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ECOLOGY OF HALOPHILIC VIBRIOS IN AN EUTROPHIC TROPICAL ESTUARY

by

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ABSTRACT

The distribution of V. parahaemolyticus and the related biotype V. alginolyticus in the urban area of the Ebrie lagoon (Abidjan, Côte d'Ivoire) was studied over a one year period. A total of 501 samples (water and water/sediment interface) were bacteriologically screened for the presence of these halophilic vibrios. Overall 16% of V. parahaemolyticus and 16% of V. alginolyticus were isolated from both surface and bottom waters. No major difference is observed between surface and bottom waters population size of halophilic vibrios. Seasonal occurrence of the species in the samples during the entire period of investigation does not show any major difference between the dry and the rainy (together with the flood) seasons. Isolation frequencies grouped into salinity ranges show that both species are recovered from waters with salinity ranging from 0 to 35% , although higher frequency occurs when salinity is over 25% . There was no correlation between V. parahaemolyticus and V. alginolyticus with E. coli and Enterococcus.

RESUME

La distribution de V. parahaemolyticus et le biotype apparenté V. alginolyticus a été étudiée dans la zone urbaine de la lagune Ebrié (Abidjan, Côte d'Ivoire) au cours d'un cycle annuel. Au total 501 échantillons (eau de surface et de l'interface eau/sédiment) ont été bactériologiquement analysés pour la présence de ces vivrions halophiles. En tout 16% de V. parahaemolyticus et 16% de V. alginolyticus ont été isolés des eaux de surface et du fond. Aucune différence majeure n'existe entre les populations des vibrions dans les eaux de surface et du fond. L'occurrence saisonnière de ces espèces dans les échantillons ne montre aucune différence majeure entre les saisons de pluies et de crue et la saison sèche. Les fréquences d'isolement groupées en classes de salinité montrent que les 2 espèces sont présentes dans les eaux dont la salinité varie entre 0 et 35% , bien que les plus hautes fréquences d'isolement s'obtiennent quand la salinité est autour de 25% . Aucune corrélation significative entre V. parahaemolyticus et V. alginolyticus avec E. coli et Enterococcus n'a été observée.

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INTRODUCTION

Since its first isolation during a food-poisoning outbreak in Japan (Horie et al. 1964, Sakazaki et al. 1963), Vibrio parahaemolyticus has been shown to be widely distributed (Davis and Sizemore, 1982). It has commonly been found in estuarine, coastal and brackish waters (Joseph et al. 1983). Sediment, suspended particulates, plankton, fish, shellfish and infrequently freshwater samples have been shown to harbor the pathogenic agent (El-Sahn et al. 1982; Joseph et al. 1983; Sarkar et al. 1985; Shiaris et al. 1987).

Isolation from the environment in temperate regions has been shown to be related to water temperature (Kaneko and Colwell, 1978); whereas, in tropical areas, the frequency of V. parahaemolyticus isolation appears to depend on season and water salinity (Bonang et al. 1974; Joseph, 1974; Nair et al. 1980).

Attemps to study the correlation of V. parahaemolyticus and its occurrence in the environment with fecal pollution indicators have been made. Several studies (Baross and Liston,1970; Horie et al. 1967; Watkins and Cabelli, 1985) showed greater concentrations of V. parahaemolyticus in polluted waters than in non polluted waters. On the other hand, Kaneko and Colwell (1973) reported no significant correlation with counts of V. parahaemolyticus with fecal pollution indicators. Coliform counts usually correlated well only with the occurrence of allochtonous bacterial pathogens and not with autochtonous, potentially pathogenic bacteria such as V. parahaemolyticus (Colwell and Karper, 1977).

Because of the similitude in their auto-ecological features, V. parahaemolyticus and Vibrio alginolyticus are more often isolated in the same samples (Joseph et al. 1983). Although its pathogenicity has been questioned (Sakazaki et al. 1968), Vibrio alginolyticus has sometimes been implicated in superficial infections of organs, which in most instances have been exposed to seawater (English and Lindberg, 1977; Hansen et al. 1979; Rubin and Tilton, 1975). Furthermore, some ecological studies have reported isolation of V. alginolyticus in water, molluscs and oysters (Kampelmacher et al. 1972).

