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Dynamics of Pollution-Indicator and Heterotrophic Bacteria in Sewage Treatment Lagoons

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The spatio-temporal dynamics of pollution-indicator bacteria and aerobic heterotrophic bacteria were studied in the sewage treatment lagoons of an urban wastewater center after 26 months of biweekly sampling at eight stations in these lagoons. Robust statistical methods of time-series analysis were used to study successional steps (through chronological clustering) and rhythmic behavior through time (through contingency periodogram). The aerobic heterotrophic bacterial community showed two types of temporal evolution: in the first four stations, it seems mainly controlled by the nutrient support capacity of the sewage input, whereas in the remaining part of the lagoon, it seems likely that the pollution-indicator bacteria are gradually replaced by other bacterial types that are better adapted to this environment. On the other hand, the pollution-indicator bacteria showed an annual cycle which increased in amplitude at distances further from the wastewater source. The main events in this cycle were produced simultaneously at all stations, indicating control of these bacterial populations by climatic factors, which act through physical and chemical factors, and also through other biological components of this ecosystem (phytoplankton and zooplankton). Finally, we use results from this study to suggest a modified design for a future study program.

A 2-year sampling program was carried out in the sewage treatment lagoons of an urban wastewater center. The purpose of this program was to verify the efficiency of the plant. We report on two aspects of this program, the elimination of pollution-indicator bacteria and the spatio-temporal dynamics of these bacteria and aerobic heterotrophic bacteria in this ecosystem. The importance of this type of study has been shown by Gloyna (12), Drapeau and Jankovic (7), Walker et al. (25), and Edeline (8).

In addition to determining how and how much of the bacteria of sanitary importance are reduced during the sewage treatment process, it is also of prime interest to ecologists to determine what role environmental bacteria play as a biological compartment in this ecosystem. The originality of this sewage treatment process resides in it being an ecosystem transforming inert organic matter into living matter through a chain primarily involving bacteria, phytoplankton, and zooplankton. In the present paper we investigate the spatial and temporal dynamics of the bacterial compartment. Other factors of the ecosystem are included in a general model elsewhere (M. Troussellier, P. Legendre, B. Baleux, and R. Sabatier, submitted for publication).

These general objectives are modulated by two kinds of constraints: (i) the sampling strategy, which is at best a compromise between statistical requirements and the physical sample handling capacity of laboratory equipment and technicians, and (ii) the very nature of this type of biological material, since it is not possible to maintain a bacterial sample isolated in situ long enough to study it without biasing its demographic parameters (species composition, growth, and mortality). Thus, the compromise sampling program described below allows one to study the community according to the spatial and temporal scales of the sampling, but it rules out the study of finer (days or shorter) or longer (cycles of several years) phenomena in time or of more finely defined gradients is space. Furthermore, the peculiarities of bacterial studies mentioned above limit the time-related phenomena that can be studied in these communities to those of tendency, periodicity, and succession. Finally, short and badly behaved (not normalized) time series call for the use of robust data analysis methods instead of the more sophisticated but more constraining methods reviewed by Fry et al. (11).

MATERIALS AND METHODS

The lagoon sewage treatment center of the city of Mèze (03°35′06″ E, 43°25′10″ N) is located on the shore of the Thau brackish water basin, which is open to the Mediterranean in the Languedoc-Roussillon area of southern France. The plant comprises three successive basins with a total surface area of 8 ha (first basin 4 ha and second and third basins 2 ha each). The average depth varies from 1.40 m (first basin) to 1.10 m (third basin). The flow of incoming waste varies from 1,200 m^3 per day in winter to 2,000 m^3 per day in summer, and the total detention time is ca. 70 days in winter and 40 days in summer.

Sampling stations, numbered 1 to 8, are shown in Fig. 1. The sampling program was started at the time of the "birth" of the system (when sewage first arrived at station 1) and was pursued for 26 months at approximately biweekly intervals between June 1980 and August 1982. Water samples were collected in sterile vials and analyzed within 3 h after preservation at 4°C. The bacterial types studied were aerobic heterotrophic bacteria (total viable count), isolated and counted by spread-plate procedure after dilution in sterile water with 9‰ NaCl on Bacto nutrient agar (Difco Laboratories), and the pollution-indicator bacteria (the fecal contamination indicators, total coliforms at 37°C and fecal coliforms at 44.5°C, both isolated and counted by spread-plate procedure on Tergitol and T.T.C. agar, Institut Pasteur Production; fecal streptococci, counted by pour-plate procedure in D. Coccosed agar, BioMerieux; and *Pseudomonas aeruginosa*, an opportunistic pathogenic bacteria, isolated and counted on cetrimidenalidixic acid medium, Institut Pasteur Production).

