CHAPTER 1

INTRODUCTION
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A. General

One of the challenging tasks of the world today is to find out ways and means to eradicate hunger and malnutrition. The rate at which human population increases makes one believe that even the expected multifold increase in the production of food from the land is unlikely to meet the requirements of mankind. Therefore, man has to depend to a great extent on marine resources.

India, with a coastline of nearly 5000 kilometers into which numerous large and perennial rivers discharge their silt-laden waters and with a number of small gulfs and bays all along the coast, offers almost an unlimited scope for the development of fisheries. In India, the marine fish landing in 1969 was 9,13,630 tons which rose to 14,15,219 tons in 1982 (Anon, 1983).
The growth and development of fishing and fish processing industry in India, during the post-independence era, have been remarkable and, this naturally, resulted in rapid and steady growth of the fishery export trade of the nation. In 1963, India exported 17,908 tons of fishery products valued at 58.6 million rupees. The quantity of exports steadily rose to 86,169 tons worth 3623.2 million rupees in 1983 (Anon, 1984). The striking feature of India's fishery export industry is that it is basically shrimp-oriented with more than 85% of the earnings coming from shrimp exports when this item constitutes only 10 to 12% of the total marine fish landings in the country.

To maintain the value of such exports it is essential that the industry is able to compete in quality, wholesomeness and price with similar products in the international market. India's fishery products must also meet the bacteriological and chemical standards stipulated by the foreign buyers. Thus, it becomes necessary for the fish processing industry in India to establish quality control procedures and take steps to improve plant sanitation and hygiene.
An organized system of pre-shipment inspection of fishery products meant for export is in operation in this country and some of the consignments have suffered rejection due to the presence of certain bacteria of public health significance such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Vibrio parahaemolyticus*. Among these organisms, *V. parahaemolyticus* has been considered as a normal flora of marine fish (Cann, 1977; Liston, 1980; Mossel, 1982; Hobbs, 1982). However, *V. parahaemolyticus* is a pathogenic organism and food poisoning resulting from consumption of fish contaminated with this organism has been recorded in majority of the countries around the world (Liston, 1973; Barrow, 1974; Barrow and Miller, 1976; Liston, 1980; Beuchat, 1982). *E. coli*, *Staph. aureus* and *Salmonella* are usually absent in fish caught from unpolluted waters, but contamination with these organisms takes place during the different stages of handling and processing depending upon the level of hygiene and sanitation in the processing establishments (Liston, 1965; Iyer et al., 1966; Cann, 1977; Hobbs, 1982). Even though the number of these organisms getting access
to the material at the time of contamination is, in most cases, far below the level necessary to cause illness, under favourable conditions, they rapidly multiply to a dangerous level in the processing factory, in the retail shop or in the kitchen. For this reason, these pathogens, even if present in very low numbers, are to be viewed seriously.

*E. coli* is a bacterium of faecal origin and its presence in fishery products indicates an unhygienic handling of the products during processing (Raj and Liston, 1963; Mossel, 1967; Hobbs and Gilbert, 1970; Cann, 1977; Mossel, 1982; Hobbs, 1983). Further, some *E. coli* strains are known to be enteropathogenic (ICMSF, 1978; Rao and Gupta, 1978). Another group of organism having sanitary significance is faecal streptococci.

*Staph. aureus*, while it is a food poisoning organism, is an indicator of workers' hygiene in processes involving human handling (Ridley and Slabyj, 1978; ICMSF, 1978; Liston, 1980; Connell and Shewan, 1980; Hobbs, 1982; Sumner *et al.*, 1982). Staphylococcal food poisoning symptoms are caused
by certain toxins called enterotoxins which are unaffected even on boiling (Bergdoll, 1970; Hobbs, 1974; Gilbert, 1974; ICMSF, 1978; Mossel, 1982). In the U.S.A., *Staph. aureus* is the second major pathogen involved in foodborne outbreaks (Centres for Disease Control, 1983). In Canada, *Staph. aureus* has been reported as the main food poisoning organism (Todd, 1978). On the basis of fundamental research carried out recently, it may be assumed that approximately 80% of *Staph. aureus* strains of human origin do possess the capacity of forming one or more toxins (Mossel, 1982). There are many publications from various countries on the incidence of *Staph. aureus* in fish and fishery products (Shewan, 1962; Appleman *et al*., 1964; Lovell and Barkate, 1969; Virgilio *et al*., 1970b; Phillips and Peeler, 1972; Cann, 1974; Hobbs, 1976; Baer *et al*., 1976; Cann, 1977; Liston, 1980; Beckers *et al*., 1981; Summer *et al*., 1982).

*Salmonella* is the most common food poisoning organism (86.6% of the total cases) in England and Wales. Out of the cases of food poisoning, 3% is due to consumption of fish and shellfish (Todd, 1978; Hepner, 1980; Turnbull and Gilbert, 1982). There are
many reports on the isolation of this organism from fish and shellfish (Bryan, 1974; Lewis, 1975; Shewan, 1977; Wyatt et al., 1979; Liston, 1980; Beckers et al., 1981; Zuberi and Qadri, 1981; Sumner et al., 1982). It is generally accepted that presence of any serotype of Salmonella at any level in a food product is pathogenic to man and animals (Jay, 1978; ICMSF, 1978; Mossel, 1982).

The presence of faecal indicator bacteria and other pathogens in fishery products is, therefore, to be considered seriously and the processor must adopt techniques to minimise the chances of their occurrence in fishery products as far as possible. In recent years, many of the importing countries have started demanding stricter bacteriological standards for fishery products exported from Asian countries (Hobbs, 1976; Anon, 1980; 1982; Sumner et al., 1982). Thus, the future of the fishery products export trade of India depends, to a great extent, on the capability of the industry to meet these rigorous standards. In order to achieve this target, the Technologists of the fishery industry must have a thorough knowledge of the source, distribution, growth pattern and behaviour of
the organisms of public health significance with particular reference to the handling and processing conditions in India. The available information does not seem to be sufficient to ensure consistent production of bacteriologically safe products. A study on these lines was, therefore, felt desirable.

