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Correlations Between Predominant Heterotrophic Bacteria and Physicochemical Water Quality Parameters in Two Canadian Rivers

C. R. BELL,† M. A. HOLDER-FRANKLIN,* AND M. FRANKLIN

Environmental Microbiology Research Laboratory, Great Lakes Institute, and Department of Biology, University of Windsor, Windsor, Ontario, Canada N9B 3P4

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The heterotrophic bacterial populations in two contrasting rivers have been examined over a period of 1 year. The populations were analyzed (i) as total heterotrophic counts, (ii) as species numbers, using numerical taxonomy, (iii) by diversity indices, and (iv) by factor analysis. Isolates were obtained by plating directly from water samples and by chemostat enrichment. Four factors emerged which profiled the bacterial community and were common to both rivers. They were, in order of decreasing importance, fermentative metabolism, inorganic nitrogen metabolism, fluorescence-oxidative metabolism, and lack of starch hydrolysis. Several factors produced significant correlations with a range of physicochemical parameters, which were also measured. The correlations suggested an intricate algal-bacterial interaction. The oxidative metabolism factor correlated with rainfall in one river, suggesting that the oxidative bacteria may be washed in from the surrounding land. In the other river, the oxidative-fermentative factor correlated negatively with sunshine. Factor analysis was the most effective method for revealing correlations between bacterial characteristics and the environmental parameters; however, the use of a variety of methods provided more insight into the ecological aspects.

The power of multivariate statistics, especially factor analysis, in elucidating the complexities of natural microbial ecosystems has been appreciated by several workers in the past decade (33, 39). Factor analysis has been used in natural habitats to examine soil bacterial populations (28, 37, 38), lacustrine bacteria (19, 20), and coastal microbial communities (7, 42). Toerien and co-workers (6, 40, 41) have made extensive use of factor analysis in areas of applied microbiology to study the microbial populations in denitrifying systems and anaerobic digesters.

This paper is the continuation of an ongoing extensive study of the heterotrophic bacterial populations in the waters of the Saint John River valley, New Brunswick, Canada (14), which was conducted at the Microbiology Research Laboratory, University of New Brunswick. Two tributaries of the Saint John River have been studied; the Dunbar, a pristine woodland stream; and the Meduxnekeag, a larger stream which received some domestic and agricultural influent. Factor analysis has already been used to successfully study the seasonal and diurnal population shifts in the Meduxnekeag River (14, 15). These population shifts have been related to the environmental changes by correlating two factor analyses, one on the bacterial test responses and one on the physicochemical data (M. A. Holder-Franklin and L. J. Wuest, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, I78, p. 94). The chemical, physical, and bacterial parameters of the Dunbar and Meduxnekeag Rivers over a period of 1 year have been reported elsewhere (1). Taxometric analysis demonstrated that the bacteria were predominantly fluorescent *Pseudomonas* spp. and psychrotrophic in both rivers. The water temperature, which maintained a low of 0°C under the ice cover and rose to 20°C in the summer, was the most influential physicochemical parameter on bacterial activity.

A multivariate statistical analysis of the data from the Dunbar and Meduxnekeag Rivers has been performed to elucidate the interactions of the bacterial populations with the physicochemical parameters of the water.

MATERIALS AND METHODS

Sampling sites. Twelve samples were taken from the surface waters of the Meduxnekeag and Dunbar Rivers (for taxometric analysis). The sampling dates were chosen to reflect seasonal differences in each river. The Meduxnekeag samples were collected during the winter of 1977 from 7 February to 7 March and during

† Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1G5.

summer and fall from 25 July to 3 October 1977. Winter samples were collected from the Dunbar during the period from 14 February to 14 March 1977, and autumn samples were collected from 12 September to 17 October 1977. In addition to these 12 samples, water samples were collected regularly from each river every alternate week between February 1977 and February 1978 and analyzed for total heterotrophic counts, chlorophyll *a*, and a range of physicochemical water quality parameters. Samples for bacterial counts were collected in the sterile 200-ml chamber of a Sorvall Omni-mixer homogenizer. Other samples were collected in Nalgene bottles and treated immediately if necessary.

Physicochemical parameters. The physicochemical water quality parameters were assayed as described previously (1) and included temperature, dissolved oxygen, turbidity, color, pH, total alkalinity, specific conductance, calcium, magnesium, potassium, sodium, ammonia, nitrate-nitrite, chloride and sulfate ions, total phosphorus, total organic carbon, total inorganic carbon, and chlorophyll *a*. The rainfall and sunshine data used in the correlations was obtained simultaneously on the day of sampling. The weekly accumulation of rainfall (millimeters) was calculated from data provided by the Fredericton Weather Office (Atmospheric Environment Service). The daily average hours of sunshine in any week was calculated from the same source.

Cultural methods. The spread plate method was used to estimate viable heterotrophs on a glucose-nitrogen minimal medium after Brown and Stanley (2), with a supplement of 0.2% (wt/vol) Casamino Acids. Plates were incubated at 20°C, and colony-forming units were counted after 1 week. Individual colonies were obscured by the colonies of spreading bacteria on longer incubation. Colonies for taxometric analysis, grown on the above agar plates, were picked from the plates at random and purified.

Chemostat enrichment. Colonies were also grown on the glucose-nitrogen minimal agar plates after chemostat enrichment. Inocula of 1,000 ml of river water were enriched in chemostats at a dilution rate of 0.027 h^{-1} at 5 and 20°C. The medium of Brown and Stanley (2) was again used with a series of inorganic nitrogen concentrations (either $N\text{-}NO_3$ or $N\text{-}NH_4$ in concentrations of 0.1, 1.0, and 10.0 μg of N ml^{-1}). The use of agar media with various nitrogen concentrations and the chemostat enrichment were designed to increase the species diversity and possibly reveal more interrelationships between the bacteria and the environment. The chemostats were left on flow for 5 to 6 days before a sample was removed for plating. A total of 1,103 strains were isolated; 559 strains (50%) were obtained from the chemostats. The chemostat enrichments were part of a much larger study which compares the chemostat populations with plate populations (C. R. Bell, M. A. Holder-Franklin, and M. Franklin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, I79, p. 94).

