APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1980, p. 48–54
0099-2240/80/07-0048/07$02.00/0

Vol. 40, No. 1

# Effects of Environmental Factors on Microbial Populations in Brackish Waters off the Southern Coast of Finland

PENTTI VÄÄTÄNEN†

*Walter and Andrée de Nottbeck Foundation, Tvärminne Zoological Station, SF-10850 Tvärminne, Finland*

The roles played by environmental factors in seasonal changes in microbial populations were investigated in the Tvärminne area, off the southern coast of Finland. Surface-layer samples were collected at 1- or 2-week intervals in 1976–78, and 14 microbiological and 10 environmental parameters were determined. Stepwise multiple regression analysis was used to explain seasonal variation in the microbiological parameters. Separate analyses were made of the data from the open-water and ice-cover periods. In analyses of data from both periods, the environmental factors included accounted for a significant proportion of the variation in the parameters for community respiration (90%) and bacterial spores (80%), and a smaller proportion (60 to 65%) of the variation in total counts of bacteria and plate counts of psychrophiles and yeasts. Lower values (40 to 55%) were obtained for the variation in the other microbiological parameters. The environmental factors with maximal contributions were organic matter, water temperature, chlorophyll *a*, and salinity, but rainfall and winds also explained part of the variation in some microbiological parameters. In the winter analysis the results differed from those obtained for the other seasons, the variation being governed by parameters indicating freshwater outflows, namely, humic matter, salinity, water temperature (positive regression coefficient), and rainfall (negative regression coefficient).

The Tvärminne archipelago, situated at the entrance to the Gulf of Finland, east of the Hanko peninsula, represents the coastal waters of the Northern Baltic Sea. From late autumn on, the influence of outflows of freshwater and sewage effluents extends to the distant parts of the outer archipelago zone, being especially evident in the low-salinity surface layer below the ice cover (15). The seaward-flowing freshwater transports sewage and microorganisms to the outer archipelago zone, sometimes carrying fecal bacteria to the outermost skerries. The effluents have caused eutrophication in the inner parts of the archipelago (15) and slight changes in the composition of the biota also in the distant parts of the outer archipelago zone (7).

In a subsequent paper (28), it will be shown that the microbial communities in the surface layer of this area are regulated by phytoplankton blooms, freshwater outflows, and water temperature. It was desired to examine in more detail the effects of some environmental parameters on selected types of microorganisms and on community respiration. Samples were collected from 1.0 m depth in the outer archipelago, at Tvärminne Storfjärd, over a period of about 2 years. Stepwise multiple regression analysis was used

to explain variations in the microbiological parameters. Separate analyses were made of the data from the open-water (late April to late December) and ice-cover (January to late April) periods to find out whether the significant environmental parameters regulating microbial populations were different in the winter, when the freshwater outflows are more abundant.

## MATERIALS AND METHODS

**Study area.** Descriptions of the hydrography and hydrobiology of the Tvärminne research area (59° 50′ N, 23° 20′ E) are available in, e.g., the papers of Luther (13), Niemi (15–17), Sarvala (22), Väätänen (24–26) and Virta (29).

**Water samples.** The water samples were collected aseptically from 1.0 m depth at Tvärminne Storfjärd, station XII, in sterile glass bottles of 5,500 ml with the sampler constructed by T. Sjölund and P. Väätänen (27). During the sampling period (29 September 1976 to 26 July 1978), a total of 71 samples were taken, 51 in the open-water periods and 20 when Tvärminne Storfjärd was covered with ice. At each of the 19 first samplings (September 1976 to June 1977), short-term fluctuations were examined by taking four consecutive single samples within 20 to 30 min. Since the four consecutive samples never differed significantly, single samples were collected thereafter. For the four-sample period, the means of the parameters of the four samples are used.

The samples were protected against temperature fluctuations and kept in refrigerators at 5 to 7°C until

† Present address: Department of Microbiology, University of Helsinki, SF-00710 Helsinki 71, Finland.

further processing, which occurred no later than 5 to 6 h after sampling.

