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INCIDENCE OF SOME PATHOGENIC ORGANISMS IN CULTURED SHRIMP (*PENAEUS MONODON*) COLLECTED FROM CHILAW

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Abstract

Samples of farm shrimp and pond water were collected from Chilaw. Total bacterial counts of coliforms and Escherichia coli *are reported for these samples. Pathogens* Vibrio parahaemolyticus, *and* Salmonella *along with qualitative microflora are also reported.*

The bacterial counts of water ranged from 5×10^2/g *to* 8.8×10^3/g *whereas that of shrimp ranged from* 2.0×10^4/g to 9.0×10^5/g. E. coli. *ranged from* 3 *to* 11/g *for prawns* 3/100ml. *to* 49/100*ml. for pond water. Two shrimp samples were positive for* V. parahaemolyticus *bacterial counts, coliforms or* E. coli *counts did not show any correlation with water salinity, which ranged from* 14-18 *ppt.*

Gram positive bacteria were predominant in shrimp and were represented by Micrococci (41.8%). Corynebacterium (19.3%) *and* Bacillus (14.2%). *Common gram-negatives were* Vibrios (19.2%) *and* Pseudomonas (5%).

Key words; Pathogenic Organisms, cultured Shrimp, *Penaeus monodon*

1. Introduction

Upto a few years ago nearly all prawns harvested in Sri Lanka were derived from natural stocks in coastal waters. However, in many east asian countries shrimp culture is already a well established industry. Recently research into shrimp culture techniques has become a very important in Sri Lanka and the production of cultured shrimp has increased considerably in the last few years. Along the west coast of Sri Lanka, low lying marshy areas are being converted into ponds. filled with brackish water and stocked with juvenile shrimp collected from hatcheries. The prawns are fed with a variety of foods, such as fish meal and rice bran, that are rich in protein. Ponds are harvested 3-4 months after stocking. Currently, most work on shrimp mariculture is focussed on methods, which include improved pond design, to develop intensive culture system, feed formulation, hatchery production or juveniles and brood stock development.

Little or no information is available on microflra of pond cultured prawns in Sri Lanka. Such information is essential since microbial activity is one of the major causes of spoilage as well as a potential cause of food-borne illness and food poisoning.

This paper reports on (a) qualitative microbial flora of shrimp, (b) presence or absence of some important pathogenic and indicator organisms in cultured shrimp, and (c) dominant bacteria prevailing in shrimp collected from a series of ponds over a period of 10 months.

2. Materials And Methods

Shrimp samples were collected from a shrimp farm located 40 miles north of Colombo in Thoduwawa. There were 34 ponds at the farm with total pond area of 10 hectares. Sampling for the study was done between January and September 1986. Shrimp samples were taken at approximately six week intervals. Five sub-samples, each of about 100g. were taken from different areas of the ponds and then pooled to constitute one sample. Sample collection was always done from ponds about to be harvested.

The samples were collected into sterile polythene bags. sealed and then cooled in ice. Water samples were also collected into sterile bottles from the same ponds. All water and shrimp samples were transported in ice to the laboratory. Samples thus collected were analysed within four hours of sampling.

Microbiological Analysis

10g. samples of sliced whole shrimp were accurately weighed aseptically into sterile stomacher bags and after addition of sterile peptone (90 ml) the contents were macerated for 30 (shrimp extract). Serial dilutions of shrimp extract were spread on duplicate Nutrient Agar (NA) plates for Total Bacterial Count (TBC) and incubated at $30 \pm 2^{0}C/48h$. After i ncubation the plates were counted and twenty random colonies were picked and purified by restreaking on NA plates till pure colonies were obtained. These were then transferred onto NA slants and stored at 4°C for further characterisation.

Biochemical characterisation to generic level was carried out according to procedures and diagnostic schemes reported by Hobbs and Hodgkiss (1960) Lee and Pfeifer (1975) and Cowan and Steel (1977).

Total Bacterial Counts (TBC) of water samples collected were carried out similarly by spreading aliquots (0.1 mL) from serial dilutions on duplicate NA plates and incubated at $30 \pm 2^{0}C/48h$.

