction was larger and 22 sites where the attached fraction was larger.
[d] N/A, Not applicable.

TABLE 4. Matrix of rotated factor loadings

Factor	Conductivity	Salinity	Elevation	pH	Seston	Particle vol	X coordinate	Turnover time			
								Glucose		Amino acids	
								Free	Attached	Free	Attached
1	0.97	0.97	−0.88	0.82	0.73	−0.69	0.65				0.49
2								0.87	0.70	0.81	0.48
3					0.52	0.54			−0.48		−0.34
4										−0.28	−0.41
5							0.55				
6											
Communality	0.95	0.96	0.87	0.73	0.84	0.79	0.79	0.78	0.73	0.77	0.75

[a] Total percent variance explained.

thoroughly a factor analysis was performed (Table 4) which produced six factors explaining 73.7% of the variance.

Factor 1 received high loadings on salinity, conductivity, pH, and seston with negative loadings on elevation, particle volume, and temperature. All these variables are characteristic of the ocean environment as is the strong west influence of the X coordinate. This first factor, responsible for 23.2% of total variance, was therefore interpreted as marine influences.

Factor 2 was characterized by high positive

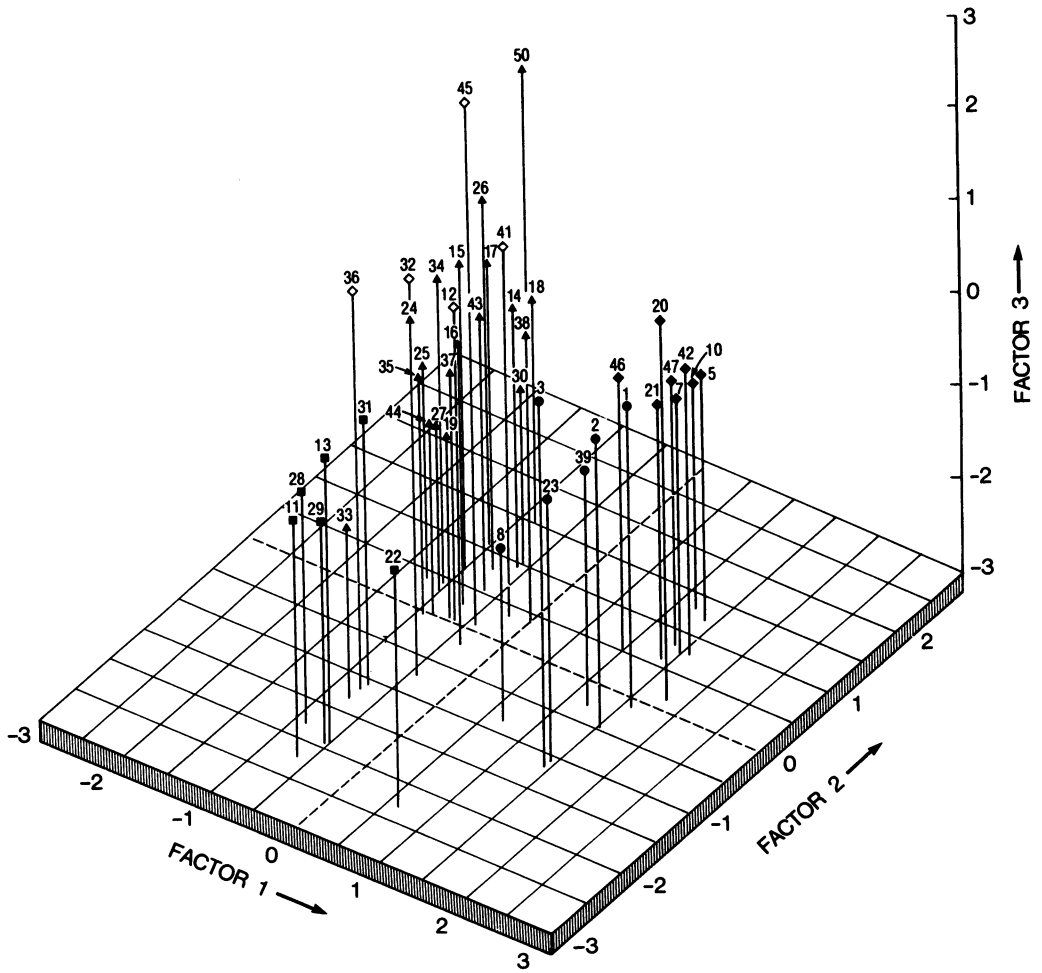

FIG. 2. Three-dimensional pin diagram of sites 1 to 50 by factor scores for factors 1, 2, and 3. Symbols: ●, cluster A; ◆, cluster B; ▲, cluster C; ◇, cluster D; ■, cluster E.

