Chapter 14
Impacts of Lagoonal Bacterial Pollution on an Aquacultural Site (Ebrié Lagoon, Côte d'Ivoire)

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14.1 Introduction

Abidjan, the economic capital of Côte d'Ivoire, has grown around Ebrié lagoon. There are significant local water pollution problems because of the waste waters that flow untreated into the natural environment (Arfi *et al.*, 1981). These anthropogenic inputs constitute a major source of lagoonal contamination by pathogenic germs such as *Vibrio cholerae*. Moreover, the physical and chemical characteristics of the lagoonal environment appear to be conducive to the survival and multiplication of halophilic or halotolerant pathogen bacteria (Lanusse and Guiral, 1988; Kouassi *et al.*, 1992). Diarrhoeal syndromes have been reported on several occasions since 1970 in the Abidjan area. The main causative agent for these epidemics has been identified as *V. cholerae*, serotype El Tor Ogawa (Duchassin *et al.*, 1973). A number of choleriform infections involving other vibrionic bacteria (*V. parahaemolyticus*, *Aeromonas* spp.) are also periodically reported in the area. Previous research on vibrios (particularly *V. cholerae*) mainly involved epidemiological studies of hospital cases (Dosso *et al.*, 1984). Little information is therefore available on the ecological conditions and incidence of these germs in the natural environment; in particular no study has been done to determine the relation between vibrio presence in the environment and the hydroclimatic conditions of Ebrié lagoon.

In the long term such pollution could undermine aquaculture in the lagoon, as *V. cholerae* can be transmitted by ingestion of contaminated fish. In 1991, during a routine check of infectious agents likely to provoke fish mortality at Layo aquaculture station on the banks of Ebrié lagoon, *V. colerae* (serotype El Tor Ogawa) was isolated in the alimentary canal of a *Heterobranchus longifilis* fingerling. As tests failed to identify sick or healthy-carrier workers at the station, the presence of this germ in the ponds would indicate a more general state of environmental contamination. This study has been done to provide more reliable information on the real risks incurred at an aquacultural site, and to describe the annual cycle of *V. cholerae* in the various hydrosystems at Layo station (lagoon, ponds and irrigation channels).

14.2 Materials and methods

Ebrié lagoon (130 km long and an area of 542 km^2) is situated along the Atlantic coast of Côte d'Ivoire. This vast body of water has an average depth of 4.8 m and is permanently connected to the Atlantic Ocean through the Vridi canal. Layo aquaculture station is situated on the northern bank of the lagoon, 45 km west of Abidjan and 3.5 km from the mouth of the Agnéby, a small coastal river (Fig. 14.1).

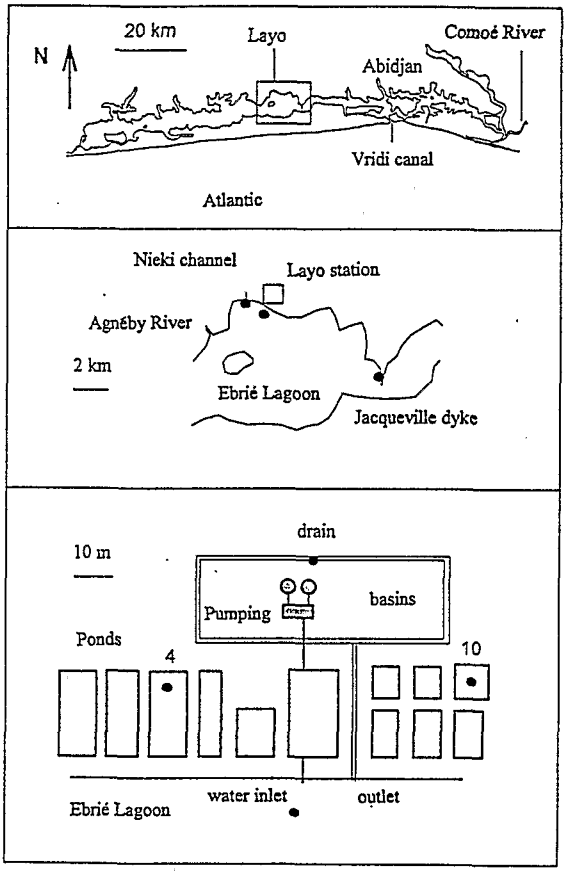

Fig. 14.1 Geographical location and map of the Layo experimental aquaculture station. The sample sites are marked with a black circle.