Numerical taxonomy. The methods for numerical taxonomy have been described previously (1). A range of 82 tests, which were primarily physiological, was selected for numerical taxonomy. The tests included ability to grow on the following compounds as sole source of carbon (0.1, wt/vol, except sugars [0.15%, wt/vol]): acetate, D-arabinose, DL-arginine, benzoate, citrate, dihydroxybenzoate, ethylene glycol, D-galactose, D-glucose, L-glutamate, DL-β-hydroxybutyrate, L-isoleucine, α-ketoglutarate, lactose, maleate, malonate, methanol, oxalate, ornithine, pelargonate, pyruvate, L-rhamnose, sorbitol, DL-serine, tartrate, L-tryptophan, carboxymethyl cellulose, and L-cystine. Ability to use inorganic nitrogen (200 and 10 μg of N ml^{-1} for $N\text{-}NO_3$ and $N\text{-}NH_4$) and ability to grow on nitrogen-free media (22, 27, 29) and to grow at pH 5.0, 6.0, 9.0, and 10.0 and at 4, 15, and 37°C were included. Tolerance to 0.5% (wt/vol) NaCl, 5.0% NaCl, 1% (wt/vol) bile salts, 0.1% (wt/vol) phenol, 0.008% (wt/vol) KCN, 1% (wt/vol) tellurite, 0.1% (wt/vol) methylene blue, and 0.1% (wt/vol) basic fuchsin and hydrolysis of casein, gelatin, Tween 80, and starch were tested. Production of catalase, oxidase, phosphatase, levan sucrase, urease, indole, fluorescein and phenazine pigments (24), arginine dihydrolase, H_2S on triple sugar iron agar, and acid or gas or both from glucose peptone water; ability to oxidize or ferment (with or without gas) glucose and lactose (16); and ability to ammonify, denitrify, reduce nitrate, reduce nitrite, grow anaerobically, and accumulate poly-β-hydroxybutyrate were tested. Methyl red and Voges-Proskauer reactions, Gram reaction, and motility were also included.

Similarity coefficients were calculated according to Jaccard (17). Seventy-five percent similarity level clusters were formed by the unweighted-pair group method with arithmetic average cluster analysis (35). Identification of the clusters to the species level was performed by reference to *Bergey's Manual of Determinative Bacteriology* (3). Sixty-five reference strains were included in the analysis (14).

The frequencies of positive responses for each test for the entire population of isolates obtained on a given sample date were expressed as percent, and the significant dimensions of variation in each data set were then determined by factor analysis. Briefly, the principal factor method of Harman (13) was used, with varimax rotation of the factors. The computer manipulations were all conducted on the package NTSYS (Numerical Taxonomy System of Multivariate Statistical Programs) of Rohlf et al. (32).

Factor scores were displayed onto a three-dimensional projection, using the program SYMVU of the Harvard University Laboratory for Computer Graphics and Spatial Analysis. These projections of the factor scores for each factor were also given for each sample date. The projections were correlated with the physicochemical parameters, using simple Pearson product moment correlations and also partial correlations. All correlations were performed with the Statistical Package for the Social Sciences.

The diversity indices of Simpson and Shannon-Weaver, outlined in Pielou (31), were calculated for each sample date. The Shannon-Weaver indices are presented; the Simpson indices were essentially the same. Clusters of four or more strains were selected. Each strain was identified to species. These indicators, together with total heterotrophic counts, and species numbers were also correlated with the physicochemical parameters.

RESULTS

The total number and percentage of bacterial types recovered throughout the year on the 12

TABLE 1. Variations in the Meduxnekeag populations between sample dates

Bacterial type	No. (%)[a]					
	Winter			Summer and Fall		
	7 Feb.	21 Feb.	7 Mar.	25 July	19 Sept.	3 Oct.
Obligate aerobes	47 (64)	84 (83)	67 (66)	64 (67)	58 (79)	59 (80)
Facultative anaerobes	26 (36)	16 (16)	18 (18)	14 (15)	11 (15)	5 (7)
Gliding bacteria		1 (1)	17 (16)		4 (6)	8 (11)
Enteric bacteria				7 (7)		
Acinetobacter sp.				10 (11)		2 (2)
Total individuals	73	101	102	95	73	74
Total species	10	7	7	11	6	6
Diversity index (Shannon)	1.90	1.44	1.21	2.12	1.09	1.02

[a] Total of individuals for each bacterial type and percentage of each type isolated on any one sample date.

sample dates (Tables 1 and 2) show considerable variability. The Meduxnekeag populations showed less seasonal change than those in the Dunbar. The samples obtained from the summer Dunbar waters were particularly prone to fluctuations in bacterial species. Generally, the species obtained in the winter from both rivers were less likely to vary than those isolated in the summer and fall, presumably due to the influence of the ice cover. Surprisingly, in these cold waters, the Meduxnekeag harbored a greater percentage of facultative anaerobes than in the summer. The Dunbar contrasted with the Meduxnekeag in the higher number of gliding bacteria, particularly *Cytophaga* spp., in its waters throughout the year. The species isolated on the various concentrations of nitrogen were essentially the same, as were the chemostat populations in this particular study except where indicated. The variations could not be related to the isolation method.

Diversity indices. The diversity indices quantified these observations more precisely and facilitated the determination of relationships with the physicochemical parameters (Tables 3 and 4). In the Meduxnekeag River (Table 3), both indices showed significant positive correlations with NH_4^+, alkalinity, and calcium. Specific conductance and color appeared to be linked to alkalinity, as indicated by the partial correlations. Alkalinity was measured as total $CaCO_3$ equivalent by titration and hence would be intrinsically related to calcium. The partial correlations showed this to be the case. NH_4^+ had no intercorrelations and so comprised the key variable along with alkalinity.

The correlations with the diversity indices of the Dunbar population (Table 4) showed slight differences depending on the index. The only significant variable correlating with Shannon's index was found to be NH_4^+.