The most probable number (MPN) of total coliforms and *Escherichia coli* for shrimp and water samples were established according to Bacteriological Analytical Manual (BAM) (1978).

Salmonella isolation and identification was based on those tests listed by Andrews *et al.*, (1978) and Collins and Lyne (1976). In the detection of *Salmonella.* Lactose Broth (LB) was used as pre-enrichment and Tetrathionate

Broth and Selenite Cystine Broth were used in enrichment. For *Salmonella* isolation Xylose Lysine Desoxycholate Agar (XLD) was used. Further biochemical tests were used for identification.

Vibrio parahaemlyticus detection was carried out according to BAM (1978) and Collins and Lyne (1976). Glucose Salt Teepol Broth was used for enrichment, Thiosulphate Citrate Bile salts sucrose agar for isolation and Triple Sugar Iron Agar for screening. For identifications, biochemical tests were used and the MPN method was used in enumeration.

3. Results

The total bacterial counts for prawn ranged from 0.2-9.1x10^5/g (Figure 1) with a mean value of 3.2x10^5/g. The total bacterial counts for pond water ranged from 0.4-8.8x10^3/g (Figure 2). A comparison of total bacterial counts for prawns and water shown in Figure 3.

The results of thc total bacterial counts, total coliforms *Escherichia/ coli* and *V. parahaemolyticus* in the prawn samples are presented in the Figure 4.

Organisms of public health significance such as total coliforms and *E. coli* were detected in very low numbers in prawns. Total coliforms were not detected in 25% of the samples. but when present, coliforms ranged from 7 to 75 coliforms!g. *E. coli* was not detected in 62.5% of the prawn samples, but when present, *E. coli* ranged from 4-11/g (Figure 4). The total coliforms for pond water ranged from 0.23-1.4/ML and *E. coli* ranged from '03-0.49/mL (Figure 4).

Vibrio parahaemolytious was detected only in two samples (Figure 5). *Salmonella* was not found. Studies of the bacterial flora showed that gram positive organisms predominated. Over 75% of the flora consisted of gram positive organisms such as *Micrococous spp.*, coryneform bacteria and *Bacillus spp.* (Figure 6). The most common gram negative organisms were *Vibrio spp.* While *Pseudomonas* and *Moraxella* were present in significant numbers and *Enterobacter Flavobacterium* and *Aeromonas* were also detected.

Conditions in the pond water were as follows: temperature ranged from 28-32°C, pH between 7.4 and 8.2 and salinity from 14-18 ppt.

4. Discussion

The mean bacterial counts of freshly caught pond-cultured prawns was 3.2x10^5 and were lower than the counts reported for marine prawns (3.9x10^6) from the Negombo Sea. The marine prawns were collected at landing sites 5-7 h after they were caught and immediately iced upon collection (Fonseka, 1985). The lower counts in the present study can be attributed mainly to the fact that immediate icing of cultured prawns after harvesting limits the likelihood of bacterial multiplication or subsequent contamination.