14.2.1 *Hydroclimatology*

Rainfall was recorded at Adiopodoumé weather station, 13 km west of Layo. The daily water flow of the Agnéby was measured at the mouth by the Hydrology Service of the Ministry of Transport and Tourism.

14.2.2 *Sampling*

Monthly samples were taken at six points between March 1991 and April 1992 (Fig. 14.1):

- At Ebrié lagoon, close to the brood stock grow-out and holding pens
- In ponds E4 and E10 at Layo station (770 and 590 m^2 in area and 1.2 and 0.8 m deep, respectively) used for the pre-grow-out of different fish species. Before each stage of the fish culture process, the ponds are emptied, treated and limed. They then gradually fill through groundwater recharge and any rainfall that may occur
- In the drainage ditch of the aquaculture station fed with lagoon water through a water tower
- In the irrigation canal of the Nieki basin, discharging 1 km from the station and fed directly by run-off waters from formerly forested, peaty, wetlands now occupied by a banana grove
- At a reference site removed from all sources of pollution, 10 km east of Layo in the central channel of Ebrié lagoon (Jacqueville dyke)

The surface water samples from the various environments were kept in sterile bottles. Samples of zooplankton and sediment were also gathered in the fishponds. Finally, underwater branches of *Salvinia molesta* and roots of *Pistia stratiotes* were also collected on the lagoon banks near Layo and in the Nieki channel. The samples were kept at ambient temperature (22–25°C) and examined within two hours of collection.

14.2.3 *Physico–chemical and biological analyses*

Temperatures were read in situ, while pH and conductivity were measured at 25°C in the laboratory using a Metrohm 605 pH meter and a Tacussel conductivity meter.

Chlorophyll *a* concentrations were measured by fluorometric analysis after methanol extraction of the phytoplankton retained on a Whatman GF/F membrane.

Microscopic bacteria counts (fixed in formaldehyde at 2% final concentration, conserved at low temperature) were conducted in epifluorescence using the Porter and Feig (1980) procedure and 4′6-diamino-2-phenyl indole (DAPI) as fluorochrome.

The abundance of *V. cholerae* in the water and sediment was estimated after decimal dilution (three tubes per dilution) and enrichment. For each triplicate, two successive cultures in salty (10 g/l NaCl), alkaline (pH 8.6), nutrient medium (biotrypticase) were incubated at 37°C for 24 hours. The surface film from the last enrichment medium was then incubated at 37°C for 24 hours on a solid thiosulphate-citrate-bile-salt-sucrose medium (TCBS Biomérieux) and applications resulting in discoloration of the medium (green to yellow) were considered positive. The average number of bacteria were then estimated on the basis of the frequency of negative tubes for the various dilutions of a given sample, using the most probable number method (Sorokin and Kadota, 1971) and calculation methodology developed by Peto (1953). The TCBS (+) bacteria densities in the water were then classified according to a logarithmic scale of relative abundance (0: less than 10 bacteria per 100 ml; 1: low abundance, 10 to 100 bacteria per 100 ml; 2: average abundance, 100 to 1000 bacteria per 100 ml; 3: high abundance, 1000 to 10 000 bacteria per 100 ml; 4: very high abundance, over 10 000 bacteria per 100 ml).

After purification of the strains (three isolations per sample), identification of the bacteria as *V. cholerae* was checked using a simplified battery of tests (examination for oxidase, urease, production of indole, fermentation of glucose and mannitol, lysin and ornithine decarboxylase, arginine dihydrolase and tryptophan deaminase). Agglutination tests were conducted on isolated strains presenting *V. cholerae* biochemical characteristics, using polyvalent *V. cholerae* 01 antiserum (Pasteur Institute, Paris).

The qualitative search for *V. cholerae* 01 was conducted on zooplankton and underwater macrophyte samples rinsed in sterilized salt water, by enrichment in alkaline nutrient medium with salinity equal to 0 and 30 g/l.

