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Distribution of *Vibrio parahaemolyticus* & Allied Vibrios in Backwater & Mangrove Biotopes at Porto Novo

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Sediments proved to be the most stable ecological niche for survival of vibrios. Changes in populations of *Vibrio* spp in samples of water, plankton and sediment, obtained during March to May 1978, could be correlated to salinity. A qualitative survey of the incidence of *V. parahaemolyticus* in freshly caught finfish and shellfish revealed that a large percentage of animals sampled harboured the pathogen.

With the advent of the 'blue revolution', backwaters and mangroves have attained paramount importance as potential areas for culture of commercially esteemed species of finfish and shellfish. It is in this context that the present investigation on the occurrence of *V. parahaemolyticus*, the etiologic agent in foodborne gastroenteritis, in the mangrove and backwater environments was undertaken.

Materials and Methods

Three hydrobiologically distinct sites (sts I, II and III with average depths of 1.4, 1.8 and 1.1 m respectively) along a 10 km stretch of backwater and mangrove environs in the vicinity of Porto Novo (lat. 11°30'N; long. 79°46'E) were established and monthly collections carried out during March to May 1978. Samples of surface water, sediment and plankton were collected using Mac Cartney bottles, Petersen grab and horizontal tow plankton net respectively. Salinity was determined by Mohr's gravimetric method[1] and dissolved oxygen estimated by Winkler's titration[2]. *p*H and turbidity were measured using a portable Elico *p*H meter (Model L1-10) and Secchi disc respectively. All samples were brought to the laboratory for bacteriological analyses within 4 hr of collection. Bacterial counts were enumerated by the 3-tubes most probable number (MPN) technique[3]. Results (Tables 1 and 2) represent mean values for 2 samples of water, sediment and plankton taken at each station.

Appropriate decimal dilutions of water, plankton (after homogenising) and sediment samples were serially prepared using sterile 50% seawater. For enumeration of total aerobic heterotrophic bacteria (THB) 1 ml portions were inoculated in triplicate into screw cap tubes containing 10 ml sea water yeast extract (SWYE) broth and incubated at room temperature (27°C) for 48 hr. Subsequently a loopful of culture from all positive tubes showing growth was streaked onto thiosulfate citrate bile salts sucrose (TCBS) agar plates and incubated at 37°C for 18 hr.

For a qualitative survey of the occurrence of *V. parahaemolyticus* in seafoods, fish, prawns and crabs were caught by cast net operation. Oysters (*Crassostrea madrasensis*) were sampled both from the oyster bed at st III and from the roots of *Rhizophora* plants. Animals were first rinsed with sterile seawater to remove adhering mud and sand particles. For isolation of bacteria different regions of each specimen were swabbed with presterilised cotton swabs. The swabs were then put into test tubes containing 5 ml glucose salt teepol broth (GSTB) enrichment medium and incubated at 37°C for 6 hr. A loopful of culture from each tube was then streaked onto TCBS agar plates.

Bacterial colonies appearing on TCBS agar plates were categorised following the presumptive identification scheme of Kaneko and Colwell[3], i.e. colonies appearing on TCBS plates were regarded as *Vibrio*-like organisms (VLO) and 'typical' green colonies as *V. parahaemolyticus*-like organisms (VPLO). All VPLO isolates selected for further characterisation were restreaked to ensure purity and maintained at room temperature on SWYE agar slants. Identification of *V. parahaemolyticus* was based on the battery of diagnostic tests recommended by Hugh and Sakazaki[4].