B. Review of literature

1. Bacterial flora of fresh fish and shellfish

Reay and Shewan (1949) have reviewed almost all the work done during the period from 1920 to 1945 on the bacterial flora of fish and their involvement in spoilage. The early research workers have shown that muscles and body fluids of a normal healthy live fish are sterile and that bacteria are normally found on the skin, outer-slime, gills and alimentary tract of a living fish. Further, the composition of the microflora of different species of fish tested has mostly been reported to be dominated by Gram-negative bacteria usually identified as Achromobacter, Pseudomonas, Flavobacterium and less
frequently Vibrios. However, there are a few reports of isolation of Gram-positive bacteria also. Since in many studies, fishes were obtained from commercial sources, the presence of Micrococcus is not unexpected. Bacterial load on the slime, gills and alimentary tract of fish has been found to vary according to the seasonal changes in the environment in which the fish live (Shewan, 1961; Shrivastava and Floodgate, 1966; Karthiayani and Iyer, 1967; Hobbs and Hodgkiss, 1982). The method of fishing has also been found to influence the bacterial flora of fish (Shewan, 1949; 1977). The qualitative and quantitative variations in the bacterial flora of fish are found to be closely associated with the feeding habits of fish (Shewan, 1961; Shrivastava and Floodgate, 1966; Hobbs, 1982).

Another comprehensive review on the microflora of fish has been made by Shewan (1977). The review clearly indicates that, in recent years, considerable amount of progress has been made towards the understanding of fish bacteriology. Advances in the bacterial taxonomy as well as the techniques for the isolation and identification of bacteria have enabled the microbiologists to identify the spoilage bacteria
with greater reliability than before. In this review, based on the studies of many previous workers, Shewan has concluded that the bacteria in the fishes from temperate waters are psychrophilic in nature whereas those in the fishes from tropical areas are mesophilic. The same opinion has been expressed by Hobbs (1982) also. Thus, these findings appear to indicate that the normal microflora of the fish from warm waters are quite different from those occurring in fishes from cold waters. Achromobacter was found to predominate in some species of fishes caught from warmer waters (Liston and Colwell, 1963; Karthiayani and Iyer, 1967). On the other hand, marine fishes caught from the warmer coastal waters of Australia have the predominance of Gram-positive organisms, especially Micrococcus (Wood, 1952; 1953; Shewan, 1977). In India, while Jadhav and Magar (1970) recorded the predominance of Bacillus and Achromobacter in white pomfret and seer fish, Banik et al. (1976) reported Proteus, Aeromonas, Achromobacter and Micrococcus to be the dominant flora in white pomfret. Studies by Surendran and Iyer (1976) revealed the presence of vibrios on fresh mackerel to the extent of 37% of the total bacterial
load. Anand and Rudra Shetty (1977) isolated Achromobacter, Vibrio and Alkaligenes from six different species of Indian fish. These observations seem to support Shewan's hypothesis that bacterial population is affected more by the environmental factors than by the species of fish. This view has also been supported by Cann (1977) and Liston (1980).

The microflora of crustaceans, like that of fish seems to vary with the temperature of water in which they live (Cann, 1977; Liston, 1980). Based on the work carried out in the different parts of the world, these authors have confirmed that Gram-negative bacteria belonging to Achromobacter and Pseudomonas (and sometimes Flavobacterium) dominate the microflora of most crustaceans from cold waters while warm water species carry mostly Gram-positive bacteria belonging to Coryneforms or Micrococcus. Sometimes, however, crustaceans from temperate zones may also carry a high proportion of Coryneform bacteria (Hobbs et al., 1971; Lee and Pfeifer, 1975). In iced Pacific shrimps (Pandalus jordani), the bacterial flora in the order of predominance, has been shown to comprise Moraxella, Pseudomonas, Acinetobacter, Arthrobacter,
Micrococcus and Flavobacterium-cytophaga (Lee and Pfeifer, 1977). Lee and Pfeifer (1975) found the same groups of organisms in fresh crab meat. In his most recent review on bacterial flora of fish and shellfish, Liston (1980) has expressed the opinion that the bacterial count of shrimps and other bottom-dwelling creatures is difficult to evaluate because these creatures tend to be contaminated with sediment material.

2. Coliforms including Escherichia coli

The bacterial quality of a processed food material depends, to a great extent, on the sanitary conditions of the processing unit. The necessity for an indicator bacterium that will indicate the extent of sanitation had long been felt and, for this purpose, Scardinger (1892) suggested E. coli. His suggestion was based on the finding of Escherich (1885) that E. coli was invariably present in human stools. The occurrence of this organism in the alimentary tract of man and animals is, by now, well accepted. Because of its faecal association, E. coli was, at first, accepted as an indicator of faecal pollution
in water supplied (Kabler and Clark, 1960; Buttiaux and Mossel, 1961). Later, this organism was accepted as an index of sanitation in many kinds of foods like meat, poultry, egg, icecream, fruits, vegetables, fish and shellfish (Jay, 1978). The basic principle in testing E. coli was that the absence of this organism indicated the likelihood of absence of enteric pathogens. In their studies on the microbiology of bivalve shellfish intended for human consumption in the raw state, Van den Broek et al. (1979) used E. coli as a key index for pathogens and as an indicator of faecal contamination. The value of E. coli as an index of pathogenic bacteria has recently been questioned (Liston, 1980; Hobbs, 1982). However, the use of E. coli as an indicator of hygiene has gained universal acceptability: tests for the presence of E. coli are now carried out to determine whether the hygienic procedures and codes of practice have been strictly followed (Liston, 1980; Hobbs, 1983). Further, the performance of these tests forms an integral part in any quality assurance programme.

Since many quick and reliable methods are available now-a-days for the direct detection of
enteric pathogens like *Salmonella* and *Shigella* in foods, the answer to the question whether *E. coli* still plays an important role as an indicator of hygiene in foods seems pertinent. For the following reasons the answer should be in the affirmative (Mossel, 1982).

i) Generally, *Salmonella* is so heterogeneously distributed in foods and feeds that a negative outcome of its detection has only limited significance.

ii) Certain enteric pathogens like hepatitis virus can be detected in foods only by certain elaborate methods beyond the scope of many food laboratories.

iii) Detection of low numbers of pathogens in presence of larger numbers of natural bacterial flora in foods is, often, difficult.

iv) The presence or absence of *Salmonella* in a particular food material has significance only for the consignment under investigation. Repeated failure to detect the enteric pathogen is, often, essential as foods manufactured in the same way can be dangerously contaminated.