Species numbers. Ammonia again emerged as the most powerful variable when correlations of species numbers and physicochemical parameters were computed (Tables 5 and 6). To obtain species numbers, only species appearing on three or more sample dates and from clusters of four or more strains were included. All species correlating with NH_4^+ did so positively, in both rivers, with one exception, *Pseudomonas fluorescens* I. Due to the dominance of *P. fluorescens* I in all of these populations (>49%),

TABLE 2. Variations in the Dunbar populations between sample dates

Bacterial type	No. (%)[a]					
	Winter			Summer and Fall		
	14 Feb.	1 Mar.	14 Mar.	12 Sept.	26 Sept.	17 Oct.
Obligate aerobes	58 (77)	80 (76)	93 (76)	64 (62)	88 (84)	36 (49)
Facultative anaerobes	15 (20)	9 (9)	4 (3)	18 (17)	12 (11)	
Gliding bacteria	2 (3)	16 (15)	25 (21)	13 (13)		33 (45)
Enteric bacteria				2 (2)	5 (5)	
Acinetobacter sp.				7 (6)		4 (6)
Total individuals	75	105	122	104	105	73
Total species	9	5	6	14	7	7
Diversity index (Shannon)	1.35	0.81	1.04	2.27	1.07	1.60

[a] Total of individuals for each bacterial type and percentage of each type isolated on any one sample date.

TABLE 3. Shannon's diversity index—Meduxnekeag River

Parameter	Correlation coefficient	Significance level (%)	Partial correlation	Significance level (%)	Variables controlled
NH_4^+	0.765	3.8	0.765	3.8	
Alkalinity	0.708	5.8	0.859	7.1	Specific conductance, color
Ca^{2+}	0.706	5.8	0.782	10.9	Specific conductance, color
Specific conductance	0.607	10.1		NS[a]	
Color	−0.604	10.2		NS	

[a] NS, Not significant.

TABLE 4. Shannon's diversity index—Dunbar River

Parameter	Correlation coefficient	Significance level (%)	Partial correlation	Significance level (%)
NH_4^+ [a]	0.853	1.5	0.853	1.5
Cl^-	−0.803	2.7		NS[b]
Temp	0.797	2.9		NS

[a] No variables controlled.
[b] NS, Not significant.

a reduction in numbers with increasing NH_4^+ concentrations may explain the observed increase in diversity indices. Most temperature correlations reflect the seasonal incidence of most of the species. *P. fluorescens* I was the exception in the Dunbar, endorsing its prevalance in this river under the ice. *Flavobacterium devorans* was the only species in the Meduxnekeag River which was not seasonal and which correlated with temperature. The correlations with sunshine are of interest because the hours of sunshine did not show an obvious seasonal pattern; therefore, correlations with sunshine must be related to changes in the angle of radiation, which does vary seasonally. The mean daily hours of sunshine per week for 1977 to 1978 were 5.6 ± 2.8 h (one standard deviation). Correlations with potassium, all negative, occurred with species recovered only during the summer months and may represent the considerable accumulation of this element under the ice cover.

Total phosphorus emerged as a strong correlating variable in the Dunbar River. Concentrations of phosphorus were lower in the Dunbar River than in the Meduxnekeag River (0.020 ± 0.030 mg [Dunbar] versus 0.033 ± 0.035 mg [Meduxnekeag]); therefore, certain sectors of the population may be limited by phosphorus availability. In this respect, the only positive correlation was observed with *Cytophaga fermentans*, suggesting that the remaining four negative correlations reflected some other unknown competitive process. Chloride has been shown

TABLE 5. Correlations between physicochemical parameters and species numbers (Meduxnekeag River)

Species[a]	Pearson product-moment coefficient (significance [%])						
	NH_4^+	Sun	Temp	K^+	SO_4^{2-}	Total P	Chlorophyll[a]
Pseudomonas aeruginosa	0.771 (3.6)	0.868 (1.2)					
Pseudomonas fluorescens I	−0.625 (9.2)	−0.678 (6.9)					
Pseudomonas alcaligenes[b]						0.931 (0.3)	
Pseudomonas solanacearum[c]	0.749 (4.3)		0.78 (3.3)	−0.866 (1.3)	−0.814 (2.4)		
Alcaligenes paradoxus[c]	0.945 (0.2)	0.789 (3.1)		−0.969 (0.1)			
Aeromonas hydrophila[c]							0.836 (1.9)
Chromobacterium lividum					0.829 (2.1)		
Flavobacterium devorans	0.761 (3.9)		0.764 (3.9)				
Acinetobacter sp.[c]	0.928 (0.4)	0.770 (3.7)	0.793 (3.0)	−0.979 (0)			

[a] Species of four strains per cluster.
[b] Isolated only in a chemostat.
[c] Species recovered only during the summer months.

TABLE 6. Pearson product-moment correlations between physicochemical parameters and species numbers (Dunbar River)

Species	Product-moment coefficient (significance [%])											
	Temp	NH_4^+	Cl^-	Inorganic C	Total P	Ca^{2+}	Alkalinity	Mg^{2+}	Chlorophyll *a*	SO_4^{2-}	DO_2 saturation	Turbidity
Pseudomonas fluorescens I	−0.760 (4)	−0.604 (10.2)	0.915 (0.5)									
Pseudomonas alcaligenes											−0.835 (1.9)	
Pseudomonas flava[a,b]	0.743 (4.5)	0.968 (0.1)	−0.758 (4.0)									
Pseudomonas palleronii[a]		0.980 (0)		0.819 (2.3)	−0.755 (4.1)					0.747 (4.4)		−0.928 (1.1)
Alcaligenes eutrophus[a]	0.824 (2.2)											
Alcaligenes paradoxus[b]				0.738 (4.7)	−0.801 (2.8)	0.797 (2.9)	0.784 (3.3)					
Aeromonas hydrophila[a]		0.799 (2.8)		0.982 (0)	−0.946 (0.2)				0.772 (3.6)			
Flavobacterium devorans[b]				0.975 (0)	−0.974 (0.1)		0.844 (1.7)	0.784 (3.3)	0.878 (1.1)			−0.962 (0.4)
Cytophaga fermentans subsp. *agarovorans*[c]						−0.820 (2.3)	−0.732 (4.9)	−0.751 (4.3)			−0.735 (4.8)	
Cytophaga fermentans subsp. *fermentans*					0.817 (2.4)	−0.887 (0.9)	−0.872 (1.2)	−0.832 (2.0)				
Cytophaga hutchinsonii		0.832 (2.0)								0.796 (2.9)		
Flexibacter succinicans[a]	0.835 (1.9)		−0.824 (2.2)									
Acinetobacter sp.[a]	0.845 (1.7)	0.845 (1.7)	−0.850 (1.6)									

[a] Species recovered only during the autumn months.
[b] Isolated only in a chemostat.
[c] Isolated only on plates.

previously (1) to be inversely proportional to temperature in the Dunbar, so correlations with chloride may be spurious. Alkalinity and inorganic carbon essentially both measure carbonate at this pH. The most noticeable trend in the correlations with alkalinity was the negative correlations of the *Cytophaga* when all other species were positive.