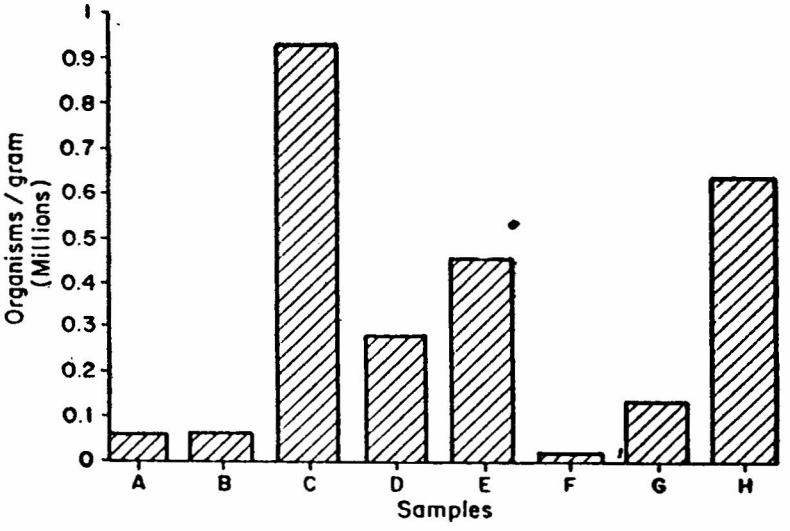

Figure 1 Microbiological data on cultured prawn -TBC

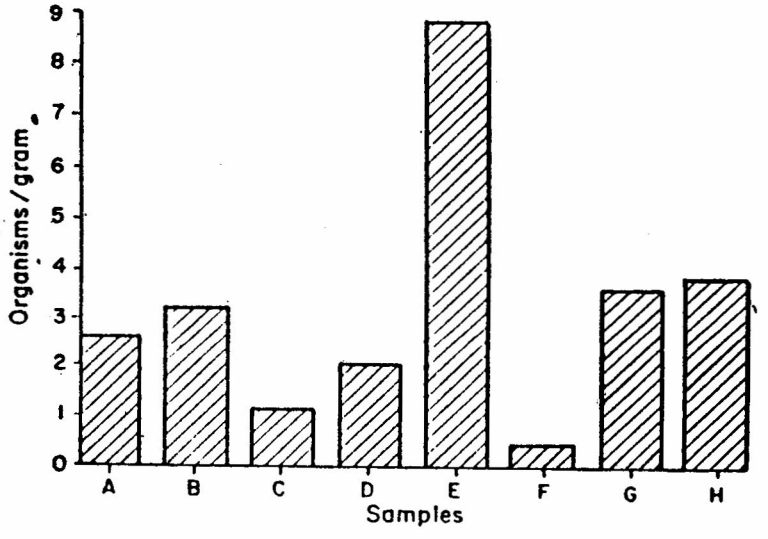

Figure 2 Microbiological data of pond water - TBC

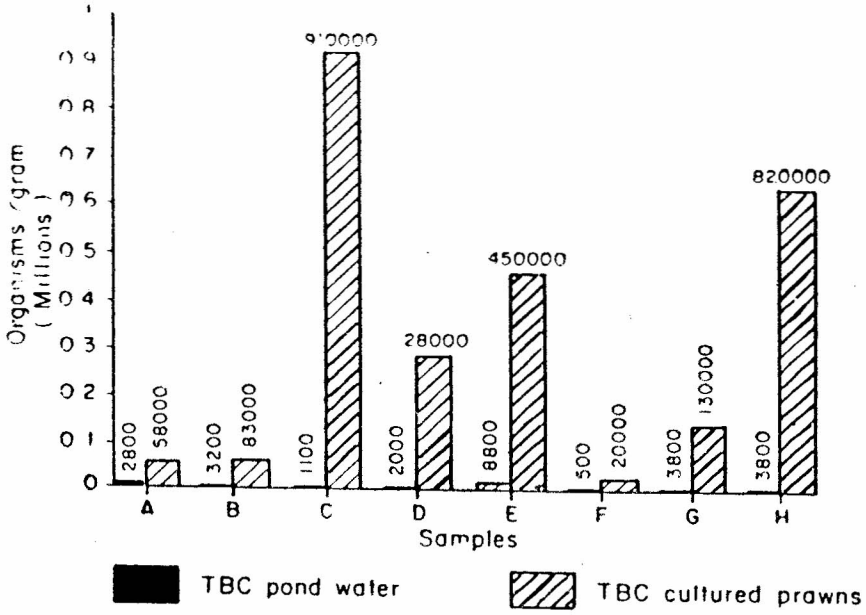

Figure 3 Comparison of TBC of prawns and water

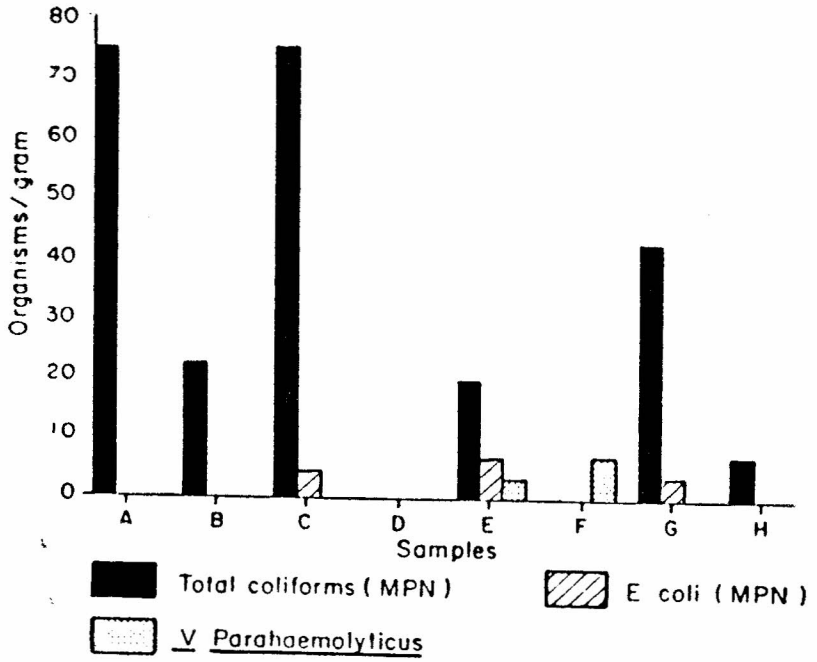

Figure 4 Microbiological data on cultured prawn

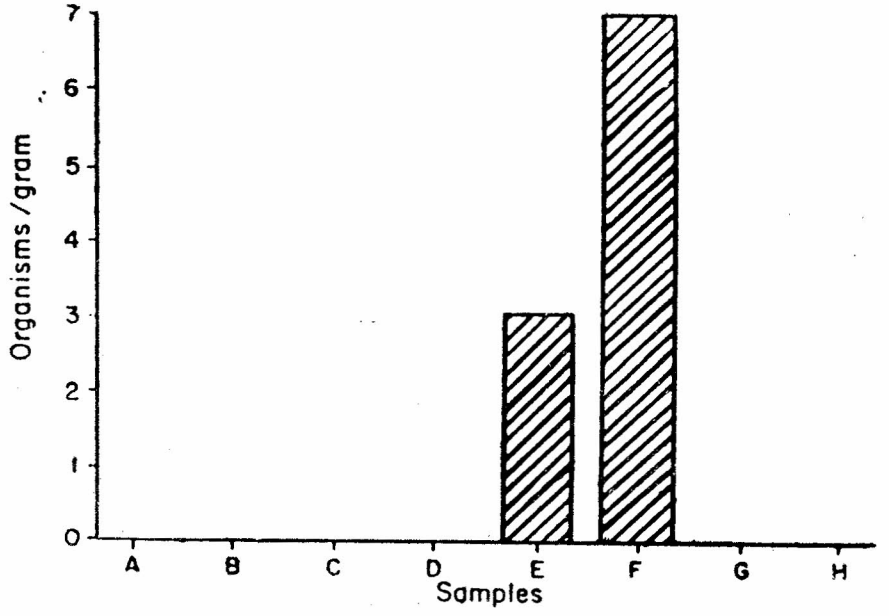

Figure 5 Vibrio parahaemolyticus in prawns

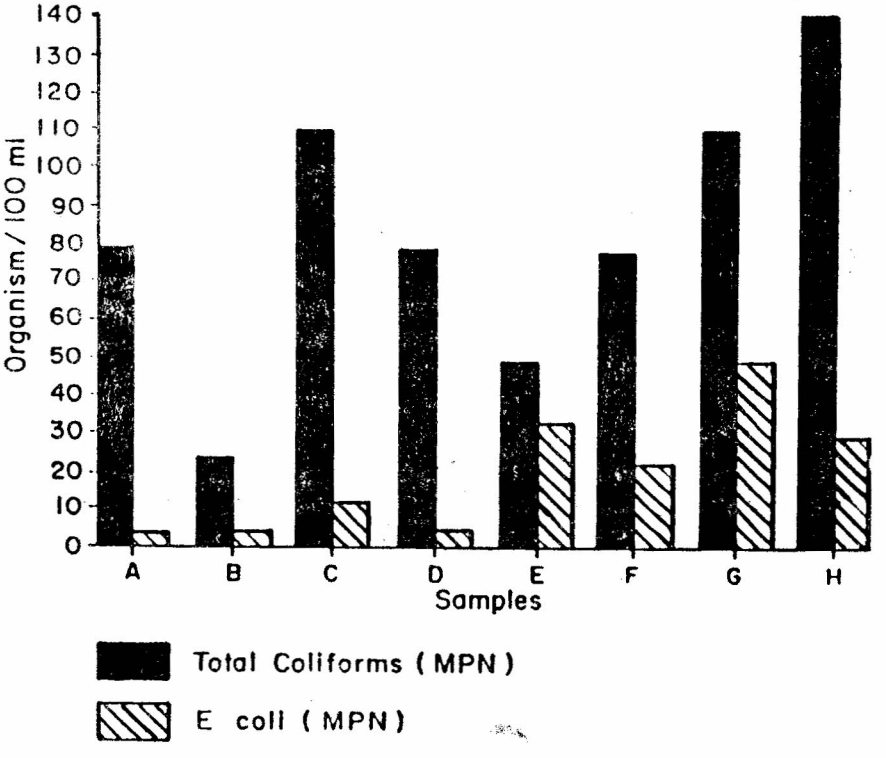

Figure 6 Microbiological data of pond water

There is evidence to support the fact that surface water carries less bacteria compared to bottom mud about 50 ft. below the surface (Williams et al., 1952). As prawns are bottom dwelling animals, the likelihood of their becoming contaminated with bacteria from the muddy substrate is always a possibility.