14.2.4 *Processing of statistical data*

Statistical analysis involved non-parametric tests (Mann-Whitney for comparison of two averages, Kruskal-Wallis for ANOVAs at 1 factor, Spearman for coefficient of rank correlation). A summary presentation of the findings grouping hydroclimatic (rainfall, temperature), physico-chemical (conductivity, pH) and biological (concentration of chlorophyll *a*, abundances of bacteria and *V. cholerae*) variables involved principal component analysis.

14.3 Results

14.3.1 *Hydroclimatic data*

Cumulative rainfall and Agnéby water flows for March 1991 to April 1992 are given in Fig. 14.2. Rainfall between April and July 1991 (main rainy season) accounted for 73% of the total, while rainfall in October and November 1991 (minor rainy season) accounted for 13%. The hydrograph of the Agnéby indicated two flooding episodes,

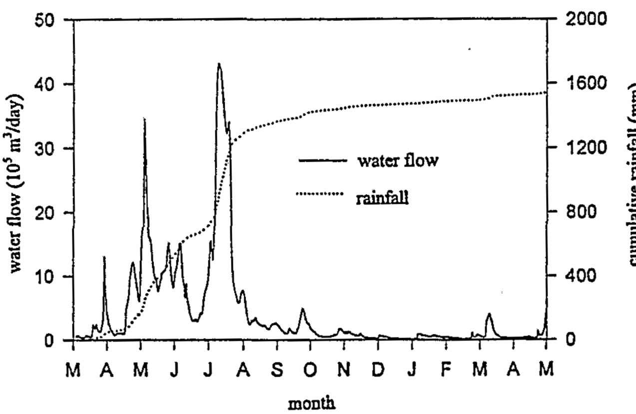

Fig. 14.2 Cumulative rainfall and daily flows of the Agnéby River between March 1991 and April 1992.

one in May and the other in July, with respective peak daily flows of 30 and $37 \times 10^5 m^3/d$. Flood recession extended until mid-August, one month after the final downpour occurred. The minor rainy season had little effect on water flow. With an annual rainfall deficit of over 750 mm (mean annual rainfall for 1980–86 was 2020 mm), 1991 was an atypically dry year. This is confirmed by the hydrology of the Agnéby (Guiral, 1992), as October flooding, usually the heaviest in the normal year, failed to occur in 1991.

14.3.2 *Physico-chemical data*

Temperature

Water temperatures during the study period varied between 22.5 and 37.2°C (Fig. 14.3), with maxima in May at the end of the long dry season. The minimum readings were in January, but the cooling was due to different causes according to the site. The drop in air temperature during the Harmattan season affected inland waters in particular, while the drop in ocean temperatures accompanying the January upwelling off the Ivorian coast mainly affected the lagoonal waters.

Conductivity

The sites under inland influence had the lowest and most stable conductivity levels. Average conductivity in the Nieki channel was 3.32 mS/cm, with a maximum of 9.43 mS/cm in September. Ponds E4 and E10 recorded averages of 6.40 and 6.97 mS/cm, respectively, and, except for the very low values recorded in June during the main rainy season, conductivity varied only slightly. The waters of the Layo drainage channel and lagoonal waters opposite the station showed identical characteristics (respective averages of 7.71 and 7.61 mS/cm, same seasonal variations) reflecting, at a more moderate level, the conditions at the reference site of Jacqueville. Average conductivity in the central lagoonal channel was 17.6 mS/cm, with low values during the main rainy season and high values between January and March with the influx of oceanic waters (Fig. 14.4).

pH

The waters of the Nieki channel were always acidic (average pH 6.1) with minimum values between 5.5 and 6.0. They can thus be considered as sourced mainly from inland, with little influence from lagoonal waters. The pond values varied more, with occasionally alkaline pH readings because of their mixed intake – groundwater and rainfall – and their intense biological activity (Fig. 14.5). Finally, pH readings for the lagoonal sites were generally close to neutral, with seasonal variations correlating closely with those for water conductivity and temperature. At Jacqueville, pH levels were higher than those of the lagoonal waters at Layo because of weaker inland influence.