In the Dunbar only *F. devorans* and *Aeromonas hydrophila*, both facultative anaerobes, correlated with chorophyll *a*. In the Meduxnekeag only *A. hydrophila*, of all species identified, correlated significantly with chlorophyll *a*.

Factor analysis. Attempts to relate physicochemical parameters to populations measured by species composition and species numbers had limitations. Because the population examined represents only those species clustered by numerical taxonomy, the investigation may be inadvertently biased. Seventy-eight percent of the total isolates clustered in groups of four or more; therefore, 22% of the strains were not included in the species diversity and species numbers sections. However, the use of factor analysis enabled the whole population of isolates, clustered and nonclustered, to be examined and the major dimensions of its variability in terms of the 82 tests used to be extracted. Each factor was a construct of the total 82 tests, and each test was loaded within this construct according to its significance in describing the factor. Each factor could then be interpreted in light of the tests and their negative or positive loadings. The usual practice of describing a factor by its most significant tests has been used. Five major factors were extracted for each river (Tables 7 and 8). The eigen value is a measure of the variance represented by each factor. There was a surprising correspondence between the four largest factors from each river (Table 9).

The projection of factor scores for each sample date as a spire on a three-dimensional graph allows the seasonal changes to be visualized more easily (Fig. 1 and 2). The three factors reflecting inability to hydrolyze starch, fluorescent psychrophilic oxidation, and nitrogen fixation were displayed.

Factor of nitrogen fixation. The three summer and fall Meduxnekeag samples showed a much higher loading on factor 1 than did the winter samples. This implied a much improved ability to "fix" N_2 or, more accurately, scavenge nitrogen in the summer. Conversely, in winter, the population had an increased capacity to utilize inorganic nitrogen, particularly to reduce NO_3. This factor also showed a significant positive correlation with chlorophyll *a* and temperature and a negative correlation with NO_3 (Table 10). These three variables demonstrated a tight intercorrelation and appeared to operate in toto (1). The partial correlations did suggest, however, that chlorophyll *a* was the primary influence.

The Dunbar populations showed a similar winter-summer divergence on the equivalent factor, number 2. Again, the ability to scavenge nitrogen was enhanced in the summer and fall, although in this river it has been shown that NO_3 utilization is greater in the summer (1). Significant correlations were again observed for this factor in the Dunbar, with temperature a negative and NO_3 a positive correlation (Table 10).

Factor of fluorescent, psychrophilic oxidation. Factor 4 in both rivers represented fluorescent, psychrophilic oxidation and seemed to be most influenced by weather conditions rather than by any physicochemical parameter in the water. In the Dunbar, this factor is negatively correlated with hours of sunshine (Table 10). In the Meduxnekeag (Table 10), rain emerged from the correlations as the predominant influence.

Factor of inability to hydrolyze starch. The height of each spire on both graphs, representing the inability to hydrolyze starch, was similar for all samples except for 21 February in the Meduxnekeag and 1 March in the Dunbar. On these dates, the incidence of starch-hydrolyzing bacteria was high. This factor in both rivers, factor 3 in the Meduxnekeag and factor 5 in the Dunbar, was correlated with oxygen (Table 10). Meduxnekeag factor 3 had a positive correlation with O_2 saturation, whereas Dunbar factor 5 was inversely correlated with dissolved O_2 concentration.

Factor of fermentative ability. An abrupt increase in fermentative ability occurred under the ice in both rivers on the same dates that starch hydrolysis was high (Table 11). The fermentative capacity was moderate in both rivers except for samples taken on 7 February and 14 February, when fermentation was extremely high.

DISCUSSION

Shifts in the predominant bacterial populations have been related to changes in the physicochemical quality of the river water. To expand the causal interpretations of correlations, the bacterial populations were measured in four distinct ways: (i) as viable heterotrophic counts, (ii) as the numbers of each species, (iii) as diversity indices, and (iv) by factor analysis. Factor analysis constituted the most thorough measure by utilizing the test response frequencies of all isolates and then mathematically reducing the significant dimensions of variation in the population to a few factors. The major factors extracted from the independent analysis of each river again showed remarkable similarities between rivers, as noted earlier (1); and were, in decreasing order of importance, fermentative metabolism, inorganic nitrogen me-

TABLE 7. Major factors representing the bacterial population (Meduxnekeag River)

Factor no.	Physiological tests	Loading	Eigen value	Interpretation
1	$N\text{-}NO_3$ reduction	−0.835	12.5	Mesophilic "N fixation" with limited ability to utilize inorganic N
	$N\text{-}NH_4$ assimilation	−0.763		
	Growth on N_2-free media			
	N20[a]	0.704		
	N21[b]	0.714		
	Growth at 37°C	0.712		
	4°C	−0.576		
	Ammonification	0.543		
2	Anaerobic growth	0.847	24.3	Fermentative and anaerobic metabolism
	Lactose fermentation	0.791		
	Lactose oxidation	−0.930		
	Catalase reaction	−0.860		
	Oxidase reaction	−0.860		
	Glucose oxidation	−0.656		
	Motility	−0.807		
	Urease	0.958		
	Methyl red reduction	0.717		
3	Starch hydrolysis	−0.961	10.8	Anaerobic growth associated with inability to hydrolyze starch and lack of nutritional versatility
	PBH accumulation[c]	−0.759		
	Growth on Cystine	−0.865		
	Arabinose	−0.847		
	Oxalate	−0.838		
	Ethylene glycol	−0.74		
	Anaerobic growth	0.506		
4	Production of fluorescein	0.830	14.3	Fluorescent, psychrophilic, oxidative metabolism
	Glucose oxidation	0.711		
	Arginine dihydrolase	0.610		
	Glucose fermentation	−0.511		
	$N\text{-}NO_3$ reduction	0.820		
	Casein hydrolysis	0.890		
	Growth at 4°C	0.630		
5	Levan production	−0.890	6.1	Nonfermentative metabolism
	Glucose fermentation	−0.740		