Results

The physico-chemical parameters measured during the investigation are presented in Table 1. The lowest and highest salinities were recorded at st I (March) and st II (May) respectively. Due to the shallow nature of

Table 1—Physico-chemical Parameters Monitored from March to May 1978

Month	Salinity (‰)	Temp. (°C)		Dissolved oxygen (ml/l)	*p*H		Turbidity (m)
		Water	Sediment		Water	Sediment	
				St I			
March	12.6	29.5	28.5	6.3	8.1	7.6	0.27
April	20.4	34.5	34	5.9	7.95	7.75	0.22
May	28.3	30.5	29.5	6	7.85	7.55	0.4
				St II			
March	22.8	29	28.5	6.8	8.15	7.55	0.48
April	28.1	33	33	5	8	7.45	0.75
May	30.1	30	29.5	6.8	8	7.5	0.51
				St III			
March	20.9	30	29	7.7	8.1	7.6	0.39
April	25.7	32	32	4.9	7.8	7.45	0.41
May	28.6	30	29.5	6.2	7.95	7.15	0.37

the sampling sites, differences between the temperature of surface water and bottom sediments were never >1°C at any one collection.

Results of bacteriological analyses (Table 2) show that the THB count of samples from all 3 stations was fairly constant. In contrast populations of VLO, VPLO and *V. parahaemolyticus* exhibited distinct patterns of abundance in space and time.

Results of bacteriological analyses of freshly caught finfish and shellfish are presented in Tables 3 and 4. Occurrence of *V. parahaemolyticus* associated with animals was consistently high. Frequency of recovery of *V. parahaemolyticus* from the different regions swabbed showed marked variations in the positive specimens of each animal group.

Discussion

Seasonal and geographical distribution of *V. parahaemolyticus* in natural habitats seem to be primarily dictated by water temperature[3,5], organic nutrient content[6,7] and abundance of zooplankton[3]. The effects of salinity, temperature, *p*H and other unidentified factors influencing the attachment to zooplankton and growth and survival of *V. parahaemolyticus* may be responsible for its distribution being restricted to estuaries and neritic environments[8]. Results of the present investigation indicate that salinity is the principal parameter governing the distribution and abundance of *V parahaemolyticus* and allied organisms in the backwater and mangrove biotopes of Porto Novo. Water samples show higher counts of VLO, VPLO and *V. parahaemolyticus* at lower salinities while at salinities >28‰ only poor counts of these bacteria are observed. The association of *V. parahaemolyticus* and

Table 2—Counts of Total Aerobic Heterotrophic Bacteria (THB), *Vibrio*-like Organisms (VLO), *V. parahaemolyticus*-like Organisms (VPLO) and *V. parahaemolyticus* (VP) in Water (per 10 ml), Sediment (per g) and Plankton (per g) Samples from March to April 1978

Month	Sample	THB	VLO	VPLO	VP
		St I			
March	Water	7.8×10^7	5.9×10^5	3.1×10^3	3.1×10^2
	Sediment	8.0×10^7	9.7×10^4	8.4×10^3	4.3×10^3
	Plankton	3.5×10^7	2.4×10^7	8.4×10^4	5.9×10^4
April	Water	9.3×10^8	1.8×10^5	3.1×10^4	5.9×10^3
	Sediment	3.1×10^8	7.9×10^6	8.7×10^4	4.9×10^3
	Plankton	9.8×10^8	1.8×10^6	9.5×10^5	3.1×10^4
May	Water	8.4×10^8	6.8×10^3	3.1×10	0.4×10
	Sediment	5.8×10^7	8.0×10^5	8.2×10^3	5.2×10^2
	Plankton	2.3×10^7	7.0×10^4	4.1×10	2.3×10
		St II			
March	Water	8.4×10^7	3.5×10^4	5.9×10^3	3.1×10^3
	Sediment	7.8×10^8	5.9×10^6	7.8×10^3	2.3×10^3
	Plankton	8.4×10^7	9.5×10^5	3.3×10^4	3.1×10^3
April	Water	5.9×10^8	7.0×10^3	9.3×10	1.2×10
	Sediment	3.5×10^8	8.7×10^5	3.3×10^4	5.7×10^2
	Plankton	9.5×10^7	6.8×10^3	9.2×10^2	5.7×10
May	Water	8.4×10^7	8.2×10^2	2.5×10	0
	Sediment	5.8×10^7	5.9×10^2	4.3×10^2	2.3×10^2
	Plankton	1.2×10^7	6.8×10^4	8.4×10	2.3×10
		St III			
March	Water	5.9×10^6	8.4×10^3	7.9×10^2	3.1×10
	Sediment	3.1×10^8	8.4×10^6	5.4×10^4	9.7×10^2
	Plankton	3.4×10^8	9.7×10^5	3.3×10^5	8.4×10^3
April	Water	9.7×10^6	9.2×10^2	5.9×10	3.3×10
	Sediment	3.5×10^8	8.0×10^4	4.9×10^3	8.4×10^2
	Plankton	9.8×10^7	9.7×10^4	6.8×10^4	4.9×10^2
May	Water	7.8×10^6	4.1×10^2	8.4×10	0.7×10
	Sediment	2.0×10^8	5.9×10^3	9.7×10^2	2.3×10^2
	Plankton	7.8×10^8	9.5×10^5	6.8×10^2	6.7×10