Some etiological studies on acute diarrheal diseases in Abidjan (Côte d'Ivoire) have reiterated that gastroenteritis caused by V. parahaemolyticus ranks first to diarrhea incidence since 1985. Futhermore, epidemiological studies have shown the relatively high incidence of carriers and implicated them in the spread of disease within the Ebrie lagoon riverside community (Dosso, 1984; Dosso, 1988). The Ebrie lagoon, characterized by high salinity variations, seems to constitute a "reservoir" of pathogenic bacteria.

The aim of this work is to study the distribution of V. alginolyticus and V. parahaemolyticus in the Ebrie lagoon urban area.

MATERIALS AND METHOD

1. Study area and sample collection

Five stations (Fig. 2), located in Abidjan (Côte d'Ivoire) area (5 N, 4 W) were bimonthly sampled for a one year period (from July 1987 to July 1988). The stations were situated at I (ST 1), 5 (ST. 2), 8 (ST. 3) 8.5 (ST.4) and 21 (ST. 5) km from the Vridi canal (Fig. 1) through which ocean waters penetrate the lagoon. Stations 1, 2, 3, and 4 are urban and receive tremendous amounts of domestic and industrial wastes. Station 5, relatively rural, is also under oceanic influence.

Surface and bottom (water/sediment interface) waters samples were collected using a Niskin bottle and aseptically transported to the laboratory in refrigerated containers and processed within two hours of collection.

2. Physical and chemical parameters

Water temperature and salinity were recorded for every sample collected using a STC (YSI) probe. Nutrients ($N\text{-}NO_2$, $N\text{-}NO_3$, $N\text{-}NH_4$, $P\text{-}PO_4$) concentrations of each sample were determined in laboratory with an auto-analyser Technicon AA2 as previously described (Strickland and Parsons, 1968).

3. Bacteriological method

Escherichia coli and Enterococci (Streptococcus D) counts were respectively determined on lactose desoxycholate medium (bioMerieux) and D coccosel (bioMerieux) by the plating methods previously described (Kouassi et al. 1990).

Nine hundreds ml of water sample were inoculated into alkaline peptone-water with 1% and 3% NaCl. Inoculated tubes were incubated at 30° C for 6 hours. Tubes manifesting growth were streaked onto thiosulfate-citrate-bile sodium (TCBS) agar plates. Streaked plates were incubated at 30° C for 18-24 hours. Appearance of green and yellow colonies on TCBS were considered as indicative of presumptive V. parahaemolyticus and V. alginolyticus respectively. Presumptive V. parahaemolyticus and V. alginolyticus were submitted to traditional tests (i.e 0/129 susceptibility, Gram reaction, growth to NaCl, motility and oxidase tests). In addition, isolates identified as V. parahaemolyticus and V. alginolyticus were characterized by using API 20 B system. Strains yielding the reactions in Table 1 were recorded as V. parahaemolyticus and V. alginolyticus.

RESULTS

Mean surface and bottom waters temperature is of the order of 28° C. No important thermal variation occurs in the studied area. Salinity is largely dependent on hydroclimatic conditions of the ecosystem. Thus, surface water salinity ranging from 10 to 30 % during the dry season rapidly decreases during rainy season and flood period to reach 0 to 2 % after which it is progressively increasing. Bottom waters temporal variations are largely influenced by the depth of the sampling stations. Similar temporal variations to surface waters, with relatively lower seasonal fluctuations occurred in sampling stations (St. 2, 4, 5) with a water depth less than five meters. On the contrary, deeper stations (St. 1, 3) with a water depth more than five meters, are characterized by a great hydrochemical stability. This is attribued to a permanent stratification, separating a salinity variable epilimnium (influenced by hydroclimatic events) and a permanently salty hypolimnium of oceanic origin.

Higher $P\text{-}PO_4$, and $N\text{-}NH_4$ concentrations are found in the waters during the rainy and flood seasons, while minimal concentrations are observed during the dry season (Fig. 3). Globally, in this environment, the concentrations of nutrients are very high (table 2).