All bacterial counts were first log-transformed (base 10),

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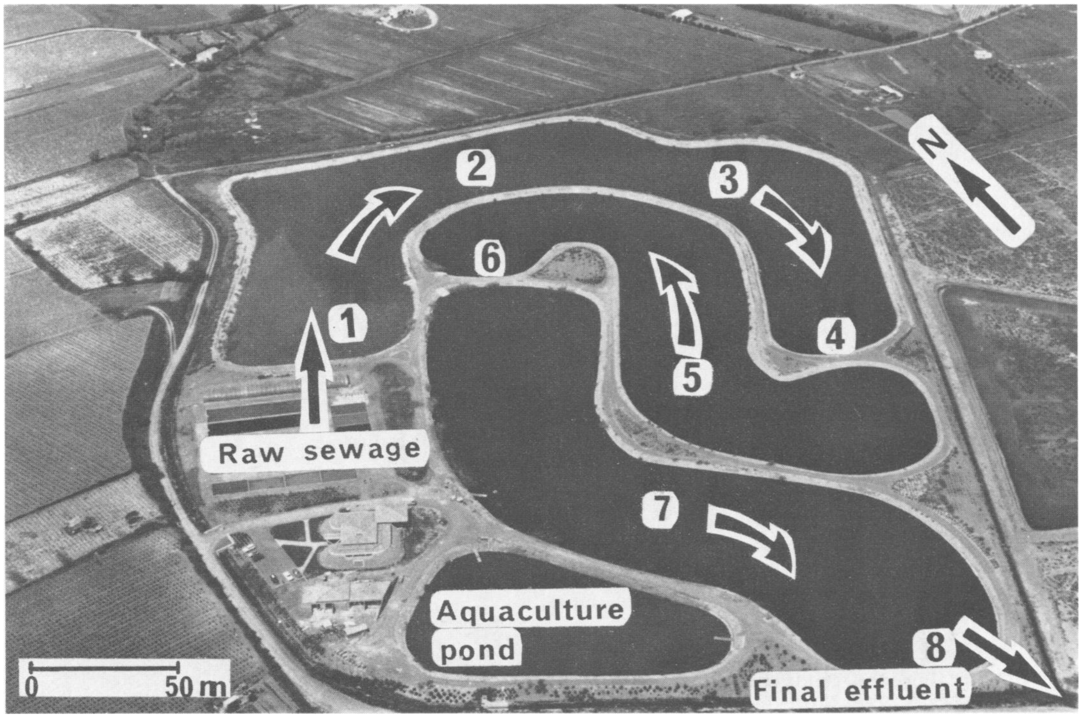

FIG. 1. Aerial photograph of the Mèze sewage treatment center showing sampling stations (1 to 8) and direction of flow (arrows).

for two reasons: (i) "bacteria often grow exponentially, consequently a logarithmic transformation makes interpretation of changes in bacterial number or activity more straightforward" (Fry et al. [11]), and (ii) this transformation eliminates a good deal of the asymmetry in the frequency distributions of the variables. This asymmetry is deleterious to reduced-space ordination methods based on a linear model, such as the principal component analysis method used below, as well as to the resolution power of Gower's similarity coefficient (Legendre and Legendre [18]).

The study of the time series of the bacterial data at each sampling station was divided into two aspects: (i) the search for homogeneous steps along the succession of events in both the aerobic heterotrophic bacteria and the pollution-indicator bacteria and (ii) the quest for periodic phenomena in the indicators of human pollution.

The first problem was tackled by using the chronological clustering method (P. Legendre, S. Dallot, and L. Legendre, Am. Nat., in press) specially designed for the identification of successional steps within a multidimensional series (multiple species) of biological samples. The method assumes that the community under study evolved by steps. An examination of the time series of log-transformed species counts, followed by principal component analyses for each station, showed that this assumption was reasonable. Chronological clustering proceeded by intermediate-link linkage agglomeration (50% connectedness was used throughout) with a constraint of time contiguity; pairs of groups were allowed to fuse only if they met a statistical criterion of cluster fusion at a predetermined α significance level based on a randomization of the between-group distance matrix. The null hypothesis was that the members of the two groups were drawn from the same statistical population (in which case the two groups were artifacts of the clustering algorithm). This corresponds to the ecological criterion of community stability within each step of the succession. This method does not make any assumptions regarding the distribution of data or the regularity of sampling; furthermore, the similarity coefficient (below) computed before chronological clustering deals with missing values. Consequently, this method can be thought of as statistically robust, since it lends itself to the idiosyncrasies of ecological, and in particular, bacteriological sampling.

Before this analysis, the resemblance matrix between samples was computed using the similarity index of Gower (14), in which the similarity between samples 1 and 2, for instance, is given by

$$S(1,2) = \frac{1}{n} \sum_{i=1}^{n} \left(1 - \frac{[y_{i1} - y_{i2}]}{R_i} \right)$$

where y_{i1} is the value taken by variable i for sample 1, and R_i is the range of variation of variable i among all samples in the study. When studying the multivariate series involving the four types of pollution-indicator bacteria, $n = 4$. In the other runs, only the variable representing the aerobic heterotrophic bacteria was used; this application allows the use of chronological clustering for segmenting other one-dimensional data series, such as are often collected in population dynamics studies.

