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Factor Analysis of the Impact of the Environment on Microbial Communities in the Tvärminne Area, Southern Coast of Finland

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Data already examined by regression analysis were subjected to factor analysis to scrutinize the effects of environmental factors on microbial populations in the brackish waters of the Tvärminne archipelago on the southern coast of Finland. Water samples were collected from 1.0-m depth at one point in Tvärminne Storfjärd, 71 times over about 2 years. Twenty-six parameters were determined on each sample, 10 of environmental and 16 of microbiological type. The correlations between the parameters were factorized using the principal axis solution, and eight factors chosen for further consideration were rotated by the varimax method. The major part of the variance (about 90% of the total communality) of the microbiological parameters was covered by five factors, interpreted as phytoplankton blooms, the periods before and after the blooms, freshwater outflows, and water temperature. Wind variables were components in the factors interpreted as freshwater outflows. Rainfall played a minor part in the total variance of the microbial community, but it washed yeasts and proteolytic bacteria from the land into the study area. The eight factors selected covered about 60 to 98% of the variance of the microbiological parameters. The highest values (above 90%) were obtained for direct counts of bacteria, for plate counts of mesophilic and polymyxin-resistant bacteria, and for the two community respiration parameters; the lowest values (60 to 75%) were obtained for H_2S-producing and proteolytic bacteria.

The uses to which factor analysis has already been put in microbiology (13, 17) include the description and comparison of microbial populations in the soil (15, 16) and lakes (12) and the evaluation of the effects on soil populations of clear-cutting of forests (10) and of crop husbandry (4). These applications were based on the use of two-state variables, not on variables measured quantitatively on a continuous scale.

Recently, Jones (5, 6) used quantitative parameters in a principal component analysis of lacustrine bacteria. Erkenbrecher and Stevenson (3) also used quantitative parameters in factor analysis of microbial populations (viable bacteria and adenosine 5′-triphosphate) in saltmarsh creeks, but the four factors given were not interpreted.

In this study, factor analysis was applied to material subjected previously to regression analysis (21) to scrutinize the influence of different environmental factors on aquatic microbial communities in the Tvärminne archipelago (59° 50′ N, 23° 20′ E). Data on fecal coliforms and enterococci from the same period were included to indicate fecal pollution and to aid in the interpretation of the factors, but results of their regression analyses are scrutinized elsewhere (P. Väätänen, submitted for publication). The outer parts of the Tvärminne archipelago are affected by freshwater outflows, by domestic effluents (11), spreading mainly in the low-salinity surface layer below the ice cover, and also by industrial effluents, some of whose components accumulate in the sediments (9).

MATERIALS AND METHODS

The study area and the collection of the water samples are described in a previous paper dealing with the same material (21).

Determinations. Water temperature, salinity, and transparency were determined as described by Väätänen (18). Total organic matter was estimated as the ultraviolet absorbance (at 254 nm; 2) of water samples brought to room temperature. Quartz cuvettes were used with a cell length of 10 cm, and empty cuvettes served for reference. Total humic matter was estimated as the absorbance at 350 nm of unfiltered water samples as above, except that deionized water was used for reference.

Chlorophyll *a* was determined by a modification of the method of Strickland and Parsons (14). Paper filters (no. 1575; Schleicher and Schüll) were used to

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concentrate the phytoplankton. The concentrates were homogenized for 1 min with an Ultra Turrax homogenizer type TP 18-10 (about 10,000 rpm) (Janke and Kunkel, West Germany) and then extracted with 90% acetone for 24 h in darkness. The extracts were filtered through double paper filters of the same quality before their extinction was measured.

Wind direction and velocity were recorded at 8 a.m., 2 p.m., and 8 p.m. with a Wild wind meter at Tvärminne Zoological Station. The wind vector along the Hanko peninsula (northeast vector) and a vector perpendicular to it (southeast vector) were taken into account.

Rainfall was recorded at Tvärminne Zoological Station.