**Determinations.** Nine environmental parameters, the concentration of chlorophyll *a*, and 14 microbiological parameters comprising numbers of selected types of bacteria, plate counts of yeasts, and two parameters for community respiration (Table 1) were determined according to the methods given by Väätänen (24, 26, 28). Polymyxin-resistant bacteria were considered to represent slow-growing bacteria from brackish water, and bacterial spores were considered to have come from terrestrial microbial populations. Fluorescent pseudomonads were thought to indicate fecal pollution. Yeasts were also selected because of an interest in their ecology.

**Analysis of the data.** All the microbial counts and chlorophyll *a* were subjected to a $\log_{10} x$ transformation. For the direct counts, a square-root transformation was applied (11). Square root transformations ($\sqrt{x} + 1$) of rainfall appeared to explain larger proportions of the variation ($R^2$%) than untransformed data. The data were analyzed by the stepwise multiple regression program at the Computing Centre of the University of Helsinki.

## RESULTS

**Variation in the microbiological parameters.** The variation in the parameters investigated is summarized in Table 1. The maxima of

TABLE 1. *Variation of parameters determined at Tvärminne Storfjärd on 71 occasions during the period 29 September 1976 to 26 July 1978*

| Parameters | Range | Date (date-month-year) for: | | Mean[a] | Coefficient of variation (%) |
| --- | --- | --- | --- | --- | --- |
| | | Minimum | Maximum | | |
| **Environmental** | | | | | |
| Temperature (°C) | −0.3–+16.7 | 1–2–78 | 26–7–78 | +4.7 | 1.6[b] |
| Salinity (‰) | 3.93–7.39 | 12–4–78 | 14–9–77 | 6.24 | 12 |
| Ice cover (cm) | 0–50 | | | | |
| NE wind vector (m s⁻¹) | −9.9–+9.1 | | | −0.8 | |
| SE wind vector (m s⁻¹) | −7.8–+9.9 | | | +0.7 | |
| Transparency (cm) | 130–790 | 17–5–78 | 18–1–77 | 490 | 25 |
| Rainfall (7 days, mm) | 0–50 | 8–2–78[c] | 6–7–77 | 14 | 85 |
| Total organic matter ($A_{254}$)[d] | 0.290–0.610 | 15–11–77 | 15–2–78 | 0.360 | 18 |
| Total humic matter ($A_{350}$) | 0.046–0.140 | 21–9–77 | 15–2–78 | 0.074 | 29 |
| Chlorophyll *a* (µg liter⁻¹) | 0.3–39.0 | 1–3–78 | 17–5–78 | 2.6 | 190 |
| **Microbiological** | | | | | |
| Direct count (10⁶ ml⁻¹) | | | | | |
| Total | 0.55–4.7 | 29–9–76 | 12–7–77 | 1.6 | 40 |
| Cocci | 0.52–2.8 | 18–1–78 | 12–7–77 | 0.95 | 36 |
| Rods | 0.12–1.6 | 12–1–77 | 12–7–77 | 0.48 | 53 |
| Curved rods | 0.011–0.56 | 21–11–77 | 17–5–78 | 0.10 | 71 |
| H₂S producers (MPN[e] ml⁻¹) | 3–3,900 | 22–11–77 | 12–4–78 | 160 | 163 |
| Mesophiles (CFU[f] ml⁻¹) | 180–430,000 | 10–11–77 | 17–5–78 | 4,300 | 276 |
| Psychrophiles (CFU ml⁻¹) | 2–320,000 | 6–9–77 | 17–5–78 | 510 | 394 |
| Proteolytic bacteria (CFU ml⁻¹) | 32–17,000 | 1–6–77 | 19–4–78 | 200 | 328 |
| Fluorescent pseudomonads (CFU ml⁻¹) | 0–90 | 12–7–77[c] | 12–4–78 | 5 | 167 |
| Polymyxin-resistant bacteria (CFU ml⁻¹) | 50–380,000 | 26–10–77 | 17–5–78 | 2,500 | 322 |
| Bacterial spores (CFU 100 ml⁻¹) | 20–1,600 | 21–9–77 | 19–4–78 | 180 | 111 |
| Yeasts (CFU 1,000 ml⁻¹) | 10–8,400 | 26–7–78 | 12–4–78 | 290 | 170 |
| Community respiration (µg of O₂ liter⁻¹ h⁻¹) | | | | | |
| Unsupplemented | 0–74 | 12–1–77[c] | 17–5–78 | 4 | 213 |
| Supplemented | 0–92 | 14–10–77 | 17–5–78 | 10 | 118 |