For the purpose of illustrating the results of chronological clustering, we searched for a compound variable synthesizing the four pollution-indicator bacterial variables, following the suggestion of St-Louis and Legendre (24). A principal component analysis of the correlation matrix of the four pollution-indicator bacteria was run on all samples of the eight stations, combined in a single data file. The result (not illustrated further) showed that all four variables had almost exactly the same contribution to the formation of the first principal component, which accounted for 83% of the variance. The synthetic variable that is used below is a slight conceptual simplification of this result: each variable was standardized (the standard deviations are 1.70 for total coliforms, 1.51 for fecal coliforms, 1.35 for fecal streptococci, and 0.70 for *P. aeruginosa*), after which the four values were summed for each sample. This synthetic bacterial pollution variable is not likely to generate noise variation since, to the contrary, variations in one component are obliterated by variations in another when they are not in phase. Missing data occurred in each series; they are represented by blank spaces in the figures.

Chronological clustering is not designed to identify cyclic components along the time axis of the sampling program. Methods specifically designed for this purpose, such as autocorrelation analysis or spectral analysis, have been recommended by Fry et al. (11) for identifying cyclic components in microbiological data. These methods of time-series analysis impose some constraints on the data, however: if the series is not long enough and reasonably well-behaved (normality requirement), the tests may be invalidated and the methods may be unable to detect the cyclic components (Legendre and Legendre [18]). These difficulties were encountered during the analysis of the bacterial variables from each sampling station when autocorrelation functions and the ARIMA models of Box and Jenkins (5) were used, because the series are both short and badly behaved. Thus, a more robust method, called the contingency periodogram (17), was used to search for the rhythms that could be seen to exist at least in some of the pollution-indicator bacterial series. For each variable submitted to analysis, this method produces a graph of the values of a contingency statistic, $H(S \cap X)$, as a function of the various periods investigated, plus a test of statistical significance for each period. To account for missing samples, a constant sampling interval of 14 days was obtained by interpolation within each data series (58 samples) before this analysis. This method of time-series analysis has proven useful with short or badly behaved data series. We will use it below to test the hypothesis of gradual emergence of ecosystem-controlled cycles as the wastewater proceeds from the source to the end-point of the treatment lagoons.

RESULTS

Figure 2 illustrates the chronological clustering of the aerobic heterotrophic bacteria represented on graphs of this variable by time at each station. The α significance level of chronological clustering is known to act as a probe of the finer details of the series: as α is increased, increasingly finer clusters are produced (Legendre et al., in press). For the aerobic heterotrophic bacteria, chronological clustering was run for several α levels, but only the results obtained with α = 10% are used. This level was selected because it delineated as many groups as were clearly discernible along the data series themselves (Fig. 2). In this figure, each step is characterized by the mean value of the log-transformed points contained in it (height of each horizontal line segment). Computing the mean on the actual untransformed numbers of bacteria would have led to slightly higher mean lines, although with the same shape; log-transformed data were preferred, however, for the reasons given above. The evolution of the shape of these graphs, as one proceeds from the beginning to the end of the sewage treatment center (two major types of shapes), is discussed below together with the main breakpoints along the series.

The chronological clustering of the four pollution-indicator bacterial variables is also presented by horizontal line segments (Fig. 3). The reference points plotted along the time axis are, in this instance, the values of the synthetic pollution-indicator variable described above, and the height of each line segment is the mean value of the points included in the given successional step. The α level selected for clustering was 5% in this instance, because the cyclic component of variability, visible in many of these graphs and analyzed in more detail below, provokes the formation of too many small clusters at higher α values. The succession steps, as well as the major breakpoints affecting several stations, are discussed below in conjunction with the establishment of cycles in this pollution-indicator bacterial community.