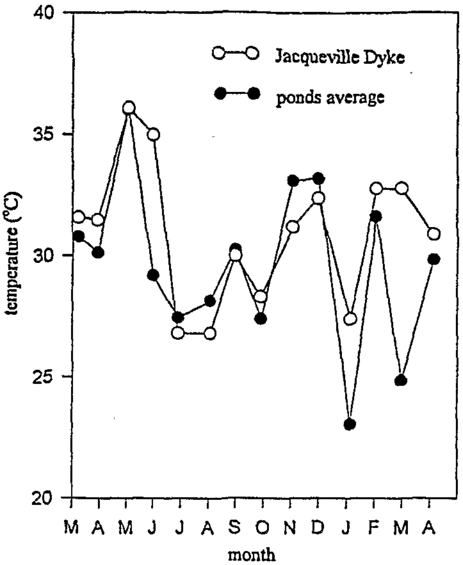

Fig. 14.3 Monthly temperature changes at Jacqueville station and average water temperatures in ponds E4 and E10.

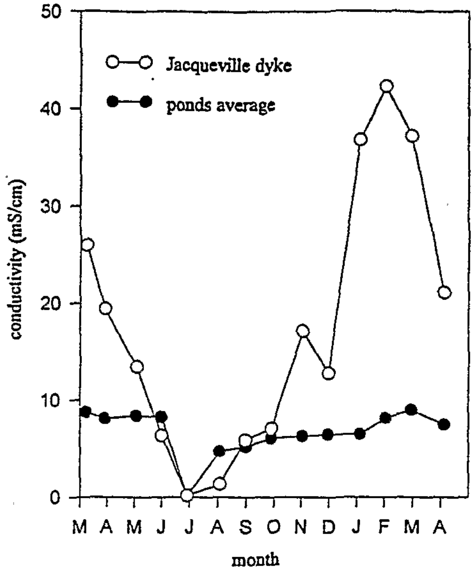

Fig. 14.4 Monthly conductivity changes at Jacqueville station and average conductivity levels in ponds E4 and E10.

14.3.3 *Planktonic data*

The highest chlorophyll biomasses occurred in ponds E4 and E10 (average values of 165.7 and 204.3 μg/l) and the lowest in the drainage channel (average of 6.9 μg/l), (Table 14.1). Pond values also varied considerably over time, with randomly recorded peak developments masking a possible seasonal trend (Fig. 14.6). These extensive fluctuations in a generally eutrophic environment were preceded by aquacultural operations (drainage and liming of pond E4 in April and harvesting of pond E10 in December). The algal blooms noted in the ponds would therefore appear to be linked to the aquacultural process rather than climatic variability. Seasonal variations at the lagoonal sites took the form of low values from July to September (end of the main rainy season) and high values in December and February (beginning of the main dry season). Finally, there was a much greater chlorophyll biomass in the Nieki channel waters than in the lagoonal waters, with occurrences of abrupt algal blooms.

14.3.4 *Bacteriological data*

Bacteria in natural waters and at the sediment surface

The greatest abundance of bacteria was always in pond waters, with average counts (10^6 bacteria per 100 ml) distinctly higher than at the other points. In the waters of the Nieki channel, the drainage channel and the lagoon near Layo, bacterial densities remained relatively stable (variation of about 30%). On the other hand, seasonal

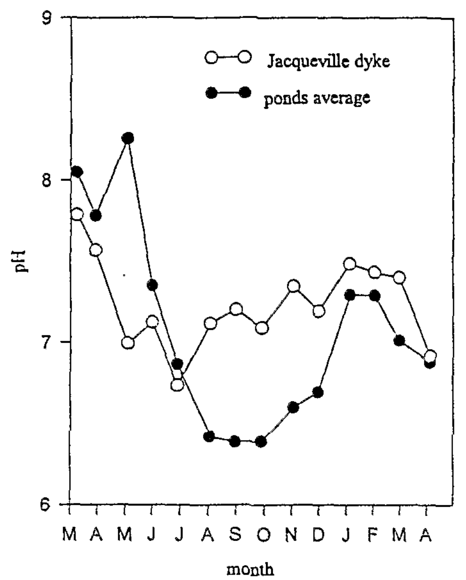

Fig. 14.5 Monthly pH changes at Jacqueville station and average pH in ponds E4 and E10.