It is generally felt, therefore, that there
is still a need for carrying out tests for the
detection of E. coli in safeguarding man's food supply.

Only limited work has been done on the growth of
E. coli in different food substrates. There is no work
on the growth characteristics of this organism in
shrimps. According to Hoff and Presnell (1963) and
Wood (1964), coliforms can multiply in stored shell
fish—particularly at temperatures above 10°C.
Gunderson and Peterson (1964) obtained similar results
in their studies on chicken gravy but found that the
organism attained maximum growth at 37°C within 5 days
and thereafter the growth declined sharply. In
general, E. coli has been reported to grow at
temperatures as low as -2°C and as high as 50°C
(Jay, 1970). In a recent study, Brown and McMeekin
(1977) observed that, at 6°C, coliforms could grow in
processed oysters after a lag period of 2 days and
reached maximum numbers in 4 days.

Some amount of work has been done on the
significance of coliforms and E. coli in potable
water. Compared to other coliforms, E. coli does not
survive longer in water (Bonde, 1968). Based on the
available information, Taylor (1958) concluded that the presence of not only E. coli type I, but also of any coliform in potable water is highly significant. This has been further confirmed by Kabler and Clark (1960) who suggested that E. coli might be considered as an indicator of recent faecal pollution in water supplies. According to Surtaj et al. (1970) the correlation among total bacterial count, coliform count and enterococci count is highly significant because an increase or decrease in one count correspondingly increased or decreased the other. However, such a correlation was not evident in the studies of Bonde (1968) and Gore et al., 1979.

The incidence of E. coli in coastal and off-shore waters has been thoroughly investigated (Buttiaux, 1962; Shuval et al., 1968; Geldreich, 1974; Gore et al., 1979). From these studies the following conclusions can be drawn.  

1) Off-shore sea water normally does not contain E. coli whereas the coastal waters are often contaminated with the organism due to sewage disposal.  

2) Coliforms including E. coli are the least resistant to destruction in the sea.
iii) The occurrence of coliforms in water or mud signifies the presence of other enteric pathogens in the same environment.

iv) The principal source of *E. coli* is land drainage and sewage discharge.

In India, *E. coli* has been isolated from beach sea water throughout the year (Ravindran *et al.*, 1978; Gore *et al.*, 1979). It is, therefore, obvious that the bacterial quality of fish and shellfish collected from these areas needs a thorough checking.

Geldreich and Clarke (1966), based on the review of a number of studies and the studies of their own, came to the conclusion that *E. coli* does not usually form a part of the normal intestinal flora of fish. The incidence of *E. coli* in fish, therefore, appears to be mainly due to external contamination during handling and processing (Liston, 1965; Cann, 1977). Several studies have indicated that contamination of fish with *E. coli* can take place onboard trawlers if the hygienic conditions of the vessels are not satisfactory or if polluted near-shore waters are used for washing the catch or the boat deck. For
instance, Spencer and Georgala (1958) observed that the wash water and filleting boards used onboard contaminated the fish caught in commercial trawlers. Buttiaux (1962) observed that the fish-hold, fish boxes and other wooden surfaces in commercial trawlers were heavily contaminated with *E. coli* from the gut contents of fish and from heavily polluted near-shore waters which were sometimes used to wash the deck surfaces. The presence of *E. coli* onboard fishing vessels has also been reported by Liston (1965) and Carroll et al. (1968). Carroll et al. (1968, b) showed that shrimps kept on such deck surfaces also got contaminated with *E. coli*. Based on his studies, Thatcher (1973) suggested that polluted waters from harbour should not be used for washing fish, because such waters will increase the load of spoilage bacteria and may also contaminate the fish with pathogens. Similar conclusion has also been drawn by Zuberi and Qadri (1981) in their recent study on the bacteria of public health significance in the fishes caught from Karachi coastal waters.

Only a limited amount of information seems to be available on the sanitary conditions of fishing
trawlers of tropical countries. In India, Iyer and his colleagues (Iyer et al., 1966) have noted that the boat-deck and fish boxes contained *E. coli* ranging from 25 to 1700 per square inch. In a more recent study, Rao and Gupta (1978) have reported that 32% and 12% of the fishing vessels being operated from the Kakinada coast are contaminated with coliforms and *E. coli* respectively.

Investigations pertaining to the sanitary conditions of fish processing establishments are meagre. In fish processing factories, the main sources of *E. coli* contamination have been found to be the utensils, water and ice used for processing and the hands of the workers engaged in the processing operation (Carroll et al., 1968, b; Iyer et al., 1969). Further, the water and ice used in the shrimp primary process centres in India have been reported to be heavily contaminated with *E. coli* (Iyer et al., 1966). Iyer and Chaudhuri (1966) regarded ice as one of the major sources of *E. coli* in the shrimp processing industry in India. These workers observed that the gunny bag and tarpaulin used in some of the processing units to cover ice contained *E. coli* ranging from 20
to 600 per square inch and this organism contaminated the ice blocks which, in turn, contaminated the shrimps during icing. In a more recent work on the bacteriological studies of the blue crab-meat processing units along the Atlantic and Gulf coasts, Phillips and Peeler (1972) noted that the picking and packing tables are the main sources of $E. \text{coli}$ in the processed product.