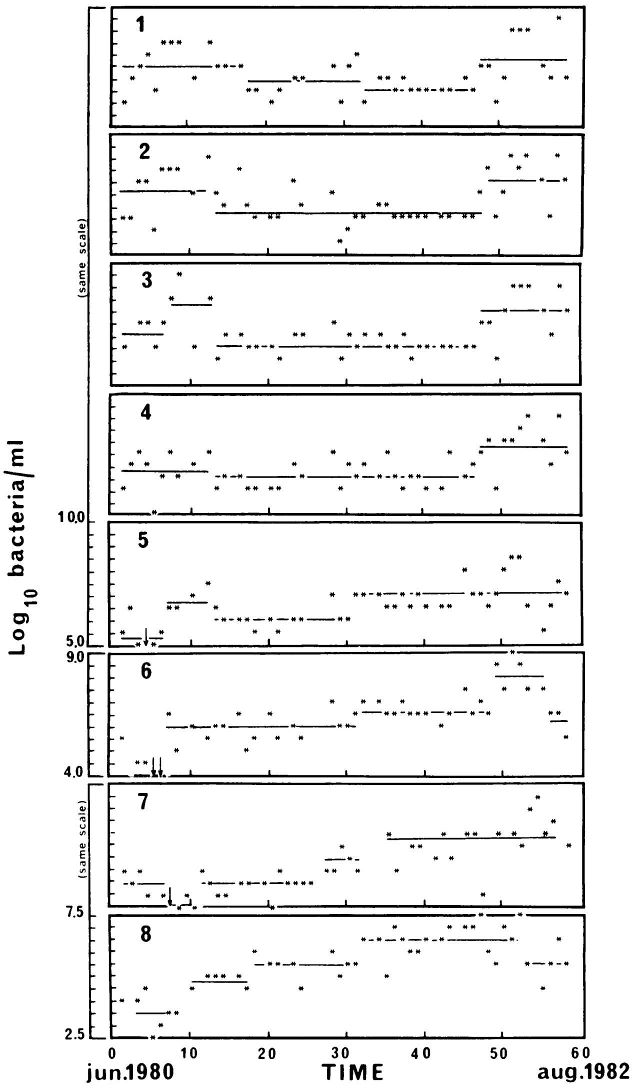

FIG. 2. Evolution of the abundance of the aerobic heterotrophic bacteria along time, at each sampling station (1 to 8, progression from the beginning to the end of the treatment process). The time axis is scaled by the successive sample numbers (average interval between successive samples, 14 days). Horizontal line segments represent the partition of each series by chronological clustering (see text). Arrows, Sample points lower than abscissa.

Four examples of contingency periodograms are presented in Fig. 4, together with the data series from which they were computed. The confidence interval for significance level α = 0.005 is plotted on each periodogram, and the most significant period is written out for convenience. Table 1, on

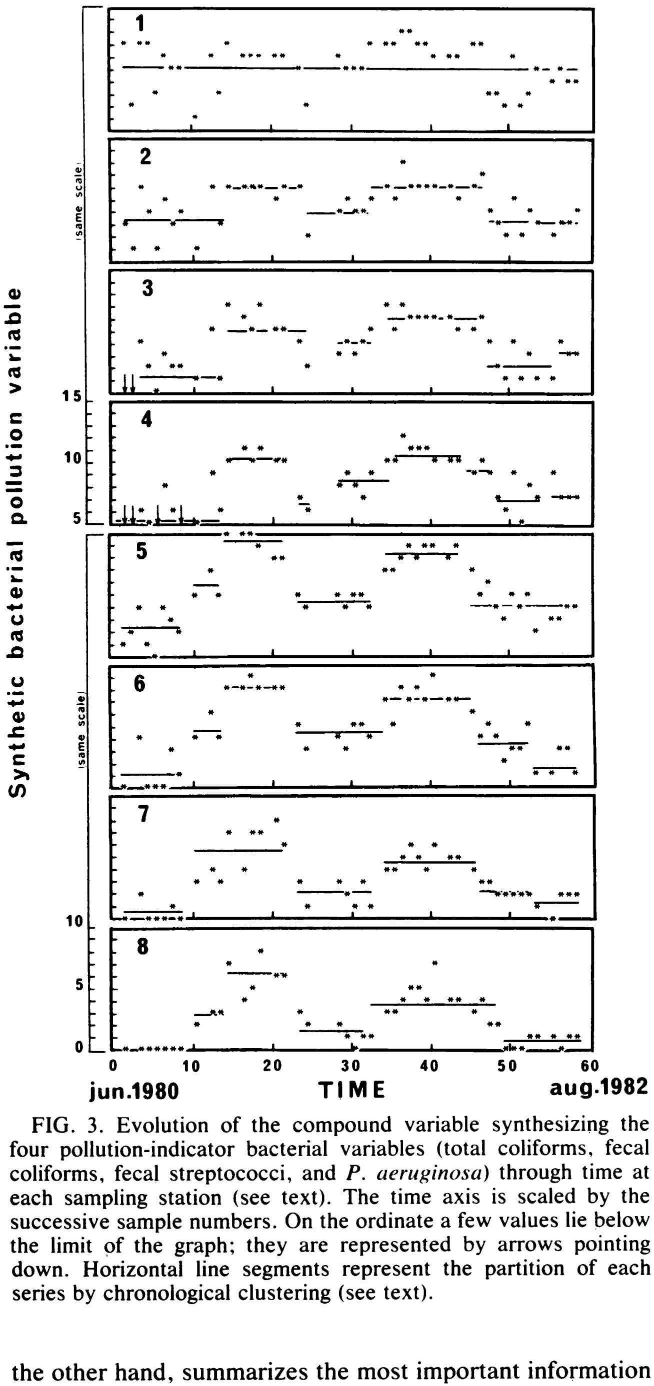

FIG. 3. Evolution of the compound variable synthesizing the four pollution-indicator bacterial variables (total coliforms, fecal coliforms, fecal streptococci, and *P. aeruginosa*) through time at each sampling station (see text). The time axis is scaled by the successive sample numbers. On the ordinate a few values lie below the limit of the graph; they are represented by arrows pointing down. Horizontal line segments represent the partition of each series by chronological clustering (see text).