Table 3—Occurrence of *V. parahaemolyticus* in Finfish and Shellfish Caught in the Study Area from March to May 1978

	Number of specimens examined	Number positive for *V. parahaemolyticus*	% positive
		March	
Fish	17	7	41.2
Prawns	13	7	53.8
Crabs	10	3	30
Oysters	16	7	43.8
		April	
Fish	19	7	36.8
Prawns	16	8	50
Crabs	12	5	41.7
Oysters	13	9	69.2
		May	
Fish	25	11	44
Prawns	14	6	42.9
Crabs	9	4	44.4
Oysters	15	10	66.7

Table 4—Distribution of *V. parahaemolyticus* in Various Regions of Finfish and Shellfish Caught in the Study Area from march to May 1978

Total No. of specimens positive for *V. parahaemolyticus*	Region of recovery in positive specimens		Per cent incidence of *V. parahaemolyticus* in different regions of positive specimens
	Fish		
25	External surface	8*	32
	Gills	12	48
	Faeces	19	76
	Prawns		
21	External surface	9	42.9
	Gills	10	47.6
	Haemolymph	17	81
	Crabs		
12	External surface	4	33.3
	Gills	8	66.7
	Haemolymph	6	50
	Oysters		
26	External surface	7	26.9
	Gills	18	69.2
	Mantle	11	42.3

*Frequency of occurrence of *V. parahaemolyticus* in the region specified

other chitinoclastic vibrios with zooplankton and their role in the recycling of nutrients via mineralization of copepod exoskeleton have been elaborately studied[3,8]. Shimidu *et al.*[9] and Kaneko and Colwell[3] have observed that in midsummer months the total viable count of plankton samples comprise almost exclusively of *Vibrio* spp. At all 3 stations, copepods form nearly 75% of the zooplankton population and this may account for the rather high counts of *V. parahaemolyticus* and related vibrios associated with plankton encountered during the March-April period (when favourable salinities prevailed). The observation that vibrios associated with plankton decreased gradually in numbers at high salinities concurs with earlier findings[8].

While populations of VLO, VPLO and *V. parahaemolyticus* in water and plankton exhibit distinct variations in abundance, the number of the above bacteria in sediments remains fairly constant. In temperate waters, it has been reported that *V. parahaemolyticus* survives the low temperatures of winter by overwintering in the sediments[3]. Hence it appears that sediments play a significant role in the annual cycle of *V. parahaemclyticus* and related organisms by serving a protective function. Whether other factors may also be involved in determining the seasonal abundance of *V. parahaemolyticus* and allied species needs further study.

Results presented in Tables 3 and 4 indicate that an alarmingly high percentage of the fish, crustaceans and oysters sampled harbour *V. parahaemolyticus*. Among these, oysters, possibly by virtue of their filter feeding habit, prove to be major reservoirs of the pathogen. From a public health view point the association of *V. parahaemolyticus* with commercially important species of finfish and shellfish is of interest since most outbreaks of gastroenteritis have been traced to consumption of contaminated seafoods. If the trends described in the present study hold good for other mangrove ecosystems as well, then any attempt to exploit the backwaters and mangroves as potential sites for aquaculture must be undertaken with utmost caution and regular bacteriological monitoring.

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