In this naturally-occurring eutrophic milieu, sewages of the city of Abidjan lead to a hyper-eutrophication of waters and to an important increase of fecal contamination, mainly in surface waters. In relation to respectively E. coli and Enterococcus, pollution is on average 500 and 70 times higher than that observed for rural estuarine area of reference (St. 5). This very important human induced pollution (ratio E. coli/Enterococcus > 40) makes the Ebrie lagoon inapropriate for bathing. Temporal variations of E coli and Enterococcus are directly coupled with the water salinity variation, which itself is a function of the importance of fluvial and rainfall inputs. Thus maximal microbial densities of E. coli and Enterococcus are observed during the rainy season, while minimal densities are noticed during the dry season.

Altogether, 501 samples were analyzed for V. parahaemolyticus and for V. alginolyticus. Overall 16.37% of V. parahaemolyticus and 16.37% of V. alginolyticus were isolated from both surface and bottom waters of the Ebrie lagoon urban area. No major difference is observed between surface and bottom waters population size of halophilic Vibrios. Seasonal occurrence of the species in waters during the entire period of investigation are presented in Table 3. Our data do not show any major difference between the dry and rainy (together with flood) seasons.

Isolation frequencies of V. parahaemolyticus and V. alginolyticus grouped into salinity

ranges are presented in Table 4. Both Vibrio species are recovered from waters with salinity ranging from 0 to 35 % . However, higher frequency occurs for V. parahaemolyticus when salinity is over 25 % . The same salinity influence in the distribution of V. alginolyticus is also found. Important isolation frequencies are noticed in waters with salinity ranging between 0 and 10 % (12.5 and 15% for V. parahaemolyticus and V. alginolyticus respectively).

The primarily bacteriological indicators used to assess the safety of coastal bathing waters are fecal coliforms and/or Enterococcus counts of waters. According to WHO/UNEP, for a water to be considered as bacteriologically safe, it should not present more than 1 000 E. coli and/or fecal Enterococci per 100 ml of water in 90% of analyzed samples. Isolation frequencies of V. parahaemolyticus and V. alginolyticus grouped into fecal coliform ranges are shown in Table 5. Overall, one might expect greater V. parahaemolyticus and V. alginolyticus recoveries when fecal coliform counts exceeded 1 000 unity forming colonie (UFC) per 100 ml. Over 20% of the samples in our study with lower counts (<10 UFC/100 ml) contained both V. parahaemolyticus and V. alginolyticus. Similar results are obtained when considering Enterococcus as fecal contamination indicator. About 28% of the samples with no detectable Enterococcus counts present 25% and 13.88% as isolation frequencies respectively for V. parahaemolyticus and V. alginolyticus (Table 6).

DISCUSSION

The present investigation shows that V. parahaemolyticus and V. alginolyticus can be isolated with relative ease from the Ebrie lagoon environment. V. parahaemolyticus is present in waters with the same occurrence during the dry and rainy and flood seasons. However, the opposite was found in Togo (West Africa) (Bonang, 1974) and in Vietman (tropical area) (Neumann et al. 1972) where the seasonal cycle of V. parahaemolyticus is correlated with rainy and dry seasons.

V. alginolyticus seems to be present in larger numbers in seawater than V. parahaemolyticus (Golten and Scheffers, 1975) and in fact, a proportional relationship between V. parahaemolyticus and V. alginolyticus has been suggested. Joseph et al (1973) found that V. parahaemolyticus showed proportionally higher numbers than V. alginolyticus during the rainy seasons in the tropical waters and seafood in Java Bay, while V. alginolyticus was more predominant in the dry season. In the results reported here, abundance and seasonal trends of V. alginolyticus and V. parahaemolyticus do not provide a distinct difference.

Although V. parahaemolyticus is considered to be a mild halophile, requiring sodium chloride for its growth and survival, the organism was detected in water with salinity close to 0% . This finding concurs with that of Sayler et al. (Sayler et al. 1976) who recovered the pathogen from water samples with low salinity.