From an early as 1904, many investigations have been carried out in the U.S.A. and Europe on the incidence of $E. \text{coli}$ in freshly caught fish. These investigations have been elaborately reviewed by Shewan (Shewan, 1970) who stated that, in freshly caught wet fish, coliforms were never present in excess of $10^2$ per gram and $E. \text{coli}$ occurred infrequently in small numbers only. Usually, samples collected from retail outlets present a different picture. Contamination is found to be more in such cases presumably due to human handling and unhygienic practices. Thus, the coliform count of iced fish retailed at Lusaka market in Zambia varied between 15 and $1.8 \times 10^5$ per gram (Watanabe and Ulstrup, 1973). However, these workers did not study the presence of $E. \text{coli}$ in the samples tested. In India,
Rao and Gupta (1978) found that the incidence of \textit{E. coli} was 12.5% in the samples collected at the landing place and 29.17% in the samples collected from the Kakinada fish market. In Karachi, while none of the samples collected from the landing places showed incidence of faecal coliforms, 29.9% of the samples from retail markets were found to be contaminated with this organism (Zuberi and Qadri, 1981). Further, Iyer \textit{et al.} (1984) have reported that 26% of fish samples collected from the retail fish markets in Bombay were contaminated with more than 20 \textit{E. coli} per gram. These results support the statement of Liston (1980) that fishes are contaminated due to mishandling after landing.

In bottom feeding fishes some workers found coliforms and \textit{E. coli} in comparatively higher numbers. For instances, in a study of 66 samples of commercial cray fish obtained from 22 sources representing the major cray fishing areas in Louisiana, Lovell and Barkate (1969) found coliforms in all the samples whereas \textit{E. coli} was detected in 92.6% of the cases. As cray fish is a bottom feeder and often burrows into mud, such a high incidence of coliforms
is not unexpected. But the presence of *E. coli* indicated pollution in these areas. Similarly, in a study of 335 units of fresh channel cat fish collected from 41 processors representing 9 states in Washington, Andrews *et al.* (1977) observed that, in these samples, total coliform and *E. coli* MPN values varied from less than 3 to $2.4 \times 10^6$ per gram and less than 3 to $2.4 \times 10^3$ per gram respectively.

With regard to the presence of *E. coli* in specific fish and shellfish products like fish sticks, fish cakes, fish fillets, minced fish and breaded shrimps, almost all the references available in the literature are from the countries of the west where such products are popular. Earlier literature on the subject has indicated that these products were often contaminated with coliforms and *E. coli*. For instance, the incidence of coliforms and faecal coliforms in the samples of commercially processed unskinned fillets tested by Spencer and Georgala (1958) were found to be 95% and 35% respectively. In the fish fillets examined by Ross and Thatcher (1958), *E. coli* ranged from nil to 250 per gram. Appleman *et al.* (1964) observed that 14% of the fish cakes analysed by them
contained *E. coli* type 1. However, recent reports, on the incidence of *E. coli* in these products show a better situation. In the U.S., for example, Baer *et al.* (1976) have reported that 95.1% of fish sticks and 90% of fish cakes contained less than 3 *E. coli* per gram. Further, only less than 1% of the 208 commercial blocks of minced fish from Massachusetts carried *E. coli* (Licciardello and Hill, 1978). In Netherlands, Van den Broek & Mol (1982) who studied 217 samples of fresh fish fillets have reported that the count in respect of *E. coli* was less than 10 per gram in 96% of the fillets tested.

The incidence of *E. coli* in frozen breaded shrimps is well documented. Gunderson *et al.* (1954), in their studies on frozen raw breaded shrimps, reported that the coliform counts ranged from $10^2$ to $10^4$ per gram. In a survey of 144 samples of commercially packed breaded shrimps representing 24 brands from the retail stores throughout the continental United States, Kachikian *et al.* (1959) noted that 98% of the samples contained coliforms upto 100 per gram. In an elaborate study by Surkiewicz *et al.* (1967) on the bacterial quality of
frozen breaded shrimps, 41.7% of the samples collected from the factories having low standard of hygiene and sanitation carried *E. coli* whereas the corresponding figure in respect of the samples obtained from the factories enforcing good hygienic practices was only 8.4%. In an examination of 164 samples of frozen raw breaded shrimps collected from the processing factories of southern United States, Carroll et al. (1968, a) isolated *E. coli* from 15% of the samples. However, the more recent reports on the microbial quality of breaded shrimps appear to indicate a definite improvement in the situation. In Canada, 99% of raw breaded shrimps and 100% of pre-cooked breaded shrimps had less than 3 *E. coli* per gram (Neufeld, 1971). In the U.S., Baer et al. (1976) have reported that 98.8% of the breaded raw shrimps tested by them contained *E. coli*. On the basis of these observations Liston (1980) concluded that, in general, the bacterial quality of breaded shrimps has shown much improvement over the years as processors have become more conscious of the need for a high standard of hygiene in their preparations. As far as it could be ascertained, there are no reports on the bacterial quality of frozen breaded shrimps from
tropical countries presumably due to the fact that this item is not popular in these countries.