Direct counts of bacteria were made by the acridine orange epifluorescence method (22) with the following improvements and modifications: (i) the formaldehyde-fixed samples were boiled for 20 min and then kept warm before staining to increase the proportion of red-fluorescing bacteria, which are easier to count by this method than green cells; (ii) the Nuclepore filters, with a pore size of 0.2 μm, were boiled for about 10 min in filtered 0.01% sodium hexametaphosphate to wet them thoroughly; (iii) the sintered glass of the 25-mm Millipore filter apparatus was wetted with the same detergent, and a wetted 25-mm Millipore filter with a pore size of 0.8 μm was then placed under the Nuclepore filter to facilitate even distribution of bacteria on the latter filter; (iv) the bacteria retained on the Nuclepore filter were stained by adding 2 ml of double-filtered acridine orange (Gurr) solution (500 mg liter^{-1}) in the filter tower for 3 min; and (v) the filters were destained with 1 ml of filtered isopropyl alcohol, and xylene was not used. Controls were made with filtered deionized water, which was always used as the sample diluent in the filter tower, to guarantee the even distribution of bacteria on the Nuclepore filters. Cocci, rods, and "curved rods" were counted separately at a magnification of 1,560×, using an ocular grid. A Leitz Dialux microscope was fitted with an HBO 50 W mercury lamp, a heat filter, a BG-38 exciter filter, a KP 500 blue exciter filter, and a K 510 barrier filter. Generally more than 400 bacteria were counted per sample, always by the same person.

Numbers of mesophilic and psychrophilic bacteria were determined on spread plates (6 to 10 replicates) of V medium (19) supplemented with 7 mg of NH_4NO_3 per liter (VA medium) (pH 7.6 to 7.8) after incubation for 28 days; mesophiles were determined at 18°C, and psychrophiles were determined at 2 to 4°C. It should be noted that the terms "mesophilic" and "psychrophilic" bacteria are used here for convenience. The former comprises all the bacteria, whether truly mesophilic or psychrotrophic, capable of forming visible colonies at 18°C. The latter includes all the bacteria, whether truly psychrophilic or psychrotrophic, growing into visible colonies at 2 to 4°C.

Polymyxin-resistant bacteria were determined on VA medium spread plates supplemented with polymyxin B sulfate (50 U ml^{-1}) and otherwise as described above for mesophiles.

Proteolytic bacteria were counted on spread plates provided with the following medium: peptone (Difco), 1.0 g; yeast extract (Difco), 0.5 g; NH_4NO_3, 7 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; Na_2HPO_4, 10 mg; agar (Difco), 12 g (all ingredients given per liter); and aged brackish water, 6‰, 1,000 ml, pH 7.6 to 7.8. Skim milk powder (Difco), 5.0 g liter^{-1}, was added after separate sterilization for 30 min at 115°C. The plates (6 to 10 replicates) were incubated for 5 days at 18°C, and the colonies surrounded by distinct clear zones were counted.

Fluorescent pseudomonads were determined with medium B (7). The salinity of the medium was adjusted to 6‰ with aged brackish water. The plates were incubated for 5 days at 18°C. The colonies with greenish fluorescence were counted in subdued daylight.

Bacterial spores were determined on VA medium plates, using the pour-plate method; 10 to 20 replicates were prepared per sample. The plates were incubated for 28 days at 18°C.

Fecal coliforms were determined on mFC medium (Difco) using presterilized Millipore filters (type HC). The plates were incubated for 24 h at 44.5°C before the blue colonies were counted.

Enterococci were determined on mE medium (8) from which nalidixic acid was omitted, using the same filter types as for fecal coliforms, after incubation for 48 h at 41°C followed by incubation for about 15 min on esculin medium at the same temperature.

Yeasts were determined as described by Väätänen (18).