Recent studies on the Na^+ requirement of V. parahaemolyticus and V. cholerae indicated that, in contrast with other marine bacteria, the quantitative requirements for Na^+ growth vary with the substrate utilized as the carbon and energy source in the medium (Sarkar et al. 1985). This would imply that, under certain specific nutritional conditions, the Na^+ requirements of V. parahaemolyticus is not mandatory and that the halophile can well survive in conditions where the salt concentrations is equal to or even lower than physiological conditions. The Ebrie lagoon estuarine area, in contact with oceanic and continental environments, is naturally eutrophic. Mineral and organic nutrients inputs, mainly from continental waters and their trapping within the estuary are the main causes of this eutrophication. Furthermore, water masses presenting great density differences, create strong horizontal and vertical gradients allowing this biological (within the biomass) and geochemical (within the deposits) immobilization (Guiral et al. 1989). The estuarine zone presents a strong temporal variation, being submitted to low frequency variations (annual variability related to hydroclimatic cycle : flood, rainfall, dry season). In this situation, especially during the rainy and flood period (i.e. when water salinity is between 0 and 10 % , one might expect the nutrients to govern the survival of V. parahaemolyticus when salinity is low. According to Aiso et al. cited by Kaneko and Colwell (1973), the optimum NaCl concentration that may be influencing the growth of V. parahaemolyticus depends on water temperature. When the temperature is 20° C, the optimum NaCl concentration is between 0.5 and 1%; at 37° C, the optimum is 3%. V. parahaemolyticus growth stops when the NaCl concentration is below 0.05%. In the Ebrie lagoon, where the temperature is always high (annual mean is 28° C), this may explain the survival of the organism at lower salinity.

V. alginolyticus, relatively more halophilic than V. parahaemolyticus, was also recovered from the Ebrie lagoon waters with salinity ranging from 0 to 35% . This is somewhat surprising and to our knowledge unprecedented. Tidal transport and/or transfer of Vibrios from sediment to the water column may be responsible for the distribution of V. alginolyticus in the Ebrie lagoon.

Besides temperature and salinity, it has also been demonstrated that seasonal cycle of V.

parahaemolyticus may be influenced by adsorption and attachment of the organism to plankton and higher organisms and to particles (Kaneko and Colwell, 1973; 1975; 1978). In the stratified zones of the Ebrie lagoon, vertical distribution of phytoplankton and bacteria and that of zooplankton population, principally dominated by Acartia clausi (Saint-Jean and Pagano, 1984), are governed by the oxycline of the environment. Since, zooplankton is absent in deeper waters, it does not seem to be directly involved in the spatial distribution of V. parahaemolyticus, as it was observed with Acartia tonsa in the Cheapeake Bay (Kaneko and Colwell, 1973; 1975). However, suspended sediments as well as Copepod chitin may indirectly be implicated in the spatial occurrence of the pathogenic agent, since proteolytic, chitinolytic and lypolytic enzymes predispose Vibrios to decompose rapidly the cellular structures of their hosts (Bockemuhl and Triemer, 1974). Thus, chitin contributes to V. parahaemolyticus growth (Watkins and Cabelli, 1985). Therefore hypolimnium of confined and anoxic zones could be, insofar as a reservoir of shells, constituting a favourable milieu for the growth of this microorganism.

The correlation of V. parahaemolyticus and its occurrence in an environment with fecal coliforms have been reported. Significant correlation with counts of V. parahaemolyticus and fecal coliform and E. coli have been signaled (Baross and Liston, 1970). However, this result is discussed on the basis of other findings. In agreement with our data, Kaneko and Colwell (1973), Sutton (1974) and Jonas et al. (1978) found no significant correlation with counts of halophilic vibrios and fecal coliforms. Similar observation is made when Enterococcus is considered as a fecal contamination indicator. These results cast doubt on the utilization of these organisms as fecal contamination test.

Because of their brackish and turbid features, and also their high temperature and eutrophication level (principally caused by waste discharges into the lagoon without any treatment), the Ebrie lagoon estuarine waters can be considered as a "reservoir" of pathogenic microorganisms such as vibrios. Consequently, epidemiological hazards related to the presence of these microorganisms, should be confirmed by the determination of the hemolytic character of isolated vibrios strains. Isolation of vibrios is more often signaled on aquatic organisms. The relationship of these bacteria with aquatic organisms should therefore be specifically studied in the Ebrie lagoon.