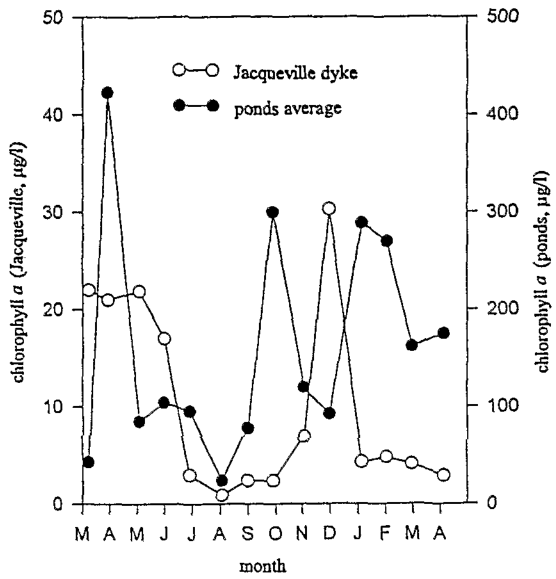

Fig. 14.6 Monthly changes in chlorophyll *a* concentrations at Jacqueville station and average chlorophyll *a* concentrations in ponds E4 and E10.

variations were more marked at Jacqueville (over 50%), with a minimum in August and a maximum in January.

There were usually high or very high *V. cholerae* counts in the waters of ponds E4 and E10 (with respective maxima of 1 and 13 × 10^6 bacteria per 100 ml). The drainage waters usually had higher counts than Ebrié lagoon, and sometimes also higher than in one of the two ponds. The bulk of the lagoon samples indicated much lower abundance levels, though there were marked seasonal variations. Though exclusively inland in origin, the waters of the Nieki channel had high *V. cholerae* counts, sometimes higher than those of the lagoonal waters in front of Layo station (Fig. 14.7). The highest counts were usually in samples with a conductivity of between 7.5 and 10.0 mS/cm. Fluctuations were greater in waters with conductivity below 5 mS/cm, but the counts were generally lower. Finally, vibrio counts were always low with conductivity above 12.5 mS/cm, with little variation (these samples corresponded mainly to the reference site of Jacqueville).

The *V. cholerae* counts, expressed in number of bacteria per 100 ml, were independent of temperature and conductivity for all the sample sites, but were inversely correlated to chlorophyll *a* concentrations in the lagoonal site waters. Despite trophic abundance and different species assemblages and community organization in each sample site, *V. cholerae* counts followed the same seasonal trends in the surface waters and sediments of ponds E4 and E10 (Spearman coefficients of rank correlation: 0.69* for water and 0.77** for sediments). In contrast, there was no relationship between variations in a given pond for pelagic and benthic *V. cholerae* communities. Bacterial

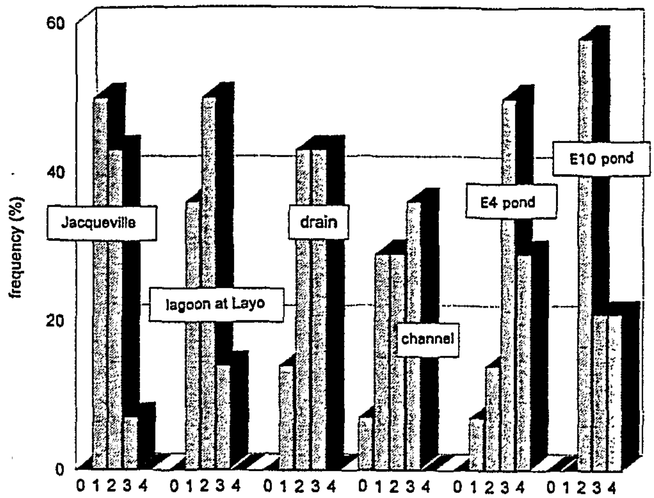

Fig. 14.7 *Vibrio cholerae* abundance frequencies at the different sites studied.

counts were similar in the surface waters and sediments of pond E10, while significantly higher in the sediments of pond E4, without any clearly discernible seasonality. There was, however, greater specificity of annual cycle in the waters, with abundances relatively low in November and December and relatively high in the long dry season.