Counts of the most probable number of H_2S producers were made from decimal dilutions of water samples, using 10 replicates for each dilution, in the following medium: cysteine, 10 mg; peptone (Difco), 1.0 g; yeast extract (Difco), 0.5 g; soluble starch (Merck), 0.3 g; glucose, 0.5 g; NH_4NO_3, 7 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; Na_2HPO_4, 10 mg (all ingredients given per liter); and aged brackish water, 6‰, 1,000 ml, pH 6.8. The medium was filtered twice through Whatman no. 4 filters before sterilization. After inoculation, the tubes were provided with sterile strips of filter paper dipped in 5% lead acetate. The tubes were incubated for 14 days at 18°C. Darkening of the dipped end of the strip was considered to indicate production of H_2S.

The community respiration in the natural and supplemented (organic substances added, 15 mg liter^{-1}) samples was determined after incubation for 20 h at 18°C by the improved Winkler method (20).

Factor analysis. The microbial counts were transformed ($\log_{10} x$) except for fluorescent pseudomonads and fecal bacteria, for which $\log_{10} (x + 1)$ was adopted, since nil results were otherwise frequent. The other data were used without transformation. Maximal correlation coefficients were used as the communality estimates. All the parameters were taken into account, since even the lowest r_{max} (0.273 df 69) was significant at the level $P = 0.05$. The analysis was run at the Computing Centre of the University of Helsinki.

Factorization was carried out according to the principal axis solution. Eight factors, whose eigenvalues ranged from 8.85 to 0.34, were accepted. Their sum (20.18) covered 102% of the total communality estimates of the 26 parameters. The sum of the factor variances corresponded to 78% of the total variance of the parameters ($n = 26$).

In addition to the factorization of all the data, factor analysis was also applied to the data from the open-

water periods alone. Here, too, eight factors were accepted for consideration. Before rotation, the eigenvalues of these factors ranged from 8.51 to 0.34, their sum (19.34) covering 105% of the total communality estimates of the 25 parameters (ice-cover parameter omitted). The sum of the factor variances corresponded to 76% of the total variance of the parameters (n = 25).

The number of observations (20) from the ice-cover periods was not considered sufficient (compared to that of the parameters, n = 26) to permit separate factor analytical treatment.

Rotation of the factors towards interpretable compositions was done by the varimax method.

RESULTS

Interpretation of the factors obtained for all the data. The eight varimax-rotated factors obtained for all the data, comprising 71 observations, are given in Table 1.

Factor A1. The first factor, A1, is characterized by high negative loadings for water temperature (−0.73) and salinity (−0.56) and by high positive loadings for the ice-cover parameter (+0.67) and total organic and humic matter. The following microbiological parameters receive high positive loadings (+0.43 to +0.83): yeasts, fecal bacteria, fluorescent pseudomonads, bacterial spores, and psychrophiles. Thus, this factor is interpreted as "freshwater outflows in late winter."

The sum of the squared loadings of each microbiological parameter, presented as a percentage in the last columns of Tables 1 and 2, is the communality term. It indicates what part of the

TABLE 1. *Rotated factor matrix A showing eight orthogonal factors obtained for all the data from Tvärminne Storfjärd (71 observations)*[a]