TABLE 4—*Continued*

NAB				Chlorophyll[a]	DOC	POC	AODC		Y coordinate	Particle size mode	Temp	Eigen value	% of variance explained by factor
Glucose		Amino acids					Free	Attached					
Free	Attached	Free	Attached										
								−0.34			−0.43	5.34	23.2
	−0.55	−0.29		−0.54		−0.32	−0.45	0.40			−0.49	3.63	15.8
		0.36	0.39	0.43	0.81	0.80	0.66	−0.50				3.37	14.6
0.64	−0.37	0.56	0.44									1.69	7.3
			−0.48						0.89			1.53	6.7
0.34			−0.34	0.34						0.89	0.34	1.41	6.1
0.63	0.53	0.54	0.69	0.65	0.71	0.78	0.65	0.53	0.86	0.87	0.56		73.7[a]

loadings on turnover with negative loadings on chlorophyll *a*, NAB, temperature, and AODC. This signified a factor of weak heterotrophic activity with correspondingly low microbial flora.

Factor 3, in contrast to factor 2, had high positive loadings on AODC, NAB, and chlorophyll *a* together with seston, particle volume, POC, and DOC. This indicated a factor concerned with high productivity, especially high particulate load.

Factors 4, 5, and 6 were not as significant as the first three factors; together they only accounted for 20.1% of total variance and were far harder to interpret. Factor 4, because of the positive loadings on the two NAB (free) variables, was believed to reflect some influence of the free-floating active bacteria. Factor 5 was obviously a location influence with a strong northwest bias, whereas factor 6 alluded to the larger particulate loads.

Cluster analysis. The first three factors appeared very relevant to the question of attached versus free-floating bacteria and associated heterotrophic uptake. These three factors accounted for 53.6% of the total variance in the population and were used as the three criteria to cluster the sites. The distance matrix (Fig. 1) contained five discrete clusters with all elements having very small distances between each other (≤2.002 units). Site 50, the primary effluent from Annacis Island sewage plant, was the only exception and was so dissimilar from the rest of the population that it was excluded. Figure 2 shows the sites drawn in three-dimensional factor space with their respective clusters indicated. Cluster A contained sites 1, 39, 2, 23, and 8, all situated in the quadrants with positive factor 1 loadings and negative factor 2 and 3 loadings. This group therefore tended to have a strong marine component with low turnover times and low particulate load. Cluster B contained sites 20, 7, 47, 21, 42, 46, 10, and 5, which all displayed positive factor 1 and factor 2, with factor 3 near zero. This implies that cluster B sites were again all marine, but with high turnover times. The largest cluster formed was cluster C, comprising 18 sites which include sites 14, 38, 18, 33, 30, 25, 27, 44, 19, 16, 43, 37, 35, 34, 24, 26, 17, and 15. These were all freshwater sites (negative factor 1) with high turnover times (positive factor 2). Sites 45, 32, 36, 41, and 12 constituted cluster D, which was the only group to receive a significant positive loading on factor 3. These sites were therefore characterized by having heavy particulate load, freshwater (negative factor 1), and a range of turnover times. The remaining cluster, cluster E, contained sites 22, 31, 13, 28, 29, and 11. They were predominantly freshwater habitats with low particulate loads, but very low turnover times (negative factor 2).

Attached and free-floating bacterial activity by cluster. Examination of the data arranged into five clusters (Table 5) indicated that the percent activity attached for both glucose and amino acids had significant differences between clusters. The order of clusters with increasing activity from the attached fraction for amino acids was A < B < E < C < D, whereas the order for glucose was A < B < C < E < D. The total turnover times for both substrates likewise showed significant differences. The ranking for amino acids and glucose was identical: E < D < A < C < B. The other variable with significant difference of variance was the total epifluorescent counts. Clusters E and D were most notable in possessing far higher bacterial counts than clusters A, B, and C.