Studies carried out in the U.K. and the U.S.A. have established that shrimps and other shellfishes collected from unpolluted waters rarely contain *E. coli* (Liston, 1965; Shewan, 1970; Ayres, 1975; Cann, 1977; Ridley and Slabyj, 1978). However, the picture, in tropical countries, has been reported to be more variable (Cann, 1977). This author has reported that freshly caught penaeid shrimps do not carry *E. coli* whereas the smaller inshore shrimps belonging to the genera *Parapeneaeopsis* and *Trachypenaeus* were consistently contaminated with *E. coli*. In Karachi, none of the samples of shrimps collected from trawlers contained *E. coli* (Zuberi and Qadri, 1981). However, contact with unclean surfaces onboard vessels (Iyer et al., 1966) and adoption of unhygienic practices (Zuberi and Qadri, 1981) are known to result in the incidence of *E. coli* in shrimps at the time of landing. This count is known to go up further if adequate care is not exercised to maintain proper hygiene and sanitation in the processing units (Liston, 1965; Iyer et al., 1966; Cann, 1977; Sumner et al., 1982).
Frozen shrimps and other shellfishes collected from processing units are found to contain \textit{E. coli} in varying intensities depending upon the sanitary precautions followed during handling and processing of the material. But it is certain that, in properly handled shrimps, the counts should be low. \textit{E. coli} is added to the material when they are handled and as this happens even at the point of capture, it is not surprising that this organism is detected in the processed product (Cann, 1977). It is further seen that the incidence of this organism has no correlation with the total bacterial count of the product and that it depends mainly on the pre-process conditions of the material (Silverman \textit{et al.}, 1961; Lekshmy and Pillai, 1964). In a study comprising 92 samples of frozen shrimps collected from the different processing units at Cochin (India), Lekshmy and Pillai (1964) observed that more than 50\% of the samples contained less than 100 coliforms per gram and \textit{E. coli} was absent in most of the samples. However, Mathen and Chaudhuri (1965) reported that 36.6\% of the commercially frozen lobster tails collected from the same area had \textit{E. coli} more than 20 per gram and they stressed the necessity for improving the sanitary conditions of
the units engaged in the production of lobster tails. Coliforms have also been isolated from pre-cooked Chilean shrimps: 35% of the samples from one factory and 60% from another carried this organism (Virgilio et al., 1970 a). In the U.K., the faecal coliform counts of frozen packaged shellfishes have been found to be comparatively low. For example, in the samples studied by Hobbs et al. (1971), 90% of frozen crab meat and 100% of frozen shrimps had less than 4 faecal coliforms per gram. Again, in a recent study on the microbiology of "Scampi", Hobbs (1983) noted that faecal coliform counts were less than 2 per gram in two factories whereas in the third factory, where there was a breakdown of hygiene, the count went up to 72 per gram in most of the samples tested. Thus, with normal hygienic precautions these organisms do not appear to pose any serious public health problem in cold countries. In a survey of the blue crab processing industry along the Atlantic and Gulf coasts, Phillips and Peeler (1972) observed that, in the plants maintaining good hygiene, the MPN counts of E. coli were less than 3 per gram in all cases. On the contrary, in the plants with poor hygiene, the corresponding figure ranged between 3 and 240 per
gram. In this study, the tables used for picking and packing were found to be the main source of contamination. These authors further observed that cross contamination between cooked products and raw crab meat might occur under commercial processing conditions. Similar observation has also been made by Webb et al. (1973) and Ray et al. (1976) in their studies on the effect of sanitation on the bacterial levels in blue crab meat processing plants. Review of literature indicates that the microbiology of crab meat has been rarely studied in tropical countries. In a bacteriological study of processed shrimps in Thailand, Cann (1974) found that the E. coli count in frozen and packed shrimps was invariably much higher than in the pre-processed shrimps. In a paper on the frozen prawn industry of Malaysia, Merican et al. (1977) reported high counts of E. coli in many batches of frozen Malaysian shrimps. Probably the most recent study on process hygiene in the prawn industry is from Sri Lanka. In this study, Sumner and his colleagues (1982) noted that most of the processed prawn samples had E. coli less than 6 per gram only even though the total bacterial load was very high. In Sri Lanka, although adequate steps
appear to have been taken to eliminate the 'transient' bacteria like E. coli, sufficient efforts have not been made for adequately chilling the material before processing and for avoiding contamination from workers (Sumner et al., 1982).

Molluscs are generally considered to be a potential health hazard as they are frequently harvested from estuarine and intertidal areas which may be exposed to pollution from human and animal sources. It is therefore a common practice to allow raw oysters and some other types of shell fishes to flush themselves in clean running water for a suitable period of time before marketing in order to ensure that these animals do not constitute a danger to health. In the United Kingdom, the suitability of molluscs for consumption is based upon the presence of E. coli within the prescribed limit. In a detailed study comprising 169 samples of oysters, mussels and clams obtained from producers and wholesalers, Ayres (1975) came to the conclusion that 95.1% of the molluscan shellfish entering English markets conformed to the accepted standard of 5 E. coli per ml tissue. Of course, such a good performance is the result of
the excellent purification practices followed in Britain. Further, in his review, Cann (1977) stated that 55% of the deep water molluscs collected from the various stages of commercial production were free from E. coli. In Southern Tasmania, the bacterial quality of oyster samples collected throughout the year was, in general, good as judged by the low level of faecal coliforms (Brown and McMeekin, 1977). In Netherlands, on the other hand, E. coli was detected in 9% of the samples of bivalve shellfishes and the generally accepted standard of 4 E. coli per gram exceeded in 5% of the samples tested (Van den Broek et al., 1979). These authors, therefore, concluded that more extensive monitoring of bivalve shellfish for microbiological safety is required.

Eventhough E. coli is a valuable indicator organism, it is generally sensitive to many of the processing conditions. Thus, in dehydrated, irradiated and frozen-stored foods, and in water subjected to common treatments, E. coli often disappears whereas various pathogens persist (Buttiaux and Mossel, 1961). Very little work appears to have been done on the viability of E. coli in fishery products during
freezing and subsequent cold storage. Further, there is no agreement between the findings of the different workers presumably due to the differences in substrates used and the experimental methods followed. It is, therefore, difficult to draw a general conclusion on the influence of sub-zero temperatures on *E. coli*. Raj and Liston (1961) reported only a 10 fold reduction in *E. coli* count in fish during a period of storage of one year at -17.8°C but the reduction in the number of this organism in Brain heart infusion broth, at the same temperature, was found to be comparatively much higher. Thus, they concluded that seafoods give considerable protection to the organism from the lethal effects of frozen storage. On the contrary, Lekshmy (1964) observed that, in fresh frozen shrimps, 90% of *E. coli* were destroyed during freezing at -40°C and the reduction during storage at -23°C at the end of 128 days was 99%. The experience of Digirolamo *et al.* (1970), on the viability of *E. coli* in frozen oysters, was different. These authors reported 95% reduction of the organism in 14 days of storage at -17.8°C. In order to have an acceptable interpretation of the significance of the incidence of *E. coli* in frozen stored fishery products, a thorough knowledge of its
viability to sub-zero temperatures is required and to accomplish this, further work on the subject is considered necessary.

Though a very sensitive organism, the ability of \textit{E. coli} to grow in competition with other organisms is well known. Studies have indicated that \textit{E. coli} is inhibitory not only to other coliforms but also to certain strains of \textit{Salmonella} and \textit{Staph. aureus} (Fredericq and Levine, 1947; Flippin and Mickelson, 1960; Dack and Lipputz, 1962; Graves and Frazier, 1963). Some \textit{E. coli} strains are, however, known to be stimulatory to \textit{Staph. aureus}. \textit{E. coli} is capable of exerting increased antagonism when the relative proportion of this organism increases in the substrate (Digiacinto and Frazier, 1966). Recently, the interactions among various microorganisms in foods have been discussed in depth by Mossel (1982) and according to him antagonistic relations between microorganisms occur in foods rather frequently. Even though it is well known for a long time that, in a mixed bacterial population, some bacteria exert antagonistic influences on the other species of bacteria, no systematic study has, so far, been
carried out on this technologically important aspect in fishery products.