[a] Microbial counts given as geometric means.
[b] Calculated from temperatures in degrees Kelvin.
[c] An example of several minima.
[d] $A_{254}$, Absorbancy at 254 nm.
[e] MPN, Most probable number.
[f] CFU, Colony-forming units.

plate counts of mesophilic, psychrophilic, and polymyxin-resistant bacteria occurred in mid-May 1978 during the second phytoplankton peak. At the same time, the direct count of "curved rods" and the community respiration values were at their highest. The direct counts (curved rods excluded) had their maxima in July 1977 in connection with a strong outflow from the inner parts of the archipelago. Proteolytic bacteria had their maximum in late April 1978 below the ice, during the onset of the vernal phytoplankton bloom, which mainly consists of diatoms (15). The maxima of $H_2S$ producers, fluorescent pseudomonads, and yeasts occurred in late winter of 1978 during the observed salinity minimum of 3.93 ‰, and the peak of bacterial spores was recorded 1 week later.

The minima of most of the microbiological parameters occurred in either the autumn or early winter, except those of proteolytic bacteria, fluorescent pseudomonads, and yeasts, the lowest numbers of which were recorded in June or July (Table 1). However, a summer peak of yeasts (710 colony-forming units per liter) was found in July 1977, in connection with the rainfall maximum of 50 mm and with a simultaneous strong outflow from the inner parts of the archipelago.

**Some intermicrobial correlations.** Bacterial spores, the majority of which are considered to represent terrestrial microbial communities in the study area, had significant positive correlations with all the microbial types during the ice-cover periods (Table 2) and with the plate counts of the various microbial types over the whole period studied. During the open-water periods, bacterial spores correlated significantly with psychrophiles, fluorescent pseudomonads, and yeasts.

Mesophilic bacteria had significant positive correlations with the direct counts (not with cocci in winter) and $H_2S$ producers; the correlation with curved rods in the winter was especially strong ($r = +0.929$).

Yeasts had significant positive correlations with fluorescent pseudomonads but a negative correlation with mesophilic bacteria during the open-water periods.

**Regression models of microbiological parameters.** Results of the stepwise regression analysis are given in Table 3. Good explanation was obtained for the variation in psychrophiles, bacterial spores, and yeasts (63 to 77%), and particularly high values were obtained for the two parameters of community respiration (about 90%). The significant independent variables accounted for approximately 50% of the variation in direct counts (not curved rods), $H_2S$ producers, mesophiles, and polymyxin-resistant bacteria. The proportion of the variation explained for curved rods, proteolytic bacteria, and fluorescent pseudomonads was only 37 to 44%.

Total organic matter and water temperature were significant for most of the microbiological parameters, having positive regression coefficients, but not for fluorescent pseudomonads, bacterial spores, or yeasts. Chlorophyll a played a major role for the respiration parameters and

TABLE 2. Some intermicrobial correlations obtained for data from Tvärminne Storfjärd

| Parameter | All data[a] | | | | Open-water periods[b] | | | | Ice-cover periods[c] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial spores | Mesophiles | Yeasts | Community respiration[d] | Bacterial spores | Mesophiles | Yeasts | Community respiration[d] | Bacterial spores | Mesophiles |
| Direct count | | | | | | | | | | |
| Total | | 0.55 | | 0.45 | | 0.61 | | 0.45 | 0.71 | 0.71 |
| Cocci | | 0.38 | | | | 0.43 | | | 0.65 | |
| Rods | | 0.57 | | 0.49 | | 0.67 | | 0.50 | 0.58 | 0.65 |
| Curved rods | | 0.69 | | 0.41 | | 0.65 | | 0.46 | 0.73 | 0.93 |
| $H_2S$ producers | | 0.48 | | | | 0.47 | | | 0.63 | |
| Mesophiles | 0.31 | | | 0.46 | | | −0.39 | 0.64 | 0.73 | |
| Psychrophiles | 0.65 | | | 0.33 | 0.41 | | | 0.53 | 0.82 | |
| Proteolytic bacteria | 0.34 | | | | | | | 0.41 | 0.79 | |
| Fluorescent pseudo-monads | 0.70 | | 0.73 | | 0.59 | | 0.67 | | 0.81 | |
| Polymyxin-resistant bacteria | 0.37 | | | 0.45 | | | | 0.60 | 0.77 | |
| Bacterial spores | | | 0.66 | | | | 0.51 | | | |