Although the turnover time per active cell exhibited no significant differences between clusters, inspection of the number of cases within clusters where the free-floating fraction was greater than the attached, and vice versa, did show interesting patterns. All of the turnover time estimates for clusters A and B showed that in the majority of sites the attached portion were larger than in the free-floating portion. This implied that the activity of the free floaters was greater in most cases. The converse applied to cluster D, where more than half the sites had

TABLE 5. Breakdown of variables by cluster

Cluster	n	% Activity attached		AODC (total) per ml[a]	Turnover time			
					Days		Per active cell per hr	
		Amino acids[a]	Glucose[a]		Amino acids[a]	Glucose[a]	Amino acids[a]	Glucose[a]
A	6	21 ± 22	13 ± 15	$2.7 \times 10^5 \pm 1.7 \times 10^5$ (F3, A3)[b]	61.9 ± 60.5 (F0, A6)	51.1 ± 25.5 (F0, A6)	0.018 ± 0.012 (F1, A5)	0.015 ± 0.015 (F2, A4)
B	8	22 ± 25	22 ± 26	$3.0 \times 10^5 \pm 2.7 \times 10^5$ (F3, A5)	158.4 ± 121.8 (F1, A7)	312.7 ± 182.2 (F1, 87)	0.842 ± 1.195 (F2, A6)	0.825 ± 1.576 (F3, A5)
C	18	55 ± 25	26 ± 26	$2.1 \times 10^5 \pm 1.6 \times 10^5$ (F9, A9)	91.4 ± 75.0 (F13, A5)	410.0 ± 492.9 (F4, A14)	1.207 ± 2.219 (F14, A4)	4.251 ± 8.982 (F6, A12)
D	5	77 ± 9	60 ± 26 (F2, A3)	$1.6 \times 10^6 \pm 4.7 \times 10^5$ (F5, A0)	33.9 ± 36.4 (F3, A2)	48.3 ± 37.4 (F3, 82)	0.080 ± 0.176 (F4, A1)	0.237 ± 0.511
E	6	42 ± 14	41 ± 12	$1.5 \times 10^6 \pm 1.3 \times 10^6$ (F4, A2)	13.6 ± 12.2 (F2, A4)	1.98 ± 16.5 (F1, A5)	0.247 ± 0.599 (F3, A3)	0.005 ± 0.008 (F4, A2)

[a] Significant differences between clusters ($P < 0.10$).
[b] (F3, A3) denotes three sites where the free-floating fraction was larger and three sites where the attached fraction was larger.

greater activities in the attached fraction. Cluster C showed a division between attached and free-floating bacteria dependent on the substrate. Turnover times for amino acids were longer for the free-floating population in approximately 75% of the sites comprising the cluster. The opposite situation occurred with glucose, where the greater activity was associated with the free-floating bacteria.

Factors influencing percent activity attached. The three-way analysis of variance tales (Tables 6 and 7) demonstrated that factor 3 had a significant influence on the percent activity attached with both amino acids and glucose. The contribution of the attached population was greater when factor 3 had positive loadings signifying high particle load. The uptake of amino acids between the free-floating and attached flora was also influenced by factor 1 (Table 6). A lower percent activity attached was recorded when factor 1 was positive, i.e., when the environments showed a strong marine character. A closer analysis of this relationship between percent activity attached and factor 1/factor 3 ratio is shown in Table 8. The grouping of factor 1 into positive and negative categories demonstrated that the reduced percent activity attached for amino acids with factor 1 positive was accompanied by a high turnover time of the attached bacteria, but no significant difference in numbers of attached bacteria, either total or active. The turnover time per attached cell, however, was significantly greater for the more marine environments. This scenario occurred again with the factor 3 categories with glucose as the substrate. When factor 3 was positive there was an increased percent activity attached and reduced turnover time per active attached cell. The relationship differed with amino acids, however, where the increased percent activity attached with factor 3 positive was accompanied by an increased number of active attached bacteria and no significant difference in turnover time per active attached cell.