Most of the isolated, purified strains identified as *V. cholerae* did not agglutinate with polyvalent *V. cholerae* 01 antiserum. The TCBS bacteria that were identified positively during the study corresponded therefore to a number of species traditionally classified as non-agglutinable *V. cholerae* (nag) or non-01 *V. cholerae*. It should, however, be noted that since the study, strains of *V. cholerae*, serotype El Tor Ogawa, from the environment of Layo station have been isolated (in the waters of the lagoon, the drainage channel, pond E10, and from zooplankton and sediments from pond E4).

The bacteria associated with zooplankton and macrophyte roots

Vibrio cholerae associations with zooplankton in aquaculture ponds have always proved positive, but the methodology has neither made it possible to determine average individual abundances, nor to compare seasonal variations in vibrio/zooplankton associations with variations in zooplankton community characteristics or *V. cholerae* densities in free-running waters and surface sediment.

Similarly, tests for *V. cholerae* on the roots of *Salvinia molesta* that colonize the lagoonal banks at Layo station have always proved positive. In contrast, and for the two ranges of conductivity tested, enrichments from *Pistia stratiotes* roots collected monthly in the Nieki channel have failed to isolate *V. cholerae*. In fact, after purification most of the strains grown on TCBS medium and characterized by sucrose oxidation had no oxidase. Thus, any contribution of floating aquatic macrophytes to the survival of *V. cholerae* in the environments studied would appear to vary according to species. However, the intermittent presence of the inland species *P. stratiotes* in the lagoonal waters and the absence of *S. molesta* from the strictly inland waters of the Nieki channel could suggest a possible role of the environment in the differences noted regarding *V. cholerae* absorption onto macrophyte roots.

Statistical analyses

Non-parametric ANOVAs showed a significant site effect for all variables tested with the exception of temperature. Sites with relatively similar averages were grouped using Tukey's test (Table 14.1). The resulting classifications confirm the specific nature of biological communities in ponds (phytoplanktonic biomasses, total bacteria and vibrio are all very high, with positive TCBS readings), whereas the environmental context is much like that of other sample sites on the aquaculture station.

Table 14.1 Average value, classification and ordination (non-parametric ANOVA and Tukey's test), for a given variable, of sites with no significant differences within a given environment, the major differences being between environments.

Temperature (°C)	Channel 28.0	E10 pond 29.6	E4 pond 29.7	Drain 30.1	Layo 30.5	Jacqueville 31.0
Conductivity (mS/cm)	Channel 3.32	E4 pond 6.64	E10 pond 6.97	Drain 7.61	Layo 7.71	Jacqueville 17.65
pH	Channel 6.09	Drain 6.89	Layo 6.91	E4 pond 7.06	E10 pond 7.12	Jacqueville 7.24
Chlorophyll *a* (μg/l)	Drain 6.9	Jacqueville 10.3	Layo 11.1	Channel 19.2	E4 pond 165.7	E10 pond 204.3
Log of bacterial densities	Drain 6.7	Channel 6.7	Layo 6.8	Jacqueville 7.0	E4 pond 8.0	E10 pond 8.1
Log of *V. cholerae* densities	Layo 3.6	Jacqueville 3.7	Drain 4.1	Channel 4.2	E4 pond 6.3	E10 pond 7.1

The first factorial design summarizes 51% of the overall information. There are four important variables for interpretation of axis 1 (28% of total variance): *V. cholerae* counts; chlorophyll *a* concentrations; bacterial counts; and pH values. Similarly, most of the information plotted against axis 2 (23% of variance) refers to temperature and conductivity. This analysis helps confirm pond specificity as compared to the other environments studied, with the observations for these two sites being projected exclusively in the same section of the factorial design. The projections are relatively similar (with the exception of January and March 1992, when pond 10 had heavy chlorophyll biomasses and a high abundance of bacteria), reflecting identical seasonal variation in the two ponds. As marked specificity could mask a possible trend at the other sites, a further analysis was made excluding the samples from the ponds.