[a] $r \geqq 0.38$, $P < 0.001$; $r \geqq 0.31$, $P < 0.01$.
[b] $r \geqq 0.45$, $P < 0.001$; $r \geqq 0.36$, $P < 0.01$.
[c] $r \geqq 0.68$, $P < 0.001$; $r \geqq 0.56$, $P < 0.01$.
[d] In unsupplemented samples.

TABLE 3. *Significant[a] independent variables in the regression models for microbiological parameters based on all the data (71 observations) from Tvärminne Storfjärd[b]*

| Dependent variable (Y) | $R^2$ (%) | Independent variables |
|---|---|---|
| **Direct count** | | |
| Total | 58 | °C(17); $A_{254}$(13); log Chl(7); $-A_{350}$(3) |
| Cocci | 49 | °C(40); -‰(21) |
| Rods | 55 | log Chl(15); °C(10); $A_{254}$(11); $-A_{350}$(4) |
| Curved rods | 37 | $A_{254}$(25); log Chl(5); °C(3) |
| H₂S producers | 45 | -‰(30); °C(7); NE winds(3) |
| Mesophiles | 51 | $A_{254}$(38); °C(3); Chl(3) |
| Psychrophiles | 63 | $A_{254}$(38); °C(3); Chl(2); SE winds(2) |
| Proteolytic bacteria | 44 | $A_{254}$(42); °C(6); $A_{350}$(2) |
| Fluorescent pseudo-monads | 41 | -°C(19); -‰(13); rainfall(3) |
| Polymyxin-resistant bacteria | 49 | $A_{254}$(37); °C(2); Chl(2) |
| Bacterial spores | 77 | -°C(31); $A_{350}$(5); -‰(1) |
| Yeasts | 64 | Fl.ps.(14); -°C(7); rainfall(5); -‰(3) |
| **Community respiration** | | |
| Unsupplemented | 89 | Chl(67); Bact.(2) |
| Supplemented | 87 | Chl(56); Bact.(6) |

[a] At the 5% risk level.

[b] The figure in parentheses is the reduction of the percentage explained if the variable is omitted. The minus sign means that the variable has a negative regression coefficient. Symbols: °C = water temperature; ‰ = salinity; $A_{254}$ = total organic matter (absorbancy at 254 nm); $A_{350}$ = total humic matter; Chl = chlorophyll $a$; Fl.ps. = fluorescent pseudomonads; Bact. = colony-forming units of mesophiles.

the number of rods and a minor role for total bacteria, curved rods, mesophiles, psychrophiles, and polymyxin-resistant bacteria. Rainfall, which may wash microbes from the land (2), appeared in the models for fluorescent pseudomonads and yeasts. Low salinity, connected with freshwater outflows (15), was especially significant for H₂S producers and fluorescent pseudomonads, and also for cocci, bacterial spores, and yeasts.

The variable that best explained the variation of yeast counts was the number of fluorescent pseudomonads.

**Comparison of regression models obtained for data from the open-water and ice-cover periods.** The independent variables found to be significant in the regression models for the two periods are presented in Table 4. With most of the parameters, the models obtained for the open-water data were either identical or similar to those for the whole study

period (Table 3). This was evidently related to the fact that most of the observations (70%) were derived from the open-water periods. For the open-water periods, the independent variables explained a satisfactory proportion of the variation in total bacteria, bacterial spores, and yeasts (61 to 68%) and a very substantial proportion of that in the respiration parameters (90%), but with the other microbiological parameters the proportion was 50% or less. For the ice-cover periods, high values (62 to 84%) were obtained for most of the parameters. The regression models for the winter seasons differed from those obtained for the open-water periods. Total organic matter was significant for many of the parameters during the open-water periods, whereas total humic matter was the dominant independent variable in the winter.