DISCUSSION

In this study we have attempted to resolve the question of the contribution of attached versus free-floating bacteria to the heterotrophic activities and bacterial numbers of a range of water bodies. The overall impression, considering all 44 sites in toto, was that the free-floating population exhibited lower turnover times than those of the attached flora, yet epifluorescent counts comparable to those of the attached flora. Even with this simple analytical approach, however, complications due to the differing responses of glucose and amino acids as substrates emerged. The insignificant difference in free-floating versus attached bacterial counts implies that there

TABLE 6. Three-way analysis of variance of percent activity attached (amino acids) by factors 1, 2, and 3

Factor	Category	n	Mean score	Multiple R^2	Source of variation	Sum of squares	df	F	Significance
1	Negative	28	56.2	0.23					
	Positive	16	28.2						
2	Negative	17	43.8	0.004					
	Positive	27	47.4						
3	Negative	30	37.1	0.22					
	Positive	14	65.2						
					Main effects	12,795	3	7.69	<0.001
					Factor 1	5,258	1	9.48	0.004
					Factor 2	19	1	0.03	0.856
					Factor 3	4,826	1	8.70	0.005
					Two-way interactions	873	3	0.52	0.668
					Factors 1 and 2	169	1	0.31	0.584
					Factors 1 and 3	809	1	1.46	0.235
					Factors 2 and 3	11	1	0.02	0.889
					Explained	13,668	6	4.105	0.003
					Residual	20,531	37		
					Total	34,198	43		

must have been an increase in the number of active bacteria or an increase in the activity per cell to explain the faster turnover times of the free-floating bacteria. Inspection of the data in Table 3 reveals that the former explanation did perhaps occur with the amino acid mix, whereas the latter explanation applied to glucose. Both of these mechanisms are commonplace and have been observed to operate in other aquatic environments (13, 25). Unfortunately, because it was impossible to determine the proportions of each amino acid taken up from the mix, no definitive comparison can be made between the amino acids and glucose.

The use of multivariate analysis, however, did prove to be a fruitful approach to pursue this phenomenon. The advantages in microbial ecology of reducing the variability of a diverse population to its most salient factors is now becoming well documented (11, 12). The formation of five distinct types of aquatic environments based on the factors of salinity, heterotrophic uptake, and particulate load was both fascinating and pertinent to this investigation.

TABLE 7. Three-way analysis of variance of percent activity attached (glucose) by factors 1, 2, and 3

Factor	Category	n	Mean score	Multiple R^2	Source of variation	Sum of squares	df	F	Significance
1	Negative	28	34.8	0.036					
	Positive	16	24.2						
2	Negative	17	34.8	0.012					
	Positive	27	28.5						
3	Negative	30	20.8	0.30					
	Positive	14	52.7						
					Main effects	11,195	3	6.93	0.001
					Factor 1	216	1	0.40	0.530
					Factor 2	1,270	1	2.36	0.133
					Factor 3	9,522	1	17.69	<0.001
					Two-way interactions	1,710	3	1.06	0.378
					Factors 1 and 2	1,575	1	2.93	0.096
					Factors 1 and 3	291	1	0.54	0.467
					Factors 2 and 3	46	1	0.09	0.770
					Explained	12,905	6	4.00	0.004
					Residual	19,918	37		
					Total	32,823	43		

TABLE 8. *t*-Test comparison of means for factor 1 and factor 3 categories[a]

Factor	Category	% Activity attached		AODC (attached) per ml × 10^{-5}	Turnover time (attached), days		NAB (attached), per ml × 10^{-4}		Turnover time per active cell (attached), h	
		Amino acids	Glucose		Amino acids	Glucose	Amino acids	Glucose	Amino acids	Glucose
1	Positive	28.2 ± 27.2[b]	N/A[c]	3.4 ± 7.8	8,083.3 ± 16,666.7[b]	N/A	15.1 ± 33.1	N/A	228.4 ± 828.8[b]	N/A
	Negative	56.2 ± 23.7[b]		3.5 ± 6.8	1,625.0 ± 7,833.3[b]		15.8 ± 25.8		4.8 ± 8.0[b]	
3	Positive	65.2 ± 27.6[b]	52.7 ± 28.0[b]	5.7 ± 9.3	208.3 ± 375.0[b]	720.8 ± 954.2[b]	24.4 ± 34.8[b]	12.9 ± 22.5	7.9 ± 19.2	16.8 ± 38.9[b]
	Negative	37.1 ± 24.0[b]	20.7 ± 21.1[b]	2.4 ± 5.7	5,750.0 ± 14,333.3[b]	12,291.7 ± 18,416.7[b]	11.4 ± 24.3[b]	7.5 ± 10.7	122.6 ± 606.9	461 ± 1,146[b]

[a] Means are given ±1 standard deviation.
[b] Significant difference between positive and negative values ($P < 0.10$).
[c] N/A, Not applicable.