Deheading of prawns has lead to reduction of bacterial counts by 75% (Fieger, 1950) and the effect of thorough washing on the reduction of microbial load of prawns has been documented by Green (1949) and Williams et al., (1952). Good post harvest practices such as thorough washing with potable water, icing, deheading and good manufacturing practices would keep the total bacterial counts of processed pond reared prawns, to a reasonably low level.

The mean total bacterial counts of pond water was found to be below the mean bacterial counts for the prawns (Figure 3). The difference was found to be about 2 log values. This is probably an indication of the satisfactory quality of pond water.

Micrococous, coryneform bacteria and *Bacillus* species predominated in pond reared prawns in this study. The marine prawns from Negombo also carried large numbers of *Micrococous* but no coryneforms were detected there (Fonseka, 1985).

The high incidence of coryneform bacteria in prawns and shellfish from tropical (Sreenivasan, 1959) and temperate waters (Cann. 1977; Walker et al.. 1970) has been reported. The work carried out on pond reared prawns (*Penaeus stylirestris, Penaeus vannamei, Penaeus setiforus* and *Penaeus aztecus*) in Texas by Vanderzant et al., (1971) and Christopher et al., (1978) further indicated the ubiquitous and common presence of coryneform bacteria. It may be possible that coryneform bacteria are closely associated with crustacean shellfish.

Two samples collected during this study were found to carry *V. parahaemolyticus* which is a halophilic, thermophilic and pathogenic organism (Qadri and Zuberi, 1977). It is essentially a coastal and estuarine organism and has only rarely been isolated from fish caught in the open sea (Barrow, 1976). It has been isolated from marine sediments, sea water, shellfish and finish (Horie et al., 1967; Aoki et al., 1967). It is reported to be highly sensitive to processing temperatures and does not survive normal good processing (Asakawa, 1967).