Water temperature appeared, with positive regression coefficients, in the winter models for many of the parameters, being highly significant for the respiration in supplemented samples. Increased water temperatures in winter indicate the presence of seaward-flowing freshwater and domestic effluents below the ice cover (16). During the open-water periods, water temperature appeared with a negative coefficient in the models for psychrophiles and bacterial spores and with a positive one in the models for nearly half of the parameters (Table 4).

Chlorophyll $a$ did not figure in the winter models for rods, curved rods, mesophiles, polymyxin-resistant bacteria, or respiration in supplemented samples.

Low salinity is connected with freshwater outflows; salinity had a significant negative coefficient in the winter models for rods, fluorescent pseudomonads, and H₂S producers, but was of no significance for the two first-mentioned parameters during the open-water periods. Salinity appeared in the open-water model for cocci but not in the winter model.

Rainfall during the open-water periods figured only in the model for yeasts, having a positive regression coefficient, whereas it appeared in half of the winter models with a negative coefficient. The model for yeasts contained fluorescent pseudomonads for both periods.

Mesophilic bacteria accounted for about 40% of the variation in the two respiration parameters during the open-water periods, but in the winter they were significant only for the respiration in supplemented samples and explained a smaller proportion of the variation (about 20%).

## DISCUSSION

Jones (12) found that about 65% of the variation in plate counts of bacteria and 75% of that

TABLE 4. *Significant[a] independent variables in the regression models for microbiological parameters based on data from the open-water (51 observations) and ice-cover (20 observations) periods at Tvärminne Storfjärd[b]*

| Dependent variable (Y) | Open-water periods | | Ice-cover periods | |
|---|---|---|---|---|
| | $R^2$ (%) | Independent variables | $R^2$ (%) | Independent variables |
| Direct count | | | | |
| Total | 61 | °C(13); $A_{254}$(11); log Chl(7); $-A_{350}$(5) | 82 | Ice(16); −rain(14); $A_{350}$(11), Chl(12) |
| Cocci | 51 | °C(24); −‰(10) | 84 | log Chl(23); ice(16), −√rain (10); $A_{350}$(4) |
| Rods | 52 | log Chl(21); °C(14) | 39 | −‰(39) |
| Curved rods | 46 | $A_{254}$(26); $-A_{350}$(10); log Chl(9) | 74 | $A_{350}$(16); −√rain(16); °C(6) |
| H₂S producers | 33 | −‰(33) | 62 | −‰(62) |
| Mesophiles | 56 | $A_{254}$(10); Chl(9); °C(8); $-A_{350}$(6) | 75 | $A_{350}$(47); −rain(14) |
| Psychrophiles | 45 | $A_{254}$(45); −°C(6) | 75 | $A_{350}$(27); −√rain(5); °C(5) |
| Proteolytic bacteria | 39 | $A_{254}$(17); °C(9) | 76 | °C(19); $A_{350}$(13); −√rain(6) |
| Fluorescent pseudomonads | 31 | −°C(29); $A_{254}$(9) | 74 | −‰(38); log Chl(18) |
| Polymyxin-resistant bacteria | 52 | $A_{254}$(14); $-A_{350}$(6); Chl(5); °C(4) | 65 | $A_{350}$(44); −√rain(5) |
| Bacterial spores | 68 | −°C(55); $A_{254}$(33) | 84 | $A_{350}$(40); °C(10) |
| Yeasts[c] | 62 | Rain(11); Fl.ps.(11); −Bact.(8) | 65 | Fl.ps.(22); −Prot.bact.(8) |
| Community respiration | | | | |
| Unsupplemented | 90 | Chl(49); Bact. (3) | 78 | °C(14); Chl(8) |
| Supplemented | 90 | Chl(48); Bact. (3) | 79 | °C(55); Bact.(7) |

[a] At the 5% risk level.

[b] Symbols and explanations are given in Table 3. Prot. bact., Proteolytic bacteria.