Parameter	A factors								Communality (% of maximum variance)
	1	2	3	4	5	6	7	8	
Temperature	−0.73	0.35		0.21			0.27		79.5
Transparency			−0.33	−0.61			−0.22		55.2
Northeast wind vector	0.34				0.21			0.38	34.5
Southeast wind vector	0.21					−0.42			25.7
Rainfall							0.52		30.8
Ice cover	0.67				0.30		−0.26	0.29	72.9
Salinity	−0.56	−0.21			−0.58		0.27	−0.32	94.9
Total organic matter	0.52	0.23		0.23	0.58		−0.27	0.38	94.3
Total humic matter	0.41			0.52	0.52		−0.23	0.27	88.7
Chlorophyll *a*			0.93						94.7
Direct count									
Total		0.92	0.21		0.24				98.3
Cocci		0.89		0.24					92.0
Rods		0.86	0.29						93.3
Curved rods		0.70	0.27						81.9
H_2S producers		0.22			0.52	0.45		0.23	62.1
Mesophilic bacteria		0.37	0.26		0.83				91.5
Psychrophilic bacteria	0.43		0.25		0.73				83.6
Proteolytic bacteria		0.22		0.32	0.67				66.0
Fluorescent pseudomonads	0.79			0.24					73.3
Polymyxin-resistant bacteria		0.29	0.24		0.85				90.4
Bacterial spores	0.80				0.28				81.5
Fecal coliforms	0.81					−0.22		0.28	86.4
Enterococci	0.67			−0.27	0.34	−0.20	−0.21	0.26	78.9
Yeasts	0.83						0.20		76.4
Community respiration									
Unsupplemented			0.92		0.23				96.1
Supplemented		0.23	0.88		0.32				94.8
Factor variance[b]	5.27	3.56	3.16	1.23	4.46	0.71	0.94	0.84	
Percent of total	26	18	16	6	22	3	5	4	
Cumulative	26	44	60	66	88	91	96	100	

[a] Plus signs and factor loadings below 0.20 are omitted.

[b] Total, 20.18.

variance of the parameters is accounted for by the common factors. Each squared loading of a factor indicates what part of the variance is explained by it. Thus, for example, the factor A1 explains $100 \times 0.83^2 = 69\%$ of the variance of yeasts.

Factor A2. Factor A2 is termed "water temperature" because the only environmental parameter for which it has a significant loading is temperature. It is further characterized by high positive loadings for direct counts and positive loadings for mesophiles and polymyxin-resistant bacteria.

Factor A3. Chlorophyll *a* has a "pure" loading (i.e., a significant loading in one factor only) in this factor, with the high value of +0.93. In addition, this factor has a negative loading for transparency and high positive loadings for the respiration parameters as well as positive loadings for various bacterial types, and is thus defined as "the vernal phytoplankton bloom."

Factor A4. Factor A4 is characterized by a negative loading for transparency (−0.61) and by a positive one for humic matter (+0.52). Furthermore, it has positive loadings for cocci, fluorescent pseudomonads, and proteolytic bacteria. It is interpreted as "summer outflows."

Factor A5. Among the environmental parameters, factor A5 obtains positive loadings for total organic and humic matter (+0.58 and +0.52, respectively) and a negative loading of −0.58 for salinity. The ice-cover parameter appears with a positive loading. High positive loadings (+0.52 to +0.85) are noted for mesophiles, psychrophiles, and polymyxin-resistant, proteolytic, and H_2S-producing bacteria. Hence, the factor may be assumed to deal with "the period before the vernal bloom."

Factor A6. Factor A6 has significant loadings only for the southeast wind vector (−0.42) and H_2S-producing bacteria (+0.45). It is probably connected with western winds and is defined as the "west winds" factor.

Factor A7. The "pure" parameter of rainfall with a loading of +0.52, a loading of +0.27 for temperature, and a negative loading for the ice-cover parameter indicate that factor A7 can be interpreted as "summer rains." A positive loading of +0.20 is shown for yeasts.

Factor A8. The last factor, A8, is characterized by positive loadings for the northeast wind vector, ice cover, and total organic and humic matter and by a negative loading for salinity. It is explained as "early winter."

Interpretation of the factors obtained for the open-water periods. The eight varimax-rotated factors obtained for the data from the open-water periods, comprising 51 observations, are presented in Table 2. Factors B2, B3, B6, and B7 appeared to be the same as factors A2 (water temperature), A3 (vernal bloom), A6 (west winds), and A7 (summer rains), respectively (Table 1), and therefore are not examined again.

Factor B1. A negative loading for temperature and higher loadings (+0.59 to +0.80) for fluorescent pseudomonads, bacterial spores, fecal bacteria, and yeasts show that the term "autumnal freshwater outflows" is justified for this factor.