The first two axes of this further analysis account for 28% and 23% respectively of the total information. The basic variance for axis 1 is attributable to conductivity and pH, while axis 2 is mainly determined by chlorophyll *a* concentrations and *V. cholerae* densities. Observation projections for a given site figure in specific sections of this design (Fig. 14.8).

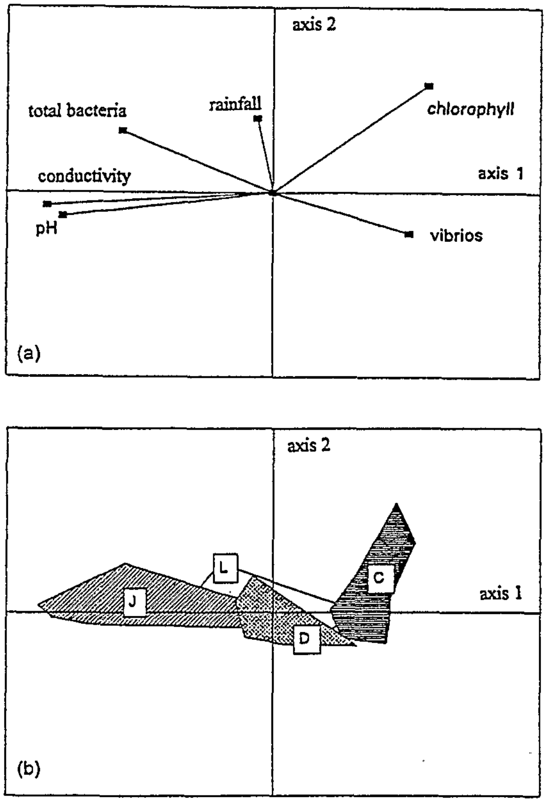

Fig. 14.8 Design 1–2 of the principal component analysis for Jacqueville (J), Layo (L), the drain (D) and the channel (C). (a) Projection of variables; (b) perimeters of observation projections.

- The observations for Jacqueville (high conductivity and alkaline pH) relate to the extreme left of the design, with clear specificity of samples for July to October, a period of low salinity and high *V. cholerae* counts.
- The observations for the Nieki channel, which is exclusively sourced from acidic inland waters, correspond to the right-hand side of the design. Seasonal changes in

this environment are little affected by changes in conductivity and are mainly conditioned by chlorophyll *a* concentrations and *V. cholerae* counts.

- The Layo lagoonal waters and those of the drainage channel occupy a central position as they have average conductivity values and pH readings midway between the estuarine waters of Jacqueville and the inland channel waters. However, the observations for the drainage channel, with a high abundance of *V. cholerae* but lower chlorophyll concentrations and total bacterial densities than in the lagoon, differ from the lagoonal observations. In general terms, the sites and/or periods with high *V. cholerae* counts correspond to waters with low conductivity and acidic pH, which also show low total bacterial abundances and modest chlorophyll concentrations.

14.4 Discussion

Comparison of the seasonal cycles of *V. cholerae* abundance in the waters of the six selected sites revealed three types of seasonal variation: the lagoonal sites (Jacqueville, Layo and the drainage waters), the site under heavy continental influence (the channel of Nieki) and the eutrophic aquacultural sites (ponds E4 and E10). These three groups have strong similarities in the type and origin of the waters that feed them and share the same bioclimatic pattern. The frequent presence of *V. cholerae* in the various environments of Layo aquacultural station and the high *V. cholerae* incidence in the ponds and drainage waters confirm the close association between these bacteria and aquacultural activities. This relationship was reported by Bruni *et al.* (1986) who found that vibrios accounted for a significant proportion of the bacterial population in waters used for aquaculture.