[c] Without Fl.ps. and Bact.: $R^2$ 36%; −°C(24); rain(18); −‰(4).

in direct counts could be explained by chlorophyll *a*, temperature, inorganic nutrients, and pH. The values obtained in the present study were lower, about 50 and 60% with organic matter, chlorophyll *a*, and temperature (Table 3). The importance of phytoplankton and temperature for these two types of bacterial counts has been shown in other aquatic biotopes (e.g., references 3, 4, 8, 10, 18, 19, 23). In the present study, temperature was found to be of significance also for cocci, rods, curved rods, H₂S producers, psychrophiles, and proteolytic and polymyxin-resistant bacteria. The significance of organic matter for the microbiological parameters (Table 3) is attributed both to autochthonous and allochthonous organic compounds, since no clear distinction between their contributions could be made. However, the role of autochthonous organic matter is indicated by chlorophyll *a* appearing in the regression models. The striking role of chlorophyll *a* in the variation of the respiration parameters (Table 3) evidently shows that microbial respiration is closely dependent on algal extracellular organic compounds. It is difficult to understand how plankton algae would be responsible for the main part of the community respiration.

In most of the investigations cited above, the population peaks for bacteria have appeared after those of phytoplankton. However, no relation between the bacterial numbers and phytoplankton biomass could be demonstrated by Goldman et al. (5), Gunkel (6), or Ramsay (21).

The correlations of plate counts of mesophilic bacteria with direct counts of bacteria (Table 2) indicate that their temporal changes were partly parallel; their variation was explained by the same environmental factors (Tables 3 and 4). In contrast to these observations, Melchiorri-Santolini et al. (14) found a negative correlation between plate counts and direct counts in Lago Maggiore. The high correlation of curved rods with plate counts of mesophiles shows that curved rods formed a considerable proportion of the colonies obtained from the winter samples.

The negative coefficient for salinity in the models not only for bacterial spores and yeasts but also for fluorescent pseudomonads, cocci, and H₂S producers (Table 3) shows that these microbial types are transported with seaward-flowing freshwater. A similar conclusion may be drawn from the negative coefficients for water temperature in the models for bacterial spores, fluorescent pseudomonads, and yeasts (Table 3),

because the effects of freshwater outflows become evident when the water temperature falls from autumn on.

Northeast winds cause the seaward flow of $H_2S$ producers, as is shown by the appearance of these winds in the regression model for $H_2S$ producers (Table 3). This bacterial type is probably derived from domestic effluents. Western winds also transport $H_2S$ producers to Tvärminne Storfjärd, in this case from the decaying *Fucus* mats in the nearby inlet Byviken (28). The model for psychrophiles contained southeast winds, which sometimes occur in connection with the seaward flow of fecal bacteria into the Tvärminne area. Hence, it may be concluded that many of the psychrophiles at Tvärminne Storfjärd originate from the inner parts of the archipelago.

The contribution of fluorescent pseudomonads to the explanation for yeasts (Tables 2 and 3) indicates that yeasts are connected with domestic effluents, since fluorescent pseudomonads often occur with sewage (1). However, because sewage and freshwater flow together into the Tvärminne archipelago, some of the yeasts might be derived from the Pojoviken proper. The presence of rainfall in the model for both these microbial types shows that they are also washed from the land. Thus, fluorescent pseudomonads are not necessarily associated with domestic effluents. Increased bacterial counts have been recorded after rainfall in lakes by Collins (2), Jones (9), and Ramsay (21).

The negative correlation of yeasts with mesophilic bacteria (Table 2) and the appearance of mesophilic bacteria with a negative coefficient in the open-water model for yeasts (Table 3) suggest that yeasts cannot compete for nutrients with bacteria, which probably have higher growth rates.