the other hand, summarizes the most important information from all contingency periodograms, the most significant period in each case and its level of significance. The following observations can be made. (i) The aerobic heterotrophic bacterial variable displays very little periodicity. When periodicity is present, it is neither highly significant nor obvious in the data series (Fig. 4b; see also Fig. 2). (ii) On the contrary, a period of almost 1 year is clearly visible among the pollution-indicator bacteria (the average value of the significant periods in Table 1 is 23.8 2-week intervals, that is, 333 days). This can be interpreted as an annual cycle, the difference possibly being due to the shortness of the data series (26 months) and to differences between years. (iii) The presence of this cycle is far from obvious in the synthetic pollution-indicator bacterial variable at station 1 (Fig. 3), in which it is also not highly significant (Table 1). The cycle builds up gradually along the four stations of the first basin (Fig. 3, Table 1), whereas in stations 4 to 8, located in the second and third basins, the annual period is obvious from both the periodograms (Table 1) and the data series (Fig. 3). (iv) *P. aeruginosa* does not show significant periodicity at the ends of the station series (Table 1) for opposite reasons. At stations 1 and 2, it is always present at an almost constant density; thus, it does not show cyclic behavior, which is the general tendency in the first basin for all pollution-indicator bacteria. In stations 7 and 8, on the other hand, it has disappeared almost completely.

To determine the "distance" between stations in terms of the bacterial community, principal component analyses were run on the aerobic heterotrophic bacteria data and on the values of the synthetic pollution-indicator variable. In each case, the various sampling times formed as many variables for the purpose of this ordination. Since the principal component analysis was carried out from the covariance matrix and the eigenvectors were normalized to length 1, the stations are then located in the rotated space at distances equal to the square root of the sum of squares of the vertical differences between their respective profiles (Fig. 2 and 3). The resulting ordinations are plotted in Fig. 5. The two principal components, plotted in Fig. 5a, account for 89% of the total variance, and those in Fig. 5b account for 97%. These ordinations are used to demonstrate the ordered behavior of the two components of the bacterial community and to suggest the most critical locations in the sewage treatment plant.

DISCUSSION

The analysis of the spatial and temporal behavior of bacterial abundances in aquatic ecosystems does not necessarily lead to evidence of coherent dynamic laws. The problem often resides in an inadequate sampling design (sampling lag, total length of the series, and regularity of sampling) or in the use of data analysis methods that impose constraints (normality, length of series, etc.) that are not met by the data set (18). These problems may well have prevented other workers from producing interpretable quantitative successional models. Robust statistical models are now available for both the analysis of periodic components in time series (even allowing the analysis of semiquantitative or qualitative data series [16]) and the study of successional phenomena without periodic component; they have been used with profit in the present study.

The study of stable ecosystems, leading to bacterial counts that vary little in time, may be deceptive, whereas perturbed systems often yield the most information about the factors which regulate bacterial dynamics. It is often during ecosystem-generated crises (red water [6]), after an acute external impact (4, 13, 21), or when studying an environmental gradient (river gradient [19], estuarine gradient [9], or freshwater bacterial community reaching sea water [1]) that knowledge on the quantitative development laws of bacterial communities may be acquired. In the present study, the sampling program focused on a particular spatial-temporal gradient. It began at the "birth" of the system (so it is a case of primary succession) and progressed to an age of 26 months, with successive volumes of sewage entering the treatment plant as the study proceeded. The transformation of the bacterial community in time, as it progressed through the treatment system, was also studied by sampling at eight stations in the treatment lagoons. The design of the sampling program along these two gradients