Vibrio cholerae is an euryhaline pathogen encountered in numerous estuarine and coastal ecosystems (Roberts *et al.*, 1982). It was observed during the course of this study within a conductivity range of 0 (strictly continental water) to 45 mS/cm (salinity approximately 26 g/l). Maximum abundance at Layo coincided with a conductivity of approximately 10 mS/cm (salinity approximately 6 g/l). Salinity was lower than that observed in two Florida estuaries (maximum density for waters with salinity between 10 and 25 g/l, Hood *et al.*, 1984) and much lower than off the coast of Japan (maximum densities for waters with average salinity of 28 g/l, Venkateswaran *et al.*, 1989). However, the maxima reported in the latter study concerned a port location subject to heavy discharge of domestic and industrial waste, and this significant abundance in high-salinity waters could also reflect both the halophilic or halotolerant nature of *V. cholerae* and the eutrophic environment.

The importance of the environmental factor in *V. cholerae* abundance at a given site was also noted during this study. The most eutrophic sites (ponds E4 and E10) had the highest *V. cholerae* counts. But the number of *V. cholerae* and chlorophyll *a* concentrations varied inversely in the Nieki channel and lagoonal waters off the aquaculture station. This phenomenon could be put down to *V. cholerae* sensitivity to phytoplanktonic excreta, as these consist largely of glycolic acid which might inhibit vibrio growth (Baumann *et al.*, 1973; Bianchi, 1976).

The seasonal variations of hydroclimatic parameters during the year had little marked impact on *V. cholerae* levels. This lack of correlation with temperature is a new finding as this parameter had been considered one of the determinants of seasonal *V. cholerae* development in estuarine and coastal environments, with the thermal optimum of 21–28°C usually occurring in a natural environment (Seidler and Evans, 1984). This lack of correlation with thermal conditions observed in Layo may stem from:

- The greater year-round uniformity of lagoon temperatures (variation of approximately 10% only) compared to other environments studied (variations of over 30% in Japanese coastal waters, Venkateswaran *et al.*, 1989).
- The high temperatures recorded at Layo, generally above the threshold of 18°C which has been seen to trigger a sharp drop in *V. cholerae* levels (Roberts *et al.*, 1984).

The high *V. cholerae* counts at the sediment surface of the ponds confirm the possible role of this interface in the survival and indeed multiplication of these bacteria, due to organic abundance and the particulate matter which increase fixation possibilities. Such absorbtion would appear in fact to result in higher levels of vibrio survival.

Epibiotic associations, particularly fixation on zooplanktonic organisms, have been proven experimentally (Huq *et al.*, 1983) for *V. cholerae* and confirmed in situ for *V. parahaemolyticus*, particularly during the benthic diapause phase (Kaneko and Colwell, 1978). However, there have been no such observations regarding epibiotic activity for *V. cholerae* in the natural environment. Research into the presence of *V. cholerae* in association with zooplankton always proved positive during the study, as did association with the *S. molesta* roots colonizing the lagoon banks. In contrast, enrichments based on *P. stratiotes* roots always proved negative, but the effect of the type of waters cannot be excluded. As experiments have demonstrated a greater survival of *V. cholerae* serotype El Tor Ogawa (and therefore an increase in proliferation capacity) with fixation on *Eichhornia crassipes* roots (Spira *et al.*, 1981), similar phenomenon might occur in Ebrié lagoon with the same implications for the health of the riverine populations.

14.5 Conclusion

The present study, which arose from the identification of *V. cholerae* serotype El Tor Ogawa at an aquaculture station on the banks of Ebrié lagoon has confirmed the abundance of *V. cholerae* in this type of environment. The majority of strains isolated at the various sites did not agglutinate with polyvalent *V. cholerae* 01 antiserum, but *V. cholerae* serotype El Tor Ogawa strains were isolated in the Layo environment.

The highest counts were recorded in environments linked to aquacultural activity (ponds and the tank water drainage channels). There also appeared to be fixation opportunities for this bacteria on suspended matter at the water–sediment interface, on zooplankton and on the roots of the floating aquatic macrophytes that colonize the lagoonal banks. Further research should now be undertaken on the significance of this fixation phenomenon and its impact on the survival of *V. cholerae*, as the studied sites appear to (1) facilitate the survival of heavy densities and (2) constitute endemic cholera foci. These environments are therefore likely to be periodically infected by pathogen vibrios and the introduction of extensive aquacultural activity therefore calls for some degree of regular monitoring of the bacteriological quality of aquacultural waters and products.

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