[a] N20, Medium of Norris (29).
[b] N21, Medium of Kawai and Sugahara (22).
[c] PBH, Poly-β-hydroxybutyrate.

tabolism, fluorescence coupled with oxidation, and starch hydrolysis.

Fermentative metabolism (Meduxnekeag factor 2, Dunbar factor 1) showed a higher loading in the Meduxnekeag River in keeping with the higher incidence of facultative anaerobes under the ice in this river. The correlation of organic carbon with viable heterotrophic counts and also with factor 2 in this river suggested that fermentative bacteria may constitute a more important proportion of the viable counts than indicated by the clustered bacteria (Table 1). Inorganic carbon was also negatively correlated to factor 2. Organic and inorganic carbon in this river produced mirror images of each other when plotted over the year. When inorganic carbon levels are low, the pH drops to 7.0 and more free dissociated CO_2 will be available for algal fixation according to the carbonic acid-bicarbonate-CO_2 equilibrium (23). The correlation of fermentative metabolism to organic C and inorganic C therefore seems to infer a connection with the primary production of the river. In the Dunbar, the relationship was more overt; chlorophyll *a* correlated positively with the lack of fermentative metabolism (negative loading). Because the Dunbar is so turbulent and well aerated, the decline in fermentative ability was probably due to the inhibiting concentrations of oxygen. Silvey and Roach (34) and then Fonden (9) demonstrated a relationship between bacteria and algae during similar seasonal studies. Kuentzel (26)

TABLE 8. Major factors representing the bacterial population (Dunbar River)

Factor no.	Physiological test	Loading	Eigen value	Interpretation
1	Glucose fermentation	−0.987	18.0	Inability to ferment and grow anaerobically or oxidative vs fermentative metabolism
	Lactose fermentation	−0.593		
	Anerobic growth	−0.907		
	Growth at 37°C	−0.730		
	Lactose oxidation	0.759		
	Catalase positive	0.862		
2	N-NO_3 assimilation	−0.988	18.1	Inability to utilize N-NO_3 or grow on N_2-free media
	N-NO_2 reduction	−0.849		
	Growth on N_2-free media[a]			
	N20	−0.954		
	N21	−0.937		
	N22	−0.814		
3	Oxidase positive	−0.920	10.1	Oxidase negative, nonaerogenic metabolism
	Methyl red reaction	−0.632		
	Gas from glucose	−0.777		
	Phosphatase	0.774		
4	Production of fluorescein	0.953	13.0	Fluorescent psychrophilic, oxidative metabolism
	Glucose oxidation	0.894		
	Growth at 4°C	0.982		
	Arginine dihydrolase	0.639		
	Lactose fermentation	−0.516		
	Levan production	−0.825		
5	Starch hydrolysis	−0.952	8.7	Inability to hydrolyze starch and reduce nitrate
	Growth on			
	Arabinose	−0.905		
	Sorbitol	−0.662		
	N-NO_3 reduction	−0.838		
	Tween 80 hydrolysis	0.612		
	Urease	0.786		

[a] N20, Medium of Norris (29); N21, medium of Kawai and Sugahara (22); N22, medium of Maruyama et al. (27).

TABLE 9. Correspondence between factors for the Meduxnekeag and Dunbar Rivers

Factor no.	Interpretation	
	Meduxnekeag	Dunbar
1	Mesophilic nitrogen fixation	Oxidative vs fermentative metabolism
2	Fermentative and anaerobic metabolism	Inability to utilize NO_3 or grow on N_2-free media
3	Inability to hydrolyze starch; anaerobic growth	Oxidase negative, nonaerogenic metabolism
4	Oxidative metabolism-fluorescence	Oxidative metabolism-fluorescence
5	Nonfermentative metabolism	Inability to hydrolyze starch

postulated that an association observed between bacteria and the *Cyanophycaea* was mediated through CO_2.

Also, three strains of bacteria isolated by Paerl (30) from *Anabaena circinalis* and *Aphonizomenon flos-aquae* (two species of freshwater blue-green algae) were identified by Holder-Franklin as *Pseudomonas marginata* and *Flavobacterium lutescens*, both commonly found in river samples (personal communication). The bacteria were found to be associated with the heterocysts of the nitrogen-fixing algae. An increase in numbers of bacteria associated with the heterocyst occurred during the peak of the bloom, which coincided with intense N_2 fixation activity.

Meduxnekeag factor 1 and Dunbar factor 2 (inorganic nitrogen metabolism) showed the most obvious seasonal differences between sum-