Factor B4. This factor is termed "vernal freshwater outflows," since it has positive loadings for the northeast wind vector, total organic and humic matter, cocci, psychrophiles, and H_2S-producing and proteolytic bacteria. The high negative loading of −0.85 obtained for salinity shows that the factor is connected with the spring, despite the positive loading obtained for temperature.

Factor B5. The positive loadings for transparency in particular and also for mesophiles, psychrophiles, and polymyxin-resistant bacteria, as well as the negative ones for salinity and yeasts, may indicate that the factor is dealing with "the breakdown of the vernal bloom."

Factor B8. The last factor of solution B is characterized by positive loadings for temperature and transparency but by negative ones for the northeast wind vector, bacterial spores, and yeasts. Therefore, it is defined as "southwest winds," which are dominant in the study area during the open-water periods.

It may be noted that the first five factors given by factorizations A and B account for nearly 90% of the total communalities (Tables 1 and 2).

Variance of the microbiological parameters covered in factorizations A and B. Examination of the communalities of the microbiological parameters, given in the last columns of Tables 1 and 2, shows that the eight factors cover more than 70% of the variance of most of the microbiological parameters. However, the percentages explained are lower (60 to 69%) in the case of cocci (factorization B), H_2S-producing bacteria, proteolytic bacteria (factorization A), and fluorescent pseudomonads (factorization B).

Apart from H_2S producers, proteolytic bacteria, enterococci (factorization A), and yeasts (factorization B), all the microbiological parameters are mainly loaded (more than 50% of the variance) in one factor. For example, the first factors (Tables 1 and 2) dealing with freshwater outflows cover 50 to 69% of the variance of fluorescent pseudomonads, bacterial spores, and fecal coliforms. The second factor, water tem-

TABLE 2. *Rotated factor matrix B showing eight orthogonal factors obtained from the open-water periods (51 observations)*[a]

Parameter	B factors								Communality (% of maximum variance)
	1	2	3	4	5	6	7	8	
Temperature	−0.55	0.43		0.32			0.23	0.32	76.1
Transparency			−0.46	−0.34	0.37	0.35	−0.26	0.23	72.6
Northeast wind vector				0.43				−0.36	41.3
Southeast wind vector	0.22					−0.43			28.1
Rainfall							0.60		37.5
Salinity		−0.28		−0.85	−0.26				92.2
Total organic matter	0.21	0.29	0.40	0.76	0.25				94.5
Total humic matter			0.39	0.69		−0.37			80.4
Chlorophyll *a*			0.91	0.22					95.1
Direct count									
Total		0.92	0.20	0.24					98.3
Cocci		0.86		0.36					60.0
Rods	−0.21	0.87	0.26		0.24				89.8
Curved rods		0.76	0.25		0.42				78.3
H_2S-producers				0.53	0.28	0.40			62.9
Mesophilic bacteria		0.40	0.35	0.29	0.70				72.0
Psychrophilic bacteria	0.22		0.32	0.32	0.72				87.9
Proteolytic bacteria		0.22		0.48	0.32		0.44		75.8
Fluorescent pseudomonads	0.78								69.4
Polymyxin-resistant bacteria		0.33	0.29	0.33	0.75				79.6
Bacterial spores	0.71		0.25	0.20				−0.34	74.3
Fecal coliforms	0.80								98.1
Enterococci	0.75				0.40				98.2
Yeasts	0.59				−0.38		0.40	−0.21	94.3
Community respiration									
Unsupplemented			0.91		0.26				95.7
Supplemented		0.23	0.90	0.22	0.26				86.0
Factor variance[b]	3.33	3.87	3.62	3.38	2.77	0.74	1.03	0.60	
Percent of total	17	20	19	17	14	4	5	3	
Cumulative	17	37	56	73	87	91	96	99	

[a] Plus signs and factor loadings below 0.20 are omitted.
[b] Total, 19.34.

perature, explains most of the variance of direct counts, and the third factor, vernal phytoplankton bloom, explains that of the respiration parameters.