When the percent activity of the free-floating and attached fraction was examined within each cluster (Table 5), the free floaters unequivocally predominated in all marine samples (clusters A and B). This finding agrees well with the data of numerous other workers who have found a preponderance of free-floating bacteria in the sea (see above). Further investigation into the effect of marine water (factor 1) on heterotrophic activity revealed that it operated only on the uptake of amino acids by increasing the turnover time per active attached cell. A probable explanation of this observation is in the effect of salinity on the chemiosmotic potential of the cell membrane (15). Unfortunately the turnover time of the marine samples were not all uniformly slow as would be expected with a chemiosmotic imbalance. Cluster A comprised estuarine sites and frontal regions within the Strait of Georgia, where turnover was comparatively fast. Why salinity should have had a selective effect on the activity of attached cells is unknown.

The cluster where the converse situation applied and attached bacteria constituted the dominant role in heterotrophic uptake was cluster D. These sites all contained heavy particulate loads and supported the later finding that factor 3 was influential in effecting percent activity attached. A substrate-specific response again occurred under the influence of factor 3 with glucose causing an increase in the activity per cell, whereas amino acids produced higher numbers of active bacteria. It is interesting to speculate that such a difference may reflect subtle changes in the bacterial populations. It seems reasonable to suggest that the population adapted to amino acid uptake in the natural environment may be engaged in proteolytic activity or the breakdown of other complex molecules such as detritus. Such bacteria would be expected to show a close association with their particulate substrate and may react to high particulate loads by increasing their numbers to promote colonization or form aggregates. Bacteria utilizing glucose, on the other hand, because of the exceptionally high liability of the compound, may find an increase in activity a more appropriate response to the transient concentrations of glucose from previous reactions, most notably phytoplankton metabolism. This argument can also be extended to explain the 46% attached activity with amino acids compared with the 31% with glucose (Table 3). The greater the complexity of the molecule transported the more likely the bacteria involved will be associated with the larger-size particulate fraction.

The most notable case where substrate-specific responses occurred was in cluster C. This large cluster comprised 18 sites, all of which were freshwater lakes or rivers with turnover

times slow enough to classify them as oligotrophic (21). Glucose again elicited a substantially faster turnover time in the free-floating fraction due to a shorter turnover time per free-floating active cell. The reverse applied to the amino acids, with faster times observed in the attached portion. This conforms to the pattern already described and lends credence to the above hypothesis. Presumably in the oligotrophic systems with the paucity of nutrients, colonization of particulates would be more critical, and hence the different response of amino acids and glucose would be more demarcated.

The partitioning of heterotrophic activity with the amino acids mix and glucose has produced consistent patterns in this selection of water bodies. The diversity of the sites sampled should ensure the significance of the results for these two substrates. However, generalizations about the contribution of particle-attached bacteria versus free-floating bacteria to the heterotrophic activity of a water body have to be made cautiously in the light of the substrate-specific nature of the uptake. Also, because the sites were sampled only once between May and November, changes in the water with time have to be ignored. Such temporal variations would obviously be important in the ecology of these water masses, but were not the topic of this project. Analyses have been confined to the influence of absolute levels of nutrients and physicochemical parameters on the partitioning of heterotrophic activity.

