A Quantitative Study of *Vibrio parahaemolyticus* (Sakazaki et al) in *Etroplus suratensis* (Bloch) and *Metapenaeus dobsoni* (Miers) from Cochin Backwater

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Seasonal variation of *Vibrio parahaemolyticus* in fish *(Etroplus sauratensis)* and prawn *(Metapenaeus dobsoni)* was monitored from March 1982 to February 1983. Analyses of total viable count, vibrio-like organisms, *V. parahaemolyticus* like organisms and *V. parahaemolyticus* showed that they occur more in prawn than in fish. In a more polluted environment, the counts of *V. parahaemolyticus* associated with fish was found to be higher than in prawn.

The presence of *Vibrio parahaemolyticus,* a halophilic marine bacterium. which is the causative agent of a type of infectious food-poisoning has been reported earlier from estuarine and marine organisms (Horie *et al.,* 1964; Kampelmacher *et al.,* 1972; Kaneko & Colwell, 1978; Van den Broek *et al.,* 1979). Investigations on this bacterium in aquatic organisms from Indian waters pertain mostly to the east coast (Chatterjee & Neogy, 1972; Chandrabose & Chandrasekaran, 1976; Natarajan *et al.,* 1979; Nair *et al.,* 1980). Presence of this bacterium in water, sediment and plankton of Cochin backwater, a major backwater complex on the west coast of India, was reported earlier (Pradeep & Lakshmanaperumalsamy, 1984). In this paper we present the quantitative variation of *V. parahaemolyticus* in one of the commercially important fish, *Etroplus suratensis,* and prawn, *Metapenaeus dobsoni,* collected from Cochin backwater.

Materials and Methods

Samples were collected from three selected stations (Stations 1, 2 and 3) in Cochin backwater from March 82 to February 83 (Fig. 1). Station 1 is least polluted by sewage outfall. Station 2 is situated in the mouth of the estuary and frequent dredging operations are carried out here for shipping purposes. Station 3 is situated opposite to the Cochin Fisheries Harbour and a number of fish-processing industries drain their waste into this area.

Fish and prawn were caught by cast net and were transferred to sterile polyethylene bags, kept in an ice box and brought to the laboratory for analysis.

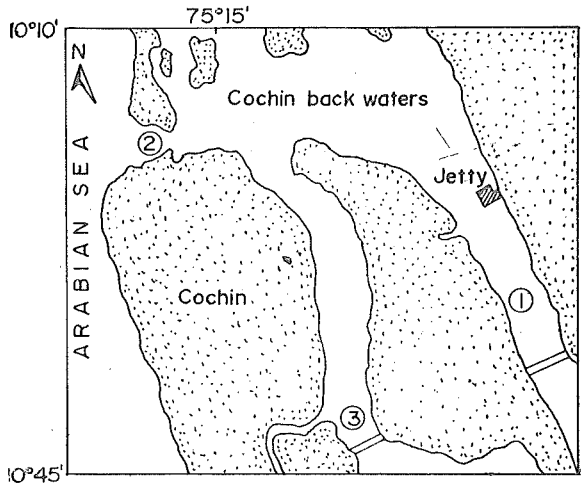

Fig. 1. Cochin backwater showing sampling stations

Samples were rinsed with sterile 50% sterile seawater to remove adhering sand and detritus and processed by FDA method (1978). From ten fish 50 g surface tissue, gill and gut were transferred aseptically into 450 ml of sterile 3% NaCl solution in an electric blender, homogenised at 8000 r.p.m. for 3 min and allowed to settle. From the supernatant solution appropriate decimal dilutions (10^{-1} to 10^{-6}) were prepared for bacteriological analyses with 3% NaCl solution. In prawn the cephalothorax was discarded and 50 g of exoskeletion, appendages,

flesh, gill and intestine were used and processed as employed in fish.

Total viable count (TVC) was enumerated by ZoBell's agar 2216 e (ZoBell, 1941), incubated at 28 ± 2°C for 7 days and colonies counted. Vibrio-like organisms (VLO), *V. parahaemolyticus* - like organisms (VPLO) and *V. parahaemolyticus* (VP) were quantified using the most probable number (MPN) technique with seawater-yeast extract (SWYE) broth (Kaneko & Colwell, 1973) as enrichment medium. A loopful of culture from 8 h incubated SWYE broth was streaked onto thiosulphate-citrate-bile salts-sucrose (TCBS) agar plates and these were incubated at 37°C for 18 h. Colonies developing on TCBS plates were considered as VLO. Typical bluish-green colonies appearing on TCBS, were considered as VPLO. Two such colonies from each plate were further purified on TCBS agar plates and inoculated into VP medium (Kaper *et al.*, 1980) for presumptive identification. Colonies which show an alkaline reaction in the slant and acid reaction in the butt were further identified by tests for identification of *V. parahaemolyticus* (Sakazaki & Balows, 1981).

Results and Discussion

Numerical analyses of various bacterial parameters are given in Figs. 2 and 3 the values are expressed in log per 100g of sample.