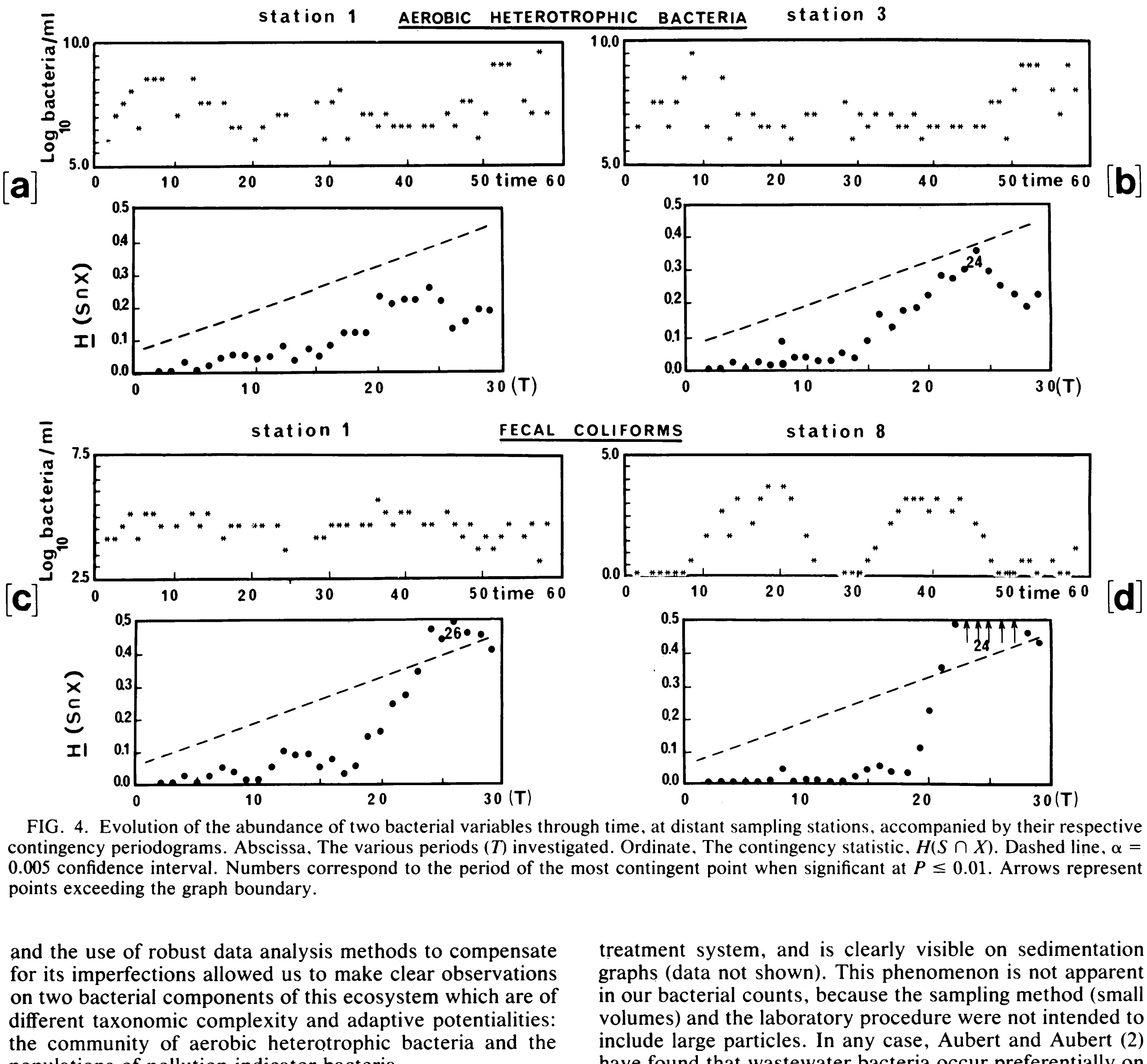

FIG. 4. Evolution of the abundance of two bacterial variables through time, at distant sampling stations, accompanied by their respective contingency periodograms. Abscissa, The various periods (T) investigated. Ordinate, The contingency statistic, $H(S \cap X)$. Dashed line, $\alpha = 0.005$ confidence interval. Numbers correspond to the period of the most contingent point when significant at $P \leq 0.01$. Arrows represent points exceeding the graph boundary.

and the use of robust data analysis methods to compensate for its imperfections allowed us to make clear observations on two bacterial components of this ecosystem which are of different taxonomic complexity and adaptive potentialities: the community of aerobic heterotrophic bacteria and the populations of pollution-indicator bacteria.

(i) Aerobic heterotrophic bacterial community. There are two types of temporal evolution of aerobic heterotrophic bacterial communities clearly differentiated in Fig. 2. They correspond to the first basin (stations 1 to 4), with a concave shape and a long low-level sequence in the middle, and to the second and third basins (stations 5 to 8), with a convex shape because the long step-shaped build up in density is followed by a slight decrease. In the first basin, relatively stable concentrations of aerobic heterotrophic bacteria through time suggest that they are mainly controlled by the sewage input, which is stable in concentration through time, and not by an eventual local ecosystem capable of reacting to seasonal variations, as is found below. To explain these synchronous and identically shaped temporal concentration sequences at several successive stations, several hypotheses were considered.

At the head of the first basin, there is an important phenomenon of settling, mainly at and immediately after station 1. Settling is to be expected in an urban wastewater treatment system, and is clearly visible on sedimentation graphs (data not shown). This phenomenon is not apparent in our bacterial counts, because the sampling method (small volumes) and the laboratory procedure were not intended to include large particles. In any case, Aubert and Aubert (2) have found that wastewater bacteria occur preferentially on small-size particles (smaller than 20 μm), which are little affected by primary settling.