For many years, several identifiable strains of *E. coli* have been known to cause infantile diarrhoea (Ewing et al., 1963). In recent years, it has become increasingly evident that *E. coli* strains can produce illness to a significant degree in adults also (WHO, 1974; Sakazaki et al., 1974; Sack, 1975; ICMSF, 1978). Strains of enteropathogenic *E. coli* have been isolated from traveller's diarrhoea (Rowe et al., 1970) and from the sick American soldiers in Vietnam (Dupont et al., 1971). The feeding of volunteers with serotypes 055:B5, 0111:B4 and 0127:B8 at the levels of $10^6$ to $10^8$ organisms per ml has been shown to produce food poisoning symptoms (Jay, 1970). It has also been convincingly demonstrated in volunteers that there exist both enterotoxin producing strains and invasive strains (Dupont et al., 1971). Two kinds of toxins exist: one is thermolabile and the other thermostable (Smith and Gyles, 1970; Saxena et al., 1984).

Enteropathogenic *E. coli* has been reported to be the etiological agent in many food poisoning
outbreaks involving a variety of foods such as cream pie, mashed potatoes, cream puffs, coffee substitute, stewed meat, roast mutton, pork, chicken and cheese (Jay, 1978; ICMSF, 1978). The widespread outbreak of gastroenteritis in the U.S.A. due to enteropathogenic \textit{E. coli} O124, on the consumption of imported cheese, is known to have affected at least 387 people (ICMSF, 1978). The probable source of infection was river water used for cleaning equipments. A food poisoning outbreak due to \textit{E. coli}, on the consumption of shell fishes, has been reported in the U.S. by Hughes \textit{et al.} (1977). In India, enteropathogenic \textit{E. coli} has been isolated from certain fishes like sardine and mackerel (Stephan \textit{et al.}, 1975). In a more recent study, Rao and Gupta (1978) have reported the isolation of two strains of \textit{E. coli} belonging to the enteropathogenic serotypes O55 and O111 from sciaenids and cat fish.

3. Faecal streptococci

Faecal streptococci are members of the genus \textit{streptococcus} which consists of Gram-positive cocci producing long or short chains and differing from most other Gram-positive cocci in being catalase-negative.
These organisms are found in the stools of man and many warm-blooded animals (Bartley and Slanetz, 1960; Kenner et al., 1960; Jay, 1978; Mossel, 1982). Ostrolenk et al. (1947) were apparently the first to show the feasibility of employing faecal streptococci as indicators of faecal pollution. In addition to their presence in faecal sources, these organisms also exist in plants, buds, insects, flowers and in soils (Mundt, 1961; 1963). Because of their isolation from many natural sources, the significance of faecal streptococci as indicators of faecal contamination is, to some extent, restricted (Mundt, 1963). However, because of their close association with the alimentary tract of man and animals, their presence is generally accepted as an indicator of faecal contamination in water (Kenner et al., 1961; Buttiaux and Mossel, 1961; Geldreich, 1974; 1976; Gore et al., 1979) and foods (Mossel, 1962; Shatlock, 1962; Niven, 1963; Mossel, 1967; Virgilio et al., 1970a; Licciardello and Hill, 1978). Many earlier workers have claimed the involvement of foods, heavily contaminated with faecal streptococci, in food borne disease but none of these workers could substantiate their point by experimental demonstrations (ICMSF, 1978). The ability of these
organisms to induce food poisoning has not, therefore, been recognised.

It is now well known that faecal streptococci do not form a part of the normal flora of fish (Geldreich & Clarke, 1966; Shewan, 1977). Incidence of these organisms in fishery products, therefore, appears to be mainly due to external contamination.

Only a limited amount of work has been done on the hygienic conditions of onboard fishing trawlers and its impact on the bacterial quality of the landed material. Contamination of fish with faecal streptococci is known to occur onboard fishing vessels from unhygienic surfaces. Faecal streptococci counts up to $2.0 \times 10^4$ per gram have been reported on wooden fish boxes (Buttiaux, 1962) and up to $3.0 \times 10^5$ per square inch on the surface of fish hold and fish boxes (Iyer et al., 1966). Liston (1965) and Iyer et al. (1966) reported that from the time the fishes were emptied on the boat-deck, till they were processed and packed, a series of contamination with faecal streptococci took place from various sources depending upon the level of hygiene and sanitation in the fishing vessel, primary process centres and processing
factories. Raj and Liston (1963) recorded a 10 fold increase in the faecal streptococci count during the initial cutting stage itself, thus pointing out substantial contamination at this stage. The water and ice used in primary process centres are known to be the major sources of contamination of the processed product with faecal streptococci (Iyer et al., 1966). These workers further reported a very heavy load of faecal streptococci on the utensils used in those primary process centres having no effective system of cleaning. Such contaminated utensils, in turn, were found to contaminate the processed product. The palms of workers handling seafoods are also known to harbour faecal streptococci (Iyer et al., 1969).