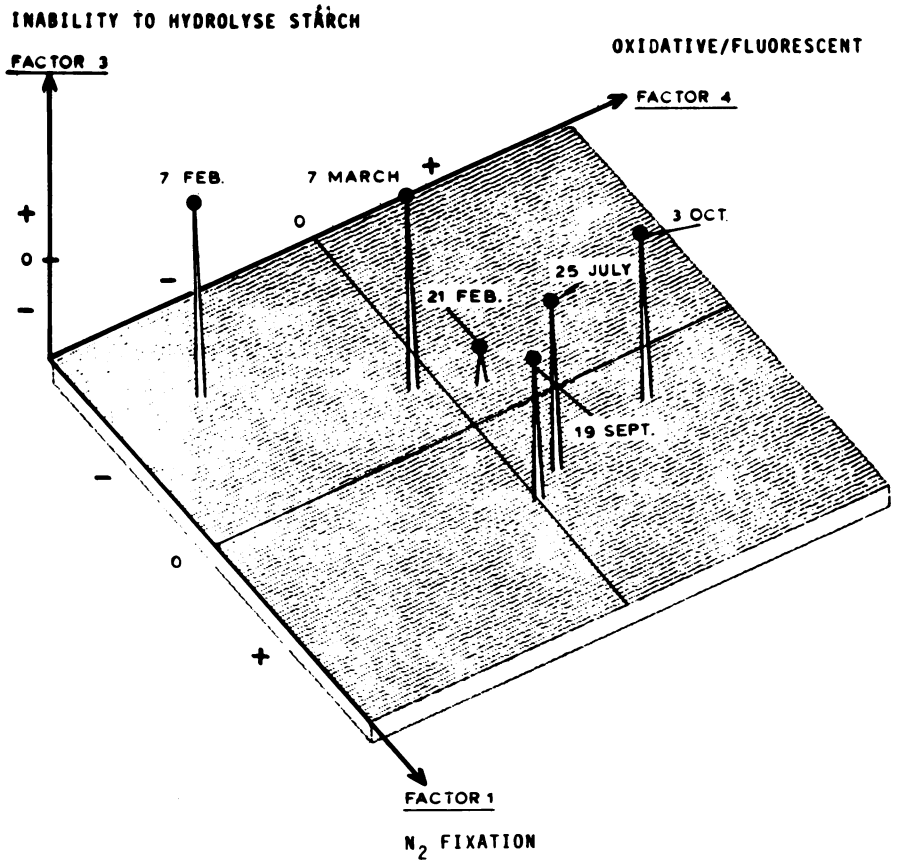

FIG. 1. Three-dimensional projection of Meduxnekeag populations by sample date. (Factor numbers as explained in Table 7.) Positive and negative projections indicated.

mer and winter. The positive correlation of factor 1 to chlorophyll *a* in the Meduxnekeag presumably reflected the scarcity of inorganic nitrogen through increased assimilation by algae. In the Dunbar, nitrogen scavenging was likewise associated with the warmer water.

The correlations of fluorescent, oxidative metabolism (factor 4) (Dunbar) to rainfall and sunshine (Meduxnekeag) was most surprising. Rainfall has been cited by previous workers as influential on the bacterial populations of lakes (4, 5, 12; M. Chen, Kansas State Univ. Dissert. Abstr. 29B, p. 1917, 1978). The relationship has always been a complex one, however, with the rainfall also affecting nutrients and other suspended solids. Rainfall in the Meduxnekeag appeared to increase color and turbidity while diluting out the ionic content, especially Na^+ and NO_3^-, as measured by specific conductance. The land surrounding the Meduxnekeag is predominantly agricultural, used for cultivating potatoes. Fertilizer is applied regularly to these crops in various ratios of P:N:K. However, with the negative correlation of factor 4 to NO_3, it seems unlikely that nutrient runoff had any effect. Indeed, the viable heterotrophic counts through the year in the Meduxnekeag showed a similar strong correlation to rainfall. Unfortunately, figures for daily discharge rates over the year in question were not available. However, data for previous years, 1971, 1972, and 1973, showed that maximum discharge always followed the spring thaw in April, May, and June. In 1978 to 1979 maximum rainfall was recorded in September–October, with a subsidiary peak in June. It is possible that rainfall could have been causing turnover of the sediment through increased discharge. However, direct examination of the sediment in September and October showed the bacterial populations in the anoxic bottom mud of the Meduxnekeag to contain only 7% fluorescent pseudomonads compared with 59% in the river water (14). Thus, it may be concluded that the fluorescent population in this river was seeded by bacteria washed in from the land.

The mean daily hours of sunshine in any week at the Dunbar River correlated negatively with

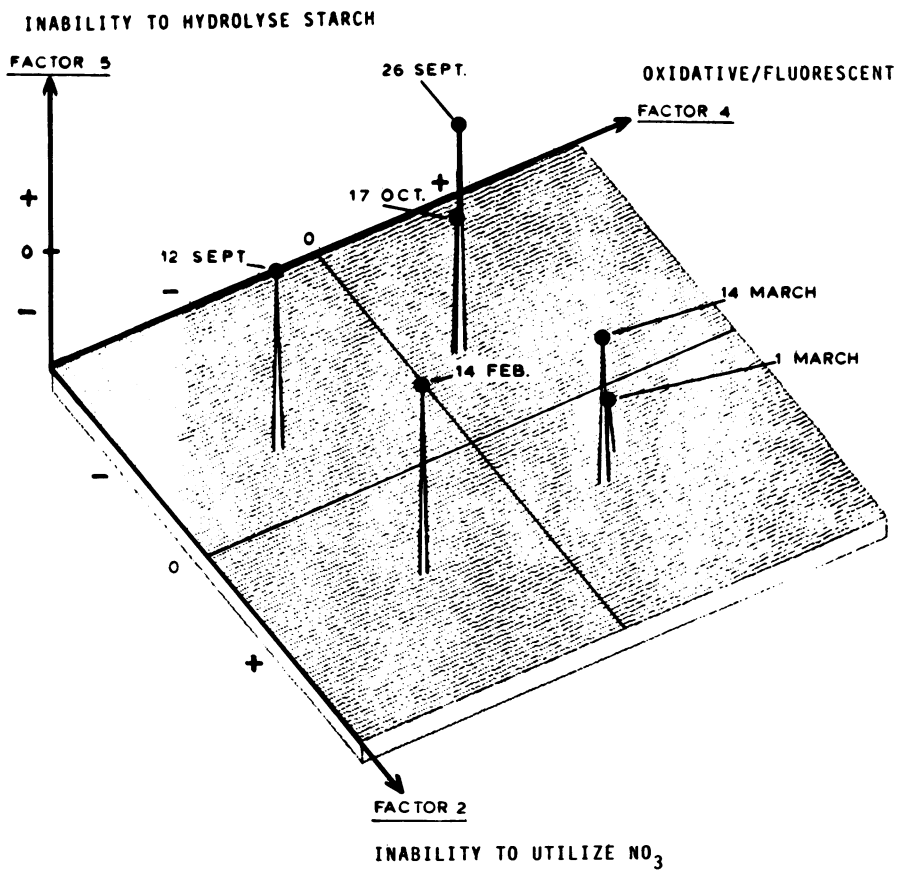

FIG. 2. Three-dimensional projection of Dunbar populations by sample date. (Factor numbers as explained in Table 8.) Positive and negative projections indicated.