TESTS	V. parahaemolyticus	V. alginolyticus
Biochemical Reactions		
Oxydase	+	+
Catalase	+	+
Nitrates	+	+
Glucose gas	-	+
Arginine	-	-
Lysine decarboxylase	+	+
Indole	+	+
Urea	-	-
H_2S	-	-
ONPG	-	-
VP	-	+
Arabinose	v	-
Gelatine	v	+
Inositol	-	-
Sorbitol	-	-
Melibiose	-	-
Rhamnose	-	-
Mannitol	+	
Amygdalin	v	-
Saccharose		
Morphological and Cultural Characters		
Growth on TCBS	G	Y
Luminescence	-	-
0/129 150 g	S	S
10 g	R	R
Growth at 30 C	+	+
37 C	+	+
42 C	+	+
Growth 0%	-	-
3%	+	+
6%	+	+
8%	+	+
10%	-	+

TABLE I. Cultural, morphological and biochemical characteristics of the halophilic vibrios. G = Green colonies; Y = yellow colonies; +, all species are positive for the biochemical reactions; -, 5-10 % of the species is positive for the biochemical tests.

		STATIONS				
		1	2	3	4	5
T C	S	27.82	27.83	28.07	27.85	28.02
	B	26.78	27.06	27.83	27.25	27.68
S °/oo	S	16.83	14.84	14.07	14.25	11.14
	B	27.01	22.59	24.49	19.55	13.12
$N\text{-}NH_4$	S	10.22	10.13	20.37	15.20	9.19
(mole/l)	B	11.79	19.93	190.02	19.73	9.88
$P\text{-}PO_4$	S	2.09	1.67	2.91	1.84	1.64
(mole/l)	B	2.06	1.96	19.66	2.09	2.60
$N\text{-}NO_2$	S	3.16	3.38	3.03	3.56	1.59
(mole/l)	B	1.30	2.66	1.50	3.50	2.41
$N\text{-}NO_3$	S	4.50	4.84	4.07	6.18	4.45
(mole/l)	B	3.68	5.40	2.60	4.94	7.03
E. coli	S	3.64	4.15	4.22	4.66	1.46
(log UFC)	B	1.87	3.03	1.51	4.06	1.61
Entero	S	1.61	2.49	2.37	3.05	0.55
(log UFC)	B	1.06	1.74	1.79	2.74	0.90

S = Surface B = Water/sediment interface; Entero= Enterococcus

TABLE II. Mean of the bio-physical-chemical characteristics of surface and bottom water samples in the Ebrie lagoon.

	rainy and flood seasons 120	dry season 381
	No positive (isolation frequency)	
V. parahaemolyticus	15 (13)	67 (18)
V. alginolyticus	18 (15%)	64 (17)

TABLE III. Isolation frequency of halophilic vibrios in surface and bottom water samples according to season.

Salinity ranges No Samples	0<X<10 120	10<x<20 129	20<x<25 149	x>25 106
	No positive (Isolation frequency)			
V. parahaemolyticus	15(13)	23(18)	19(13)	25(24)
V. alginolyticus	18(15)	16(12)	24(16)	24(23)

TABLE IV Isolation frequency of halophilic vibrios in surface and bottom water samples according to salinity.

UFC/100 ml No samples	0<x<100 124	100<x<2000 102	2000<x<10^4 103	x>10^4 172
	No positive (Isolation frequency)			
V. parahaemolyticus	22(18)	18(18)	19(19)	23(13)
V. alginolyticus	23(19)	14(14)	18(18)	27(16)

TABLE V. Isolation frequency of halophilic vibrios in surface and bottom water samples according to E. coli counts. UFC (Unity forming colony).

UFC/100 ml No samples	x = 0 144	0<x<100 101	100<x<10^3 144	x>10^3 106
	No positive (Isolation frequency)			
V. parahaemolyticus	37(26)	18(18)	19(18)	23(13)
V. alginolyticus	23(19)	14(14)	18(17)	27(16)

TABLE VI. Isolation frequency of halophilic vibrios in surface and bottom water samples according to Enterococcus counts. UFC (Unity forming colony).