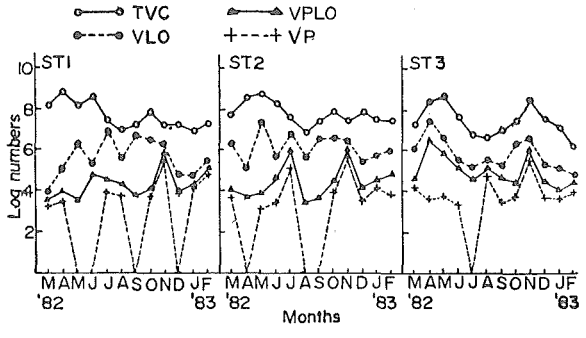

Fig. 2. Distribution of total viable aerobic heterotrophic bacteria; Vibrio like organisms, *Vibrio parahaemolyticus* like organisms and *Vibrio parahaemolyticus* in 100g of fish

The number of TVC varied between log 7.18 and log 9.53 in fish and log 7.42 and log 9.60 in prawn. TVC of fish and prawn did not show a common pattern of distribution in all the stations. However a decline of TVC associated with prawn was noticed at all stations during September and in fish during August. The peak values of TVC associated with prawn were recorded during June, May and November at stations 1, 2 and 3 respectively, while in fish it was in

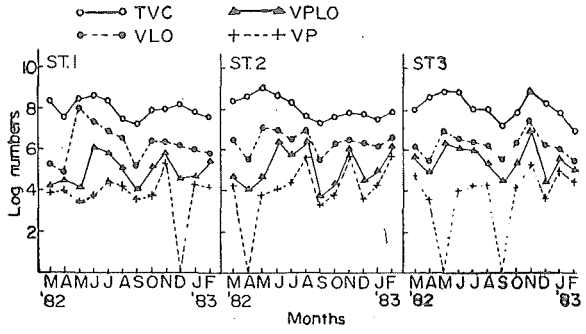

Fig. 3. Distribution of total viable aerobic heterotrophic bacteria, vibrio like organisms, *Vibrio parahaemolyticus* like organisms, and *Vibrio parahaemolyticus* in 100g of prawn

April at station 1 and in May at stations 2 and 3. TVC associated with fish showed a bimodal trend at station 3 with a smaller peak in November. Similarly the distribution of TVC in prawn at station 3 also showed more or less a bimodal trend though the peaks did not coincide with that of fish.

The maximum counts of VLO were found to be associated with prawn. VLO populations of fish varied between log 4.2 and log 8.1 and in prawn between log 5.0 and log 8.5. Similarities in distribution pattern of VLO among fish and prawn were not observed. In prawn the maximum was recorded during May at stations 1 and 2 and in November at station 3, whereas in fish it was seen in July at station 1, in May at station 2 and in April at station 3. The highest VLO density in fish was recorded at station 2 (log 8.1) and in prawn at station 1 (log 8.5).

Among the three stations the highest peak of VPLO was recorded for prawn and fish at station 3. The range of fluctuations of VPLO was between log 3.8 and log 7.0 in fish and log 3.8 and log 7.6 in prawn. In November the peak density of VPLO was recorded in fish at stations 1 and 2 and in prawn at station 3. In the rest of the samples, a peak in VPLO population, though not the highest was recorded in this month. At station 3 a marked increase in TVC was noticed in fish and prawn during this month.

Fluctuations of VPLO generally coincided with fluctuations of VLO in prawn. Such a similarity was not observed in fish.

VP was consistently present in higher numbers throughout the investigation in prawn at stations 1 and 2 and in fish at station 3. In fish caught from the least polluted areas (station 1), VP was below a detectable level in May, June, September and December. Similarly in fish at station 2 VP was at this level in April, August and September. Among the samples in which VP was consistently present in higher numbers the range was between log 3.5 and log 6.5. In all the samples maximum VP was recorded in November. In general the fluctuations of VP followed closely those of VPLO.

The mean density of various bacterial parameters such as TVC, VLO and VPLO was found to be higher in prawn than in fish (Table 1). The same was true for VP at stations 1 and 2, whereas at station 3 higher numbers of VP were found to be associated with fish than with prawn.

Table 1. *Mean density of various bacterial parameters in fish and prawn*

Organism	Fish			Prawn		
	St 1	St 2	St 3	St 1	St 2	St 3
TVC	8.27*	8.32	7.87	8.47	8.56	8.63
VLO	6.04	6.6	6.13	6.55	6.72	6.54
VPLO	4.75	4.87	5.31	5.16	5.49	5.8
VP	3.0	3.41	3 96	3 93	4 35	3.87

* values in log/100 g

Bacteria prefer more or less a constant environment for their normal activity and growth. Due to the apparently stable environment prevailing in the higher forms of life in the sea (fish, prawn etc), these organisms can act as shelter for various types of microorganisms in tiding over or escaping from adverse seasonal conditions. Such a shelter will be of importance to those microorganisms which lack built-in mechanism for surviving adverse conditions. This is especially true for non-spore forming bacteria like vibrios.

The International Commission for Microbiological Specifications for Foods has recommended maximum allowable limits of VP in marine products for export as 10^2/g (1974). To initiate gastroenteritis in man 10^6 cells of viable VP are sufficient (Sakazaki, 1972). The consistent presence of VP in fish and prawn makes it necessary that strict precautions have to be taken during transportation, storage and consumption of fish and prawn.

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