factor 4 and also with the viable heterotrophic counts. Sunshine also arose as a significant variable in the Meduxnekeag, being negatively correlated with the numbers of *P. fluorescens* I (Table 5). Analysis of the sunshine figures showed negligible differences between summer and winter. Although the angle of the sun varied over the year, it seems unlikely that sunshine was masquerading as a seasonal influence. The harmful effects of UV and visible light on bacterial suspensions are well documented (8, 18, 25). The germicidal qualities of light are known to reside in the UV wavelengths (200 to 300 nm). In waters with low levels of suspended organics, penetration of UV light can be appreciable. Gameson and Saxon (11) reported that sunlight could have lethal effects on coliform bacteria down to a depth of 4 m. It is pertinent to note that sunshine appeared to be more influential in the very shallow, turbulent, yet clear Dunbar where penetration would be enhanced. The Dunbar ice cover was never complete, so UV light could still penetrate the water in winter. The ice surface of the Meduxnekeag was complete, yet remained blown free of snow; ice is an important attenuator of the blue-violet spectrum. Penetration may have occurred through the clear ice. It is possible, on the other hand, that these correlations may represent some phototactic response of the fluorescent bacteria.

It is incongruous that in one river the viable heterotrophs were influenced by rain whereas in another they were affected by sun. Sunshine was originally selected in the Meduxnekeag as a negatively correlating variable to expand the numbers of environmental influences studied (Table 12). Partial correlations showed it to have an inverse relationship to rain. The catchment area of the Dunbar represents an area only 8% the size of the Meduxnekeag. The volume of surface and groundwater runoff after rainfall will be much smaller in the Dunbar river. Sunshine exerts a more powerful effect where the volume of runoff is smaller. The Dunbar is more tree-shaded than the Meduxnekeag, which is wider and more exposed, complicating the conclusions even further. Values for heterotrophic viable counts for the Dunbar were as follows: NH_4^+—

TABLE 10. Correlation coefficients (percent significance) of physicochemical parameters correlated with factor scores

River	Factor		Physicochemical parameter					
	No.	Interpretation	NO_3	Color	Temp (water)	Turbidity	Na^+	SO_4^{2-}
Meduxnekeag	1	N_2 fixation	−0.857 (1.5)		0.863 (1.3)		−0.705 (5.9)	−0.663 (7.6)
	2	Fermentative-anaerobic metabolism						
	3	Inability to hydrolyze starch						
	4	Oxidative metabolism	−0.671 (7.2)	0.683 (6.7)		0.649 (8.2)	−0.671 (7.2)	
	5	Levan production			No correlations			
Dunbar	1	Oxidative vs fermentative metabolism						
	2	Inability to grow on N-NO_3 or N_2-free media	0.929 (0.4)		−0.921 (0.5)			
	3	Oxidase negative nonaerogenic phosphatase production		0.777 (3.4)			−0.812 (2.5)	
	4	Oxidative-fluorescent						−0.614 (9.8)
	5	Inability to hydrolyze starch						

correlation coefficient, 0.419 (significance, 2.6%; sun—correlation coefficient, −0.439 (significance, 2.1%). There were no interfering variables.

The positive correlation of Meduxnekeag factor 3 (inability to hydrolyze starch) to O_2 saturation inferred that starch hydrolysis would tend to be low over the winter. The O_2 saturation curve for this river (1) remained below 100% until November, when it stayed saturated through January. In February, after 2 months of ice cover, the percent saturation dropped sharply, causing an increase in hydrolysis (represented by the short spire in Fig. 2). Oxygen had the reverse effect in the Dunbar populations. The negative correlation of Dunbar factor 5 with dissolved oxygen ensured that starch hydrolysis was higher over the winter period when oxygen concentrations of 14.0 mg liter^{-1} were measured. There was, however, a sudden increase in dissolved oxygen on 1 March to 16.5 mg liter^{-1}, causing the short spire on that day. The significance of these spurts in starch hydrolysis is obscure unless a cyclical event was occurring.

The use of diversity indices in bacteriology is gaining popularity (14). Gamble et al. (10) used indices to assess the diversity of denitrifying bacteria in soil. Kaneko and Atlas (21) used them to show differences in bacterial diversity between geographical locations in the Beaufort Sea. It is commendable to describe a bacterial population without the subjectivity of speciation.

In this study, ammonia emerged as the indisputable variable related to species numbers. Tables 5 and 6 suggest that the influence of ammonia was to decrease the relative number of *P. fluorescens* I, which encouraged other species to proliferate. The nutritional versatility of *P. fluorescens* I is legendary (14, 36). The mechanism inhibiting the growth of *P. fluorescens* I can only be speculated upon. However, it seems inconceivable that ammonia itself would be inhibitory. The viable heterotrophic counts in the Dunbar throughout the year were likewise correlated to ammonia. Fonden (9) observed identical correlations between inorganic nitrogen and total bacterial counts in two Swedish lakes. Chlo-

TABLE 10.—*Continued*

Physicochemical parameter											
Carbon		Oxygen		Chlorophyll *a*	Rain	Sun	Specific conductance	Alkalinity	NH_4^+	Mg^{2+}	Total P
Organic	Inorganic	% Saturation	Dissolved								
			−0.704 (5.9)	0.870 (1.2)							
0.924 (3.8)	−0.869 (6.5)										
		0.781 (3.3)									
					−0.705 (5.9)		−0.648 (8.2)				
				0.618 (9.5)							
	−0.729 (5.0)			−0.773 (3.6)							
			−0.767 (3.7)	−0.738 (4.7)				−0.802 (2.7)		−0.786 (3.2)	−0.842 (1.8)
						−0.854 (1.5)			−0.695 (6.3)		
			−0.616 (9.6)								

rophyll *a* concentrations were also correlated to total inorganic nitrogen in the Dunbar. These findings corroborate earlier impressions that nitrogen limits microbial productivity in the Dunbar River (1).