The available information on the incidence of faecal streptococci in frozen seafoods is limited. There are a few studies from India, but no work on the incidence of these organisms has been reported from other Asian countries. Compared to *E. coli*, the incidence of faecal streptococci has been found to be more in fishery products, evidently due to their wide distribution in nature. In Massachusetts, Kachikian et al. (1959) examined 144 samples of frozen breaded
shrimps and found enterococci to be present in all the samples, their numbers ranging from a few organisms to $1.35 \times 10^4$ per gram. Almost a similar observation has been recorded in respect of frozen shrimps in India, with the counts ranging from 43 to 18,080 cells per gram in 70% of the samples tested (Lekshmy and Pillai, 1964). Frozen lobster tails processed in India were also found to be contaminated with faecal streptococci. For instance, 71% of the frozen lobster tails processed in India carried faecal streptococci with the counts ranging from nil to $1.0 \times 10^5$ per gram (Mathen and Chaudhuri, 1965). In Mississippi, Carroll et al. (1968a) have recorded the presence of faecal streptococci in 84.7% of the samples of frozen raw breaded shrimps. About 94% of the fresh crayfish of Louisiana has been found to be contaminated with faecal streptococci (Lovell and Barkate, 1969). The pre-cooked frozen Chilean shrimps are also known to carry faecal streptococci: 69.1% and 45.3% of the samples from factory A and B respectively were contaminated with these organisms their counts ranging from 100 to 1000 per gram (Virgilio et al., 1970a). A more recent work by Vanderzant et al. (1973) on processed breaded shrimps has recorded faecal
streptococci ranging from 9 to 11,000 per gram. In Thailand, these organisms were invariably present in all the samples of factory-processed shrimps but in very low numbers only (Cann, 1974). The commercial bivalve shellfish entering British markets has been reported to be in a better position with regard to the presence of faecal streptococci: almost 70% of the samples had less than 100 faecal streptococci (Ayres, 1975). Besides, the recent report on commercially frozen minced fish blocks is, in general, satisfactory as 90% of the samples contain less than 1000 faecal streptococci per gram (Licciardello and Hill, 1978).

Compared to other bacteria of public health significance, faecal streptococci are more resistant to processing operations, freezing and storage at -17.8°C (Raj and Liston, 1961; 1963; Lekshmy, 1964; Thatcher and Clark, 1968). Since faecal streptococci are highly resistant to freezing and cold storage, Larkin et al. (1956), Zaborowski et al. (1958), Raj and Liston (1963), Lekshmy (1964), Thatcher and Clark (1968) and Licciardello and Hill (1978) regarded them as better indicators of faecal contamination in fish processing factories than E. coli. However, according
to Varga and Anderson (1968), faecal streptococci in fish originate from improperly sanitized working surfaces and their numbers should be used to reflect plant sanitation rather than faecal contamination.

The use of faecal streptococci as an indicator of the possible presence of pathogenic bacteria in fish has not been well accepted despite their worldwide use (Hobbs, 1982). There is indeed an increasing amount of evidence to show that there is no good correlation between enteric pathogens and faecal indicators (Hobbs, 1983). However, the value of these organisms as an indicator of hygiene in fish processing units is unquestionable (ICMSF, 1978; Liston, 1980; Hobbs, 1983).

4. *Staphylococcus aureus*

From the public health point of view, another important bacterium to be considered in fishery products is *Staph. aureus*. The genus *Staphylococcus* belongs to the family **Micrococccaceae** and is an asporogenous non-motile Gram-positive cocci occurring singly, in pairs or in the form of irregular clusters resembling bunches of grapes. Since 1930, it is known that contamination of food with *Staph. aureus* could cause gastroenteritis of sudden onset because
the organism growing in food secretes an exotoxin (Jay, 1978). This toxin is generally termed as enterotoxin as it affects the intestinal mucosa.

It is now well established that staphylococcal food poisoning is caused only by certain well defined strains of Staph. aureus and such strains are found to produce coagulase (Dack, 1962; Bryan, 1968; Minor and Marth, 1972; Hobbs, 1974; Jay, 1978). These strains are, therefore, termed as coagulase-positive staphylococci. However, not all coagulase-positive staphylococci are capable of causing food poisoning (Jay, 1978). Food-borne outbreaks due to coagulase-negative staphylococci have also been seldom reported (Omori et al., 1960; Breckinridge and Bergdoll, 1971). The role of Staph. aureus as a food poisoning organism has been reviewed by Munch-Peterson (1960), Bryan (1968) and Minor and Marth (1972). The inter-relationship between enterotoxin production and other physiological and cultural characteristics of Staph. aureus, such as production of coagulase, formation of a heat-stable deoxyribonuclease and fermentation of mannitol have been studied but no single property or combination of
properties is an absolutely reliable index of enterotoxigenicity (Bergdoll, 1970).

*Staph. aureus* can grow vigorously in 10% sodium chloride solution and can withstand drying at room temperature (Bryan, 1968). Though mesophilic in nature, *Staph. aureus* has been reported to grow at a temperature as low as 5°C (Munch-Peterson, 1960) and to grow and produce toxin even in vacuum-packed bacon (Thatcher et al., 1962). Studies by Lovell and Barkate (1969), on the other hand, point out that the organism cannot grow in cray fish substrate at 5°C. Again, Vanderzant et al. (1973) observed no significant increase in *Staph. aureus* count in breaded shrimps at 5.5°C; at 10°C, however, there was good growth. The growth rate of *Staph. aureus* in different foods has been reviewed by Minor and Marth (1972). According to the information detailed in this review, the number of staphylococci inoculated onto the surface of chicken increases 10,000 fold within 8 hours at ambient temperature and further increase in temperature, within the range of 35.5°C to 42.5°C, did not appreciably alter the growth rate. However, at 45°C the number of staphylococci increased 1000
fold within 8 hours, but at 47°C, they did not grow at all. Luxurient growth of the organism occurred in canned meat held at 22°C and 37°C for up to 60 days. The above review has also reported that the minimum temperature for the growth of staphylococci in chicken gravy was between 5°C and 10°C and the growth reached maximum stationary phase in 48 hours and 15 days at 20°C and 10°C respectively. So far, no studies on the growth pattern of Staph. aureus in pre-cooked shrimps appear to have been reported. In such a substrate, where the competing microorganisms are less, a study of the growth characteristics of Staph. aureus is particularly relevant.