Its growth is not only temperature dependent but is also influenced by the presence of organic nutrients such as those present in sewage (Liston, 1973). Thus, although its incidence in water in the coastal zone is usually seasonal, in estuarine waters rich in organic nutrient it is possible to detect it throughout

the year (Barrow, 1973). While ingestion of a small number of this organism apparently harmless, in as little as two hours they can multiply, resulting in a population capable of causing food poisoning (Liston, 1973), and is therefore considered a food poisoning agent. Food poisoning due to V. *parahaemolyticus* has been reported from many countries (Mitwani & Takeda, 1976; Aoki et al., 1967; Qadri & Zuberi, 1977).

The low counts of *V. parahaemolyticus* detected in two samples of prawns in this study, would not constitute a major threat to the quality of pond reared prawns as subsequent washing. processing, freezing, cold storage or cooking within a short period of harvesting which will ensure elimination of the organism and thereby prevent any chance of food poisoning.

Reilly et al., (1984) working on brackish water prawns reported that *V. parahaemolyticus* was present commonly as part of the natural flora, but not isolated after processing.

The presence of Vibrios in pond reared shrimps (Christopher et al., 1978; Vanderzant et al., 1971) and fish and shellfish from estuarine and marine waters is well documented (Liston, 1957; Simidu et al., 1969; Surrendran and Gopakumar, 1982; 1957; Simidu et al., 1969; Surrendran and Gopakumar 1982; Durairaj et al., 1983; Reilly, 1984). Vibrios were the next prominent group of gram negative bacteria detected in this study.

No clear relationship could be established between the variation of bacterial load of prawns and pond water characteristics such as salinity, temperature and pH. Some pond characteristics such as oxygen concentration and salinity can be controlled by management practices. The environmental conditions, feed type, rate of feeding, fertilisations of pond and aeration, probably would have contributed to slight variation in type and levels of microflora over the period of the study.

Coliform organisms, faecal coliforms and *E. coli* are primarily used to indicate some degree of potentially hazardous contamination based on the assumption that the natural habitat of the family (*Enterobacteriaceae*) to which these bacteria belong, is the faeces of man and other mammals, thereby indicating faecal contamination. *E. coli* is considered the most positive indicator of human faecal contamination. Presence of *E. coli* in a food implies the possibility of finding one or more of a wide diversity of enteric pathogens which may also have gained access to the food and hence introduce human health hazards. There is evidence to show that lagoon and inshore waters carry *E. coli* regularly. Cann (1977) reported that freshly caught penaeid shrimps do not carry *E. coli* but smaller inshore shrimps of *Parapeneopsis spp.* collected at sea were contaminated with *E. coli.*

In this study 50% of the prawn samples had coliforms at less than 20 (MPN/g while the rest of the samples carried coliforms below 75 (MPN)/g. *E. coli* was not detected in 82.5% of the samples. Even the *E. coli* positive

samples had low counts. Counts of coliforms and *E. coli* in pond water were also found to be less than those in prawns. There are no microbiological standards for water used in prawn culture in Sri Lanka, however, the coliforms standard for approved shellfish waters, described in the Manual of Operations of the USA National Shellfish Sanitation program. indicate a necessity for maintaining values so that: "The coliform median HPN of water does not exceed 7r/100ml and not more than 10% of the samples ordinarily exceeds an MPN of 230/100 ml". The coliform median MPN in this tudy is 79/100 ml and non eof the samples had the coliform MPN over 140/100ml which is well below 230/100 ml.

It should be pointed out here that though the coliform median MPN of pond watsr observed in this study was slightly above 70/100ml, the particular American standard applies to molluscan shellfish, which feed by pumping large quahtities of water using a filter mechanism. Thus this feeding process concentrates bacteria and plankton in the digestive tract of the animal. As such, exceeding the microbiological limits of 70/100 for coliforms by a small value in pond water samples should not be considered adverse in prawn culture.

It is evident from these findings that the cultured prawns and pond water do not carry very high total bacterial counts. Coliform counts and *E. coli* in these samples were not very high. while *V. parahaemolyticus* which is considered to be one of the important food pathogens in seafood, is not found in sufficiently large numbers to cause any problems. *Salmonella* was not detected in any of the samples. Under proper management and harvesting practices 'pond reared prawns can be used to produce a good quality wholesome product as good as the conventionally caught marine product.

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