The aim of this study has been to compare methodologies for elucidating the interrelationships between the biotic and abiotic spheres in an aquatic econiche. The complexities of any econiche at our present state of knowledge make a complete analysis impossible; therefore, the tendency to utilize simplistic probes and indicators is understandable. The more elementary analyses used here, such as heterotrophic counts and enumeration of species, cannot reveal more than the method implies; yet, even this type of information becomes meaningful when linked to the environmental parameters. Proceeding to the next level, which would be taxometric, a relatively modest data base will provide useful information. Species diversity, albeit based on taxometrics, is even more effective because the relative numbers of the predominant population are resolved into an index. Armed with a manageable mathematical index, relationships with the physicochemical parameters can be more accurately defined. This type of correlation can be meaningful with an analysis of a few water samples and allows seasonal and diurnal changes to be detected.

Ideally, when an aquatic econiche is exam-

TABLE 11. Fermentative ability

River/factor	Date	Score
Dunbar, factor 1 (inability to ferment)	14 Feb.	9.8
	1 Mar.	58.8
	14 Mar.	64.8
	12 Sept.	55.6
	26 Sept.	55.6
	17 Oct.	55.6
Meduxnekeag, factor 2 (ability to ferment)	7 Feb.	90.4
	21 Feb.	41.2
	7 Mar.	37.4
	25 July	44.6
	19 Sept.	46.4
	3 Oct.	40.2

TABLE 12. Correlations between physicochemical parameters and viable heterotrophic counts (Meduxnekeag River)

Parameter	Correlation coefficient	Significance level (%)	Partial correlation	Significance level (%)	Variables controlled
Specific conductance	−0.336	8.0		NS[a]	
Color	0.797	0.1		NS	
Alkalinity	−0.377	5.6		NS	
Cl^-	−0.500	1.5		NS	
Organic C	0.759	0.1	0.732	0.1	SC,[b] alkalinity, Cl, inorganic C
Inorganic C	−0.335	8.7	0.405	7.5	SC, alkalinity, Cl
Dissolved O_2	−0.436	3.1		NS	
O_2 saturation	−0.407	4.2	−0.333	10.0	Organic C
Rain	0.713	0.1	0.450	6.1	Cl^-, organic C, dissolved O_2, sun
Sun	−0.312	9.7		NS	

[a] NS, Not significant.
[b] SC, Specific conductance.

ined, many parameters must be included and variations within the water environment must be clearly identifiable. Factor analysis is an effective tool for reducing the incredible data base accumulated in a study in which the frequency of positive responses in an 82 × 1,300 matrix is compressed into a series of descriptors or factor constructs. The advantages of this type of analysis are many: the variance is a recognizable mathematical entity, but the interpretation is biological—a profile of the most important biological features. The entire body of isolates is represented and is not limited to taxa or clustered strains.

A comparison of the various methods should begin with a detailed examination of the heterotrophic count data, and it is apparent that these data provided an incomplete picture of the microbial ecology. The true significance of the results cannot be fully understood even with the expanded analysis of this study. In the Meduxnekeag the positive correlations of heterotrophic counts with rain, color, and organic carbon and the negative correlations with sun, O_2, specific conductance, alkalinity, and chloride indicated a stimulus of heterotrophic bacteria by rain and nutrients which was not entirely seasonal.

Changes in the species numbers were primarily stimulated by ions, the most important being ammonium ion, a result of the negative correlation of *P. fluorescens* with this ion. Another ion with a strong negative correlation was potassium; the negative aspect is not clear as it is unlikely that this implies an inhibitory effect. Carbonate and chloride ions also were influential. The species diversity indices underscored the correlations of the simpler methods. Certain ionic concentrations, in particular, NH_4, CO_3, and Ca, were the strongest influence overall. In the Dunbar, temperature had a high positive correlation and chloride had a high negative correlation.

A summary of the significant correlations recorded in detail in the results is shown in Table 13 so that the importance of each method can be observed at a glance.

TABLE 13. Summary of significant correlations[a]

Analytical method	Positive	Negative
Viable heterotrophic bacterial counts	Organic C, O_2, rain, NH_4	Sun, Cl
Species no. by numerical taxonomy	Sun, water temp, K, SO_4, P, chlorophyll *a*	NH_4
Species diversity index, Shannon/Weaver	Specific conductance, color	
Principle factor analysis Meduxnekeag	Chlorophyll *a*, water temp, organic C, O_2 saturation, turbidity, color, rain	NO_3, dissolved O_2, Na, SO_4, inorganic C, specific conductance
Dunbar	Chlorophyll *a*, NO_3, color	Temp, inorganic C, chlorophyll *a*, alkalinity, Na, Mg, total P, sun, SO_4 NH_4, DO_2[a]

[a] Significance detailed on preceding tables.

The simpler methods considered up to this point cannot provide well-supported conclusions regarding the primary influences. The factor analysis presents a firmer picture where the most important parameters of temperature, oxygen, and nutrient levels emerge more consistently as influential. The major metabolic characteristics of the populations are defined not only qualitatively but also statistically, ensuring that the most important variables of the population are represented. In analyzing the strengths and weaknesses of the various methods, the factor analysis, although satisfactory, is limited by the size of the data base. In previous studies, we have utilized a much larger set of tests on more isolates and thus have overstated the factors. There is a critical number of variables for each of the expected major factors for the factor analysis to be most effective. As the methodology progresses in microbial ecology, the selection of the variables and the numbers of variables for each econiche will be clarified. The real test of the analysis aside from the mathematical stringencies is interpretability and the consistency of correlations with the environmental influences. For instance, the oxidative-fluorescent factor correlated positively with rain and color and negatively to ions. This was consistent with the negative correlation for sun and ions within the same factor from the Dunbar.

The four approaches adopted to examine the microbiology of these two rivers all demonstrated many interactions between the bacteria, the algae, and the environment. However, without the power of the multivariate techniques used, the complexities of the ecosystems would have been underestimated or even overlooked.

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