DISCUSSION

The application of factor analysis was fruitful since the environmental parameters included allowed an interpretation of the major part of the variance of the microbial populations. The five main factors given by the two factorizations, namely, the vernal phytoplankton bloom, the period before the bloom, the breakdown of the bloom, water temperature, and freshwater outflows, covered about 90% of the factor variance in the populations and also the major part of the variance of most of the microbiological parameters (Tables 1 and 2). Similar factors, such as chlorophyll *a* and temperature, play important roles for lacustrine bacteria (5).

As already revealed by regression analysis (21), factor analysis also indicated that fluorescent pseudomonads, bacterial spores, yeasts, and fecal bacteria are transported into the Tvärminne area by freshwater outflows (factors A1 and B1). Thus, most of them are allochthonous types.

The significance of the vernal bloom (factor 3), mainly consisting of diatoms (11), for the respiration parameters was also shown by the regression analysis. Factor 3 also indicates that plate counts of mesophilic, psychrophilic, and polymyxin-resistant bacteria, as well as the "healthy" types of direct counts, namely, rods and curved rods, are associated with the vernal phytoplankton bloom. Evidently, they utilize al-

gal extracellular organic compounds.

The very strong influence of water temperature (factor 2) on direct counts of bacteria was not so evident for rods and curved rods in the regression analysis (21). The effect of temperature on various heterotrophic bacteria was also revealed by regression analysis, which, however, did not indicate the influence of temperature on community respiration.

The interpretation of factor A5 (Table 1) was rather difficult. However, because maximal numbers of proteolytic bacteria occur in the Tvärminne area during the onset of the vernal phytoplankton bloom below the ice (21), this factor may be dealing with the onset of the phytoplankton bloom. Regression analysis did not give any indication of this period.

The reason why mesophilic, psychrophilic, and polymyxin-resistant bacteria were connected more with the onset of the bloom (factor A5) than with the peak of the bloom (factor A3) is that the influence of freshwater outflows is strong during the onset of the bloom below the ice, as revealed by the negative loading of salinity in factor A5. No such involvement of freshwater outflows was evident during the breakdown of the vernal bloom (factor B5), which was of significance for the three bacterial types. This period was not indicated, however, by the regression analysis (21).

Factors 6 to 8 (Tables 1 and 2) did not contribute much to the factor variances. However, the loading of H_2S-producing bacteria in factor 6 may indicate that western winds cause transport of this bacterial type to Tvärminne Storfjärd. Western winds, which drive the surface layer eastwards and cause westward movements in the deeper layers, may detach bacteria from the dense, decaying, loose-lying *Fucus* communities in the nearby inlet Byviken, about 2 km from the sampling site. On the other hand, regression analysis revealed that H_2S-producing bacteria may also be transported to Tvärminne Storfjärd from the inner archipelago with freshwater outflows caused by northeast winds (21).

Proteolytic bacteria and yeasts may be washed into the Tvärminne archipelago from the land by rain, as is shown by their positive loadings in factor 7 (Table 2). The effect of rain on the numbers of proteolytic bacteria was not revealed by the regression analysis. Heavy rains are known to increase bacterial counts in lakes (1).

None of the parameters proved unnecessary for the interpretation of the factors, which indicates that the selection of the environmental parameters was satisfactory. However, in subsequent studies, it would be advisable to include inorganic nutrients, because they proved to be useful in multivariate analysis of freshwater bacteria (6). It should be noted that the present study comprised two normal winters with solid ice. In years without an ice cover, the importance of phytoplankton may be expected to increase owing to the earlier development of the vernal bloom, and the role of freshwater outflows may then diminish.

Although regression and factor analyses of the same material gave the same information in many cases, the application of the latter was useful. The vernal periods revealed by factor analysis as important for microorganisms were not shown by the regression analysis. Furthermore, the environmental processes affecting microbes were as a rule easier to identify with the factor analysis than with regression analysis.

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