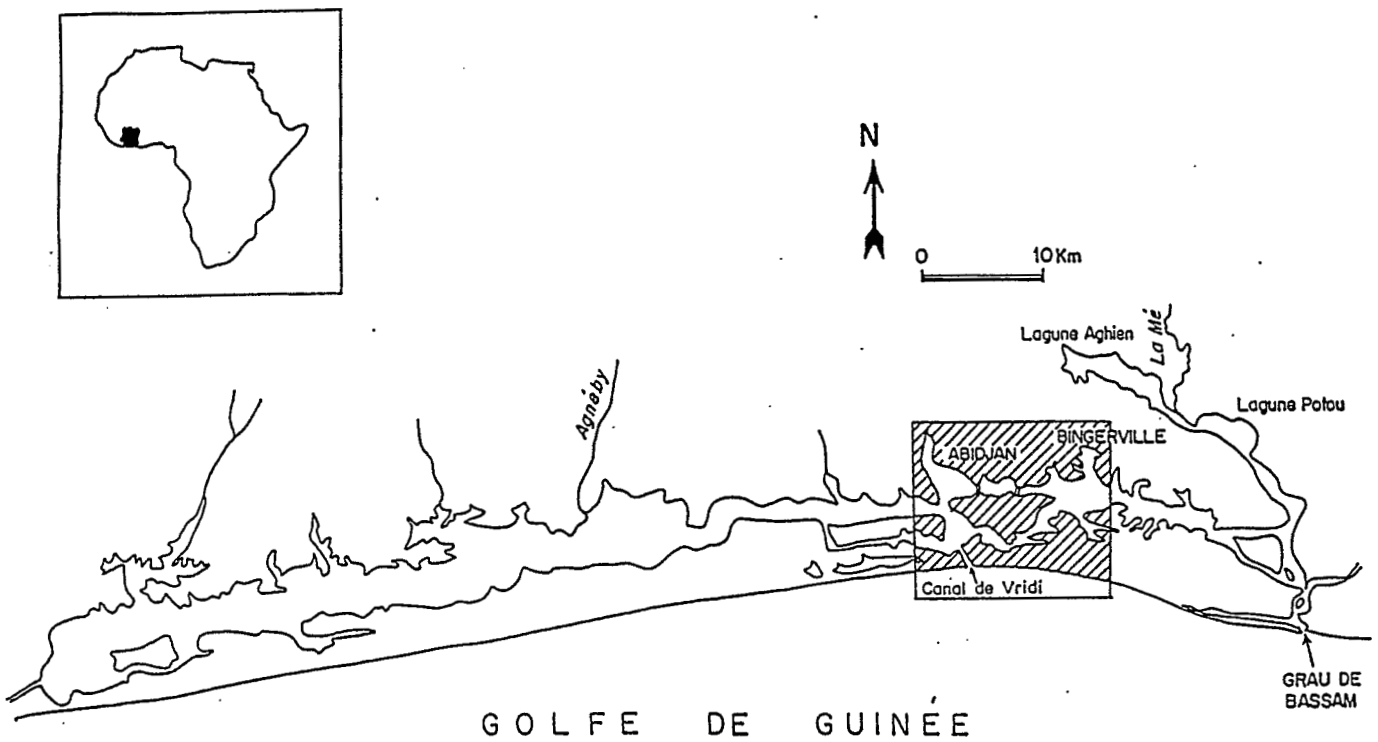

Figure 1. Ebrie lagoon ecosystem.