TABLE 1. Most significant period of each contingency periodogram[a]

Bacterial variable	Most significant period[b] for station:							
	1	2	3	4	5	6	7	8
Aerobic heterotrophic			24[c]	20[c]				
Total coliforms	22[c]	24[c]	26[d]	24[d]	24[d]	24[c]	24[d]	24[d]
Fecal coliforms	26[d]	25[d]	25[d]	24[d]	24[d]	24[d]	24[d]	24[d]
Fecal streptococci	24[c]	22[d]	26[d]	26[c]	25[c]	24[d]	22[d]	22[d]
P. aeruginosa		23[c]	22[d]	21[d]	22[d]	21[c]		
Synthetic variable	24[c]	24[d]	24[c]	25[c]	24[d]	23[d]	24[d]	24[d]

[a] Periodograms were computed for each bacterial variable and each station. Periods are listed only when statistically significant.

[b] Number of 2-week intervals (see the text).

[c] $P \leq 0.01$.

[d] $P \leq 0.005$.

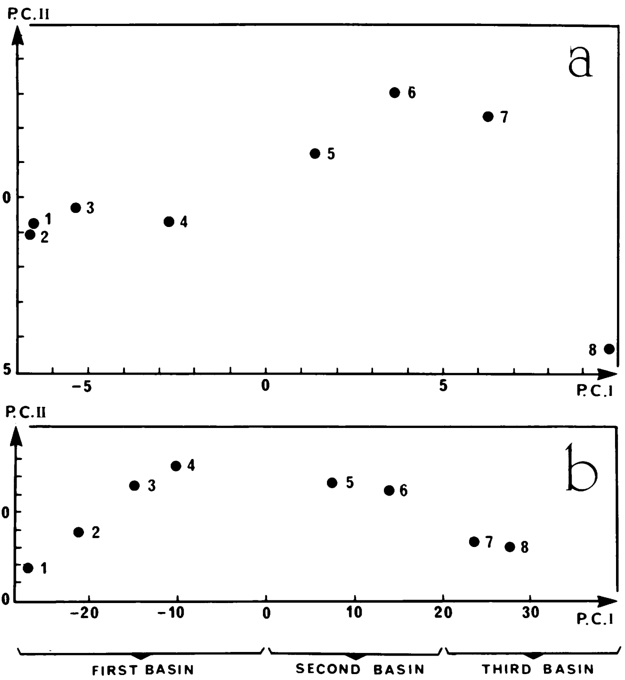

FIG. 5. Reduced-space ordination of the eight sampling stations (1 to 8) by principal component analysis based on (a) the values of the aerobic heterotrophic bacterial variable in the series of samples, and (b) the values of the synthetic bacterial pollution-indicator variable in the series of samples.

The counts of aerobic heterotrophic bacteria are high in the first basin and roughly constant from one station to the next and through considerable time lapses, despite large fluctuations of the pollution-indicator bacteria (below) which represent a fraction of the aerobic heterotrophic bacteria (compare Fig. 2 and 3 for stations 1 to 4). This phenomenon may be interpreted by the following hypothesis: the high nutrient support capacity found in dissolved and particulate organic matter makes it possible for the bacterial community to develop in this basin an optimal demographic strategy, producing permanently high and stable abundances.

In basins 2 and 3 (stations 4 to 8), abundances of aerobic heterotrophic bacteria seemed to depend on other phenomena. The overall stability found in the first basin through time is broken. In stations 5 to 8, there is a marked stepwise increase of bacterial concentrations through time until a maximum is reached, followed by a slight decrease. This happens despite cyclic fluctuations in pollution-indicator bacteria (Fig. 3 and below) included in aerobic heterotrophic bacterial counts, indicating that, here again, the pollution-indicator allochtonous bacteria are replaced by other bacterial types that are better adapted to this environment. The general tendency towards higher counts may be the consequence of the immigration of bacteria from the outside environment, which proceeds at a roughly constant rate in basins 2 and 3, in which bacteria adapt themselves to an environment that becomes more and more enriched with organic matter produced extraneously (wastewater) or in situ (bacteria, phytoplankton, and zooplankton). This tendency reaches an asymptote, however, showing the presence of factors limiting bacterial development (limited resources or grazing, autoinhibition, etc.)

Furthermore, a decrease in bacterial concentrations appears simultaneously in stations 6 to 8, at the end of the sampling period. Several hypotheses may be considered. The decrease may be the sign of a stabilization of bacterial abundances, or it may mark the beginning of other types of chronological phenomena (see below). It may be hypothesized that both cases could be the result of a decrease in available nutrients and probably also of an increase in the pressure exerted upon bacteria by other biotic components of the ecosystem (antibiosis and predation) when these other components increased their concentration or diversity or both.