ACKNOWLEDGMENTS

We acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. **Azam, F., and O. Holm-Hansen.** 1973. Use of tritiated substrates in the study of heterotrophy in seawater. Mar. Biol. **23**:191–196.
2. **Azam, F., and R. E. Hodson.** 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. **22**:492–501.
3. **Bell, C. R., and L. J. Albright.** 1981. Attached and free-floating bacteria in the Fraser River Estuary, British Columbia, Canada. Mar. Ecol. Prog. Ser. **6**:317–327.
4. **Berman, T., and M. Stiller.** 1977. Simultaneous measurement of phosphorus and carbon uptake in Lake Kinneret by multiple isotopic labelling and differential filtration. Microb. Ecol. **3**:279–288.
5. **Burnison, B. K.** 1975. Microbial ATP studies. Verh. Int. Ver. Limnol. **19**:286–290.
6. **Ferguson, R. L., and P. Rublee.** 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. **21**:141–144.
7. **Geesey, G. G., and J. W. Costerton.** 1979. Microbiology of a northern river: bacterial distribution and relationship to suspended sediment and organic carbon. Can. J. Microbiol. **25**:1058–1062.
8. **Goulder, R.** 1976. Relationships between suspended solids and standing crops and activities of bacteria in an estuary during a Neap-Spring Neap tidal cycle. Oceologia **24**:83–90.
9. **Goulder, R., A. S. Blanchard, P. L. Sanderson, and B. Wright.** 1980. Relationships between heterotrophic bacteria and pollution in an industrialized estuary. Water Res. **14**:591–601.
10. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescent microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
11. **Holder-Franklin, M. A.** 1981. The development of biological and mathematical methods to study population shifts in aquatic bacteria in response to environmental change. Scientific series no. 24. Inland Waters Directorate, Environment Canada, Ottawa, Canada.
12. **Holder-Franklin, M. A., M. Franklin, P. Cashion, C. Cormier, and L. Wuest.** 1978. Population shifts in heterotrophic bacteria in a tributary of the Saint John River as measured by taxometrics, p. 44–50. *In* M. W. Loutit and J. A. R. Miles (ed.), Microbial ecology. Springer Verlag, New York.
13. **Hoppe, H. G.** 1976. Determination and properties of actively metabolizing heterotrophic bacteria in the sea, investigated by means of autoradiography. Mar. Biol. **36**:291–302.
14. **Jannasch, H. W.** 1956. Vergleichende Bakteriologische untersuchung der Adsorptionswirkung des Nil-Treibschlammes. Ber. Limnol. Flusst. Freudenthal Munden. **7**:21–27.
15. **Konings, W. H., and H. Veldkamp.** 1980. Phenotypic responses to environmental change, p. 161–192. *In* D. C. Ellwood, J. N. Hedger, M. J. Latham, J. M. Lynch, and J. H. Slater (ed.), Contemporary microbial ecology. Academic Press, Inc., London.
16. **Paerl, H. W.** 1974. Bacterial uptake of dissolved organic matter in relation to detrital aggregation in marine and freshwater systems. Limnol. Oceanogr. **19**:966–972.
17. **Paerl, H. W.** 1975. Microbial attachment to particles in marine and freshwater ecosystems. Microb. Ecol. **2**:73–83.
18. **Pomeroy, L. R.** 1974. The ocean's food web, a changing paradigm. Bioscience **24**:499–504.
19. **Riemann, B.** 1978. Differentiation between heterotrophic and photosynthetic plankton by size fractionation, glucose uptake, ATP and chlorophyll content. Oikos **31**:358–367.
20. **Roper, M. M., and K. C. Marshall.** 1979. Effects of salinity on sedimentation and of particulates on survival of bacteria in estuarine habitats. Geomicrobiol. J. **1**:103–116.
21. **Seki, J., K. S. Shortreed, and J. G. Stockner.** 1980. Turnover rate of dissolved organic materials in glacially-oligotrophic and dystrophic lakes in British Columbia, Canada. Arch. Hydroiol. **90**:210–216.
22. **Sieburth, J. M., R. D. Brooks, R. V. Gessner, C. D. Thomas, and J. L. Tootle.** 1974. Microbial colonization of marine plant surfaces as observed by scanning electron microscopy, p. 418–432. *In* R. R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
23. **Strickland, J. D. H., and T. R. Parsons.** 1968. A practical handbook of sea-water analysis. Bull. Fish. Res. Bd. Can. **167**:185–192.
24. **Wiebe, W. J., and L. R. Pomeroy.** 1972. Microorganisms and their association with aggregates and detritus in the sea: a microscopic study. Mem. Ist. Ital. Idrobiol. Dott Marco de Marchi Pallanza Italy **29**(Suppl.):325–352.
25. **Wright, R. T.** 1978. Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. **36**:297–305.