Below the ice cover, all the parameters were affected by freshwater outflows. This was evident from the appearance of either humic matter or salinity in the winter regression models (Table 4) and from the winter correlations of microbes with bacterial spores. The appearance of temperature with a positive regression coefficient in the winter models for psychrophiles, proteolytic bacteria, and the respiration parameters (Table 4) is connected with the effects of freshwater outflows, since increased temperatures below the ice cover indicate freshwater outflows in the Tvärminne area (16). However, it can also be attributed to an increase in microbial metabolism with increasing temperature. Low rainfall (negative coefficient) appearing in the winter regression models is often linked with rising atmospheric pressure, which also increases the

freshwater flow seawards in the Tvärminne area. Hence, it also indicates effects of freshwater outflows. The reason why the percentage of the variation explained was in most cases highest in the winter was that freshwater outflows governing the populations were characterized well by the environmental parameters chosen, whereas during other seasons other properties than those determined became more characteristic.

The role of freshwater outflows in the variation of polymyxin-resistant bacteria indicates that these bacteria did not represent slow-growing bacteria from brackish water. The identity of the regression models of mesophilic and polymyxin-resistant bacteria shows that similar bacterial types were determined in many cases. Thus, polymyxin mostly failed to select slow-growing bacteria in the Tvärminne area.

Sampling frequency has been found to affect the degree to which population changes can be explained (11). At the beginning of the present study, samples were also collected at short intervals (about 5 min), but they did not reveal significant changes in any of the parameters. The subsequent sampling intervals of 1 or 2 weeks, on the other hand, may have been too long during the phytoplankton blooms. An intermediate frequency with daily samplings proved satisfactory in connection with the vernal bloom (P. Väätänen, J. Appl. Bacteriol., in press). However, in regression models for microorganisms the percentage of the variation explained will be increased by phytoplankton only when there is considerable overlapping of microbial and algal peaks, which is not always the case. Unfortunately, the breakdown of phytoplankton blooms shown to be of importance for microbial populations (28) invariably escapes the regression analysis, because no parameters unambiguously characterizing the breakdown are available.

It seems that understanding of temporal changes in the microbial populations in the Tvärminne area might be increased by taking into consideration inorganic nutrients and pH, since these undergo strong seasonal variation in this area (15, 16). Measures of microbial interactions, e.g., predation (11), might also contribute to their explanation. Examination of the effects of filter-feeding zooplankton on microbial populations might also further our understanding of their fluctuations.

navigation. Helpful criticism of the manuscript was given by Veronica Sundman, Hans Luther, Seppo Niemelä, and Åke Niemi, Helsinki.

## LITERATURE CITED

1. **Bonde, G. J.** 1977. Bacterial indication of water pollution, p. 273–364. *In* M. R. Droop and H. W. Jannasch (ed.), Adv. Aquatic Microbiol., vol. 1. Academic Press, London.

2. **Collins, V. G.** 1960. The distribution and ecology of gram-negative organisms other than Enterobacteriaceae in lakes. J. Appl. Bacteriol. **23**:510–514.

3. **Coveney, M. F., G. Cronberg, M. Enell, K. Larsson, and K. Olofsson.** 1977. Phytoplankton, zooplankton and bacteria—standing crop and production relationships in a eutrophic lake. Oikos **29**:5–21.

4. **Fondén, R.** 1969. Heterotrophic bacteria in Lake Mälaren and Lake Hjälmaren. Oikos **20**:344–372.

5. **Goldman, C. R., M. Gerletti, P. Javornicky, U. Melchiorri-Santolini, and E. de Amezaga.** 1968. Primary productivity, bacteria, phyto- and zooplankton in Lake Maggiore: correlations and relationships with ecological factors. Mem. Ist. Ital. Idrobiol. **23**:49–127.

6. **Gunkel, W.** 1968. Die Bakterien und ihre Beziehungen zum Plankton in den Tümpeln der Helgoländer Düne nach der schweren Sturmflut im Februar 1962. Mitt. Int. Ver. Limnol. **14**:31–42.

7. **Hällfors, G., P. Kangas, and A. Lappalainen.** 1975. Littoral benthos of the Northern Baltic Sea. III. Macrobenthos of the hydrolittoral belt of filamentous algae on rocky shores in Tvärminne. Int. Rev. Gesamten Hydrobiol. **60**:313–333.

8. **Henrici, A. T.** 1938. Studies of freshwater bacteria. IV. Seasonal fluctuations of lake bacteria in relation to plankton production. J. Bacteriol. **35**:129–139.