A decrease in the concentrations of aerobic heterotrophic bacteria may result from two different phenomena, inasmuch as this community is a good indicator of the evolution of the ecosystem through time. In the first explanation, the lagoon wastewater treatment system may have gone through the various steps of evolution (birth, maturity, senescense) very quickly, so that its life span would be much shorter than expected. The second explanation is that the decrease shows the beginning of endogenous cyclic oscillations, not linked to seasonal rhythms, which can be found in ecosystems reaching maturity (10, 20) and, in particular, in bacterial communities reaching stability in open environments (22). These questions could only be answered by pursuing the study through a few more annual cycles, although not necessarily with the same sampling lag.

(ii) Pollution-indicator bacterial populations. The evolution of pollution-indicator bacterial populations through space (stations 1 to 8) and time (Fig. 3) is different from that of the aerobic heterotrophic bacterial community (Fig. 2). The chronological clustering results show station 1 to be relatively stable, whereas the other stations present several successional steps whose average values have a rhythmic behavior. Differences between the lower and higher steps grow stronger from station 2 to station 8. The contingency periodogram results confirm the increasing significance of the observed periodicity as distance from the wastewater source increases.

The breakpoints between successional steps are almost simultaneous when comparing sampling stations (Fig. 3). One would expect them to be shifted in time at successive stations if they were controlled by differences in the quality of the affluent. This suggests that the control lies instead with external seasonal factors acting indirectly on the pollution-indicator bacterial community, possibly through other biotic components of the ecosystem such as phytoplankton and zooplankton. Their development is determined by climatic variables (maximum in summer) in a way opposite to the development of pollution-indicator bacteria (maximum in winter). Antagonism phenomena described in the literature, such as the competition between bacterial species (3), the inhibitor action of phytoplankton species on fecal bacteria (23), or the predation of bacteria by zooplankton (15) usually put forward to explain the self-purifying capacity of aquatic environments, could intervene in lagoon wastewater treatment systems to regulate the dynamics of pollution-indicator bacterial abundances. On the other hand, knowledge of the nature (e.g., sine function) and mathematical parameters (period and position of maximum) could help users of such treatment systems to predict the level of bacterial pollution in the effluent of the system.

For sanitary purposes, it is important to relate the cyclic nature of bacterial purification to the type of use made of the pollution-receptor environment. For instance, a maximum reduction of pollution-indicator bacteria in summer is desirable near summer sport beaches. However, in shellfish breeding areas (as is the case here) it is more desirable to reduce pollution-indicator bacteria in the water during all seasons. Our findings suggest that it may be necessary to increase pollution-indicator bacterial monitoring in winter and to consider the possibility of treating the effluent further.

One should remember that the periodic behavior observed during two years of sampling does not allow us to extrapolate to the bacterial purification performance of the future. This information can only be acquired by further sampling as the ecosystem matures through time. The seasonal periodicity may retain its present wave shape, or it may evolve and stabilize at around its present summer average (increased purification performance) or its present winter average (reduced performance) or at some intermediate level.

(iii) Sampling design. Analysis of the present data may provide information on the best way to design the sampling of future studies. Examination of the distances between sampling stations in the space of the aerobic heterotrophic bacteria and the pollution-indicator bacterial variables suggests a possible simplification of the sampling. The ordinations in reduced space shown in Fib. 5a and b and the actual distance between stations in these graphs clearly show the three basins as forming three distinct bacterioecological units. This result suggests that a maximum amount of information per unit of sampling effort could be obtained by sampling only, for instance, at the wastewater input (station 1), before the water flows out of the first (station 4) and second (station 6) basins, and again at the exit of the sewage treatment center (station 8).

(iv) Further ecological considerations. The observations reported above suggest two important considerations on dynamic aspects of the bacterial community in a lagoon wastewater treatment center. First, if one compares the spatial and temporal dynamics of the aerobic heterotrophic bacteria with those of the pollution-indicator bacteria, this study has revealed two types of strategies for bacteria living in such a newly formed eutrophic aquatic ecosystem. A slow but regular strategy of gradual occupation of ecological niches is suggested in the aerobic heterotrophic bacterial community, which is characterized by a multiple species structure representing many different ecological functions (niches). In addition, the pollution-indicator bacteria grown in a very closed environment (digestive tract) and characterized by its stability, to which they are well adapted, are transplanted into a new, open environment in which they can at best survive. These allochtonous bacteria show large fluctuations in population abundances.

These cyclic fluctuations lead to a second consideration. Indeed, since the cycle is annual and is not controlled by the quality of the affluent (it is not found in the affluent and builds up gradually as the distance from station 1 increases), it seems to characterize an increasingly disrupted group of populations; this is to be hoped for with pollution-indicator bacteria passing through a wastewater treatment system. Further observations have suggested that the seasonal influence acts on these bacterial populations through physical, chemical, or other biological channels. These aspects will be further developed in another study (Troussellier et al., submitted for publication), analyzing causal relationships between the bacterial variables and various other environmental factors (physical and chemical variables, phytoplankton, and zooplankton) which have been measured simultaneously with the bacterial data reported here.

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