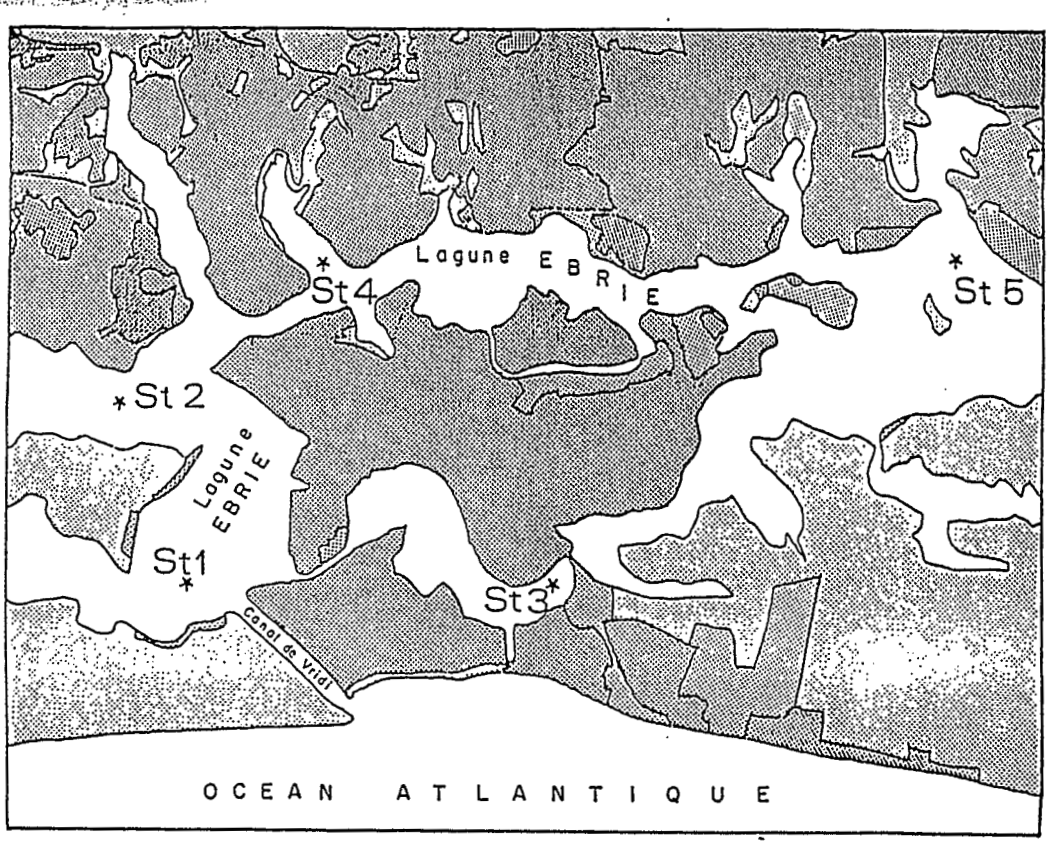

Figure 2. Ebrie lagoon estuarine area. The grey scales are proportional to the rate of soil occupation (area of high urbanization rate; area of spreaded accomodation; agricultural or forested area (low urbanization rate); Ebrie lagoon.

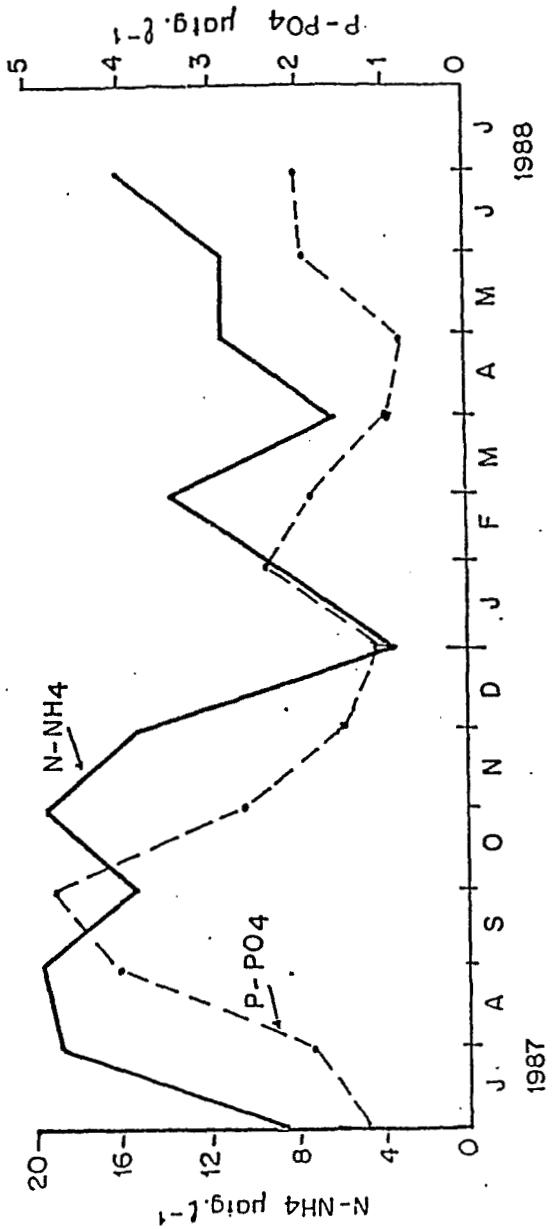

Figure 3. Seasonal variations of P-PO4 and N-NH4 concentrations in the Ebrie lagoon.

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