9. **Jones, J. G.** 1971. Studies on freshwater bacteria: factors which influence the population and its activity. J. Ecol. **59**:593–613.

10. **Jones, J. G.** 1972. Studies on freshwater bacteria: association with algae and alkaline phosphatase activity. J. Ecol. **60**:59–75.

11. **Jones, J. G.** 1977. The effect of environmental factors on estimated viable and total populations of planktonic bacteria in lakes and experimental enclosures. Freshwater Biol. **7**:67–91.

12. **Jones, J. G.** 1978. The distribution of some freshwater planktonic bacteria in two stratified eutrophic lakes. Freshwater Biol. **8**:127–140.

13. **Luther, H.** 1951. Verbreitung und Ökologie der höheren Wasserpflanzen im Brackwasser der Ekenäs-Gegend in Südfinnland. I. Allgemeiner Teil. Acta Bot. Fenn. **49**:1–232.

14. **Melchiorri-Santolini, U., R. Bertoni, G. J. M. Maestroni, and A. A. Mariazzi.** 1975. Distribution of pe-

lagic bacterial populations and related ecological factors in a deep lake, p. 385–394. *In* T. Hasegawa (ed.), Proceedings of the First Intersectional Congress of IAMS, vol. 2: Developmental Microbiology Ecology. Science Council of Japan, Tokyo.

15. **Niemi, Å.** 1973. Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. I. Dynamics of hydrography, nutrients, chlorophyll a and phytoplankton. Acta Bot. Fenn. **100**:1–68.

16. **Niemi, Å.** 1975. Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. II. Primary production and environmental conditions in the archipelago and the sea zone. Acta Bot. Fenn. **105**:1–73.

17. **Niemi, Å.** 1978. Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. III. Environmental conditions and primary production in Pojoviken in the 1970s. Acta Bot. Fenn. **106**:1–28.

18. **Niewolak, S.** 1973. Seasonal changes in numbers of some physiological groups of microorganisms in Ilawa lakes. Pol. Arch. Hydrobiol. **20**:349–369.

19. **Overbeck, J.** 1968. Prinzipielles zum Vorkommen der Bakterien im See. Mitt. Int. Ver. Limnol. **14**:134–144.

20. **Pratt, D., and J. Reynolds.** 1974. Selective media for characterizing marine bacterial populations, p. 258–267. *In* R. R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.

21. **Ramsay, A. J.** 1976. Heterotrophic bacteria and their relationship with plankton in a New Zealand freshwater lake. N. Z. J. Mar. Freshwater Res. **10**:77–90.

22. **Sarvala, J.** 1971. Ecology of *Harmothoe sarsi* (Malmgren) (Polychaeta, Polynoidae) in the northern Baltic area. Ann. Zool. Fenn. **8**:231–309.

23. **Schegg, E.** 1968. Beziehungen zwischen Planktonentwicklung und Bakterien im Vierwaldstättersee und Rotsee. Schweiz. Z. Hydrol. **30**:289–296.

24. **Väätänen, P.** 1976. Microbiological studies in coastal waters of the Northern Baltic Sea. I. Distribution and abundance of bacteria and yeasts in the Tvärminne area. W. & A. de Nottbeck Foundation Scientific Report no. 1, Tvärminne.

25. **Väätänen, P.** 1977. Effects of composition of substrate and inoculation technique on plate counts of bacteria in the Northern Baltic Sea. J. Appl. Bacteriol. **42**:437–443.

26. **Väätänen, P.** 1979. Microbial activity in brackish water determined as oxygen consumption. Arch. Hydrobiol. Ergeb. Limnol. **12**:32–37.

27. **Väätänen, P.** 1979. A device for aseptic collection of large samples from surface water. Finn. Mar. Res. **246**:135–142.

28. **Väätänen, P.** 1980. Factor analysis of the impact of the environment on microbial communities in the Tvärminne area, southern coast of Finland. Appl. Environ. Microbiol. **40**:55–61.

29. **Virta, J.** 1977. Estimating the water and salt budgets of a stratified estuary. Nord. Hydrol. **8**:11–32.