Available information on the behaviour of Staph. aureus during freezing and subsequent storage is scanty. Raj and Liston (1961) observed that the organism showed a seven fold reduction in numbers in fish homogenate during the first 130 days of storage at -17.8°C followed by little change thereafter till the end of the study which continued up to 393 days. No other work seems to have been done on the behaviour of Staph. aureus at sub-zero temperatures and certainly, there is a need for a detailed study on this important aspect.
According to the current epidemiological information, the main reservoir of *Staph. aureus* is man: hands, face, nasal cavities, throat, eargum and post-nasal drips of man contain this organism in considerable numbers (Elek, 1959; Bryan, 1968; Hobbs, 1974; Gilbert, 1974; ICMSF, 1978; Mossel, 1982). About 30% of human population is known to be nasal carriers of this organism (Williams, 1963; Bryan, 1973; Gilbert, 1974). Skin of 30% of food handlers are known to inhabit *Staph. aureus* (Bryan, 1968). Jay (1970), Hobbs (1973), Gilbert and Wieneke (1973), Lee and Pfeifer (1975) and Hobbs (1982) maintained that food handlers are the main source of contamination of the processed product with *Staph. aureus*. Therefore, this organism while it is itself a food poisoning organism, is a useful indicator of hygiene in a process involving human handling (Shelton et al., 1962; Cann, 1977; ICMSF, 1978; Liston, 1980; Connell and Shewan, 1980; Hobbs, 1982). Shrimp peeling is a good example of such a situation (Sumner et al., 1982; Hobbs, 1982). According to Hobbs (1983) the presence of *Staph. aureus*, even in smaller numbers, is an excellent indicator of human hygiene.
The number of *Staph. aureus* in foods at the point of contamination may be very low but when handled unhygienically and exposed to ambient temperatures it may multiply rapidly as this organism can grow exponentially between 6.7°C and 45.5°C (Angelotti *et al.*, 1961; Michener and Elliot, 1964; Bryan, 1973) and produce toxin (Gilbert, 1974; ICMSF, 1978). The toxin, once formed, will not be materially affected at 100°C even though the organism will be killed at this temperature (Munch-Peterson, 1960; Hobbs, 1974; ICMSF, 1978; Mossel, 1982). Therefore, the normal cooking temperature, though sufficient to kill *Staph. aureus*, could leave the toxin unaffected. It is thus evident that, once sufficient quantity of toxin is formed in a food material before its consumption, food poisoning can follow even though the material is cooked. Foods most likely involved in staphylococcal food poisoning are cooked and processed foods having low numbers of competing microorganisms (Hodge, 1960; Hobbs, 1974; ICMSF, 1978; Mossel, 1982). Large numbers of *Staph. aureus* (usually more than one million organisms per gram of food material) must be present at one time to produce enough enterotoxin and to cause symptoms. Thus, in
the study of Hobbs (1960), the counts obtained in the incriminated foods were usually less than 5.0 x 10^5 per gram. Counts of *Staph. aureus* in 39 incidents of food poisoning varied between 7.5 x 10^5 and 9.0 x 10^9 per gram (Gilbert et al., 1972). Almost a similar observation has been reported by Gilbert (1974), Hobbs (1974) and Mossel (1982). Further, Bergdoll (1973) has concluded that less than one microgram of enterotoxin is sufficient to cause illness in a sensitive individual. Ingestion of food containing sufficient quantity of enterotoxin results in the onset of symptoms within 2 to 4 hours, although in certain cases it takes only 30 minutes (Bryan, 1968). Nausea, vomiting, abdominal pain, diarrhoea, absence of fever and sub-normal blood pressure are the usual symptoms and although there is high morbidity, the rate of mortality is low (Gilbert, 1974).

*Staphylococcal* food poisoning symptoms are caused by antigenically distinct polypeptides which function as emetic toxins known as enterotoxins. Five specific enterotoxins called enterotoxins A, B, C, D and E have been reported by Bergdoll (1970) and Gilbert (1974). Recently, Mossel (1982) has reported
the identification of eight enterotoxins designated as $A, B, C_1, C_2, D, E, F$ and $G$. The toxin most commonly implicated in food poisoning is enterotoxin $A$ while those strains which produce enterotoxin $B$ are only rarely found (Gilbert and Wieneke, 1973; ICMSF, 1978). According to Olsvick et al. (1981) enterotoxin $A$ is mostly associated with human food poisoning. On the basis of fundamental research work carried out recently, it may be assumed that approximately 80% of the $Staph. aureus$ strains of human origin do possess the capacity of forming one or more toxins (Mossel, 1982).

Until recently, the methods for the detection of staphylococcal enterotoxin involved intraperitoneal or intravenous injection of cats and kittens and feeding young rhesus monkeys. As these procedures are expensive and not entirely reliable, they have been largely replaced by serological techniques. Several methods for the laboratory production of enterotoxin have been comparatively evaluated by Simkovicova and Gilbert (1971). The cellophane sac-culture dialysis methods of Casman and Bennett (1963) and Donnelly et al. (1967) have been used
successfully in a number of laboratories in Europe and North America: the method of Casman and Bennett produces larger amounts of toxin but, that of Donnelly et al. is easy to perform (ICMSF, 1978). The cellophane-over-agar method (Hailender, 1965; Robbins et al., 1974) has also been used successfully. A number of methods which employ specific antibodies have been used in various ways for the serological detection and measurement of the enterotoxins. These include the Duchterlonly plate technique, the fluorescent antibody test, reversed passive hemagglutination and radio immuno assay (ICMSF, 1978).

Reports on food poisoning caused due to consumption of various fishery products, including staphylococcal food poisoning due to canned shrimps, canned smoked cod, sprats in oil, North African sardines in oil, Moroccan sardines in oil, kippers, fish sausage, fish pudding, boiled salmon, light salted smoked mackerel, weakly salted herring, frozen fish sticks and fish cakes have been reviewed by Shewan (1962). In many of these cases, the contamination during handling and processing coupled with favourable time-temperature conditions, in the
factory before processing has been shown to be the cause of the poisoning. Generally, subsequent faulty heat processing has been found to increase the chances of food poisoning. In most cases, where canned fish were incriminated in food poisoning, the tins were not bulged as *Staph. aureus* neither formed gasesous products nor did it produce any abnormalities in appearance, odour or taste of the product (Shewan, 1962). Of the 175 outbreaks of staphylococcal food poisoning in England and Wales, 17 were due to consumption of fish (Anon, 1963; 1964). Canned fishes, canned prawns and frozen shrimps were the main products incriminated in these food poisoning outbreaks. In his review on the food poisoning outbreaks for the period 1967 to 1969, Bryan (1973) stated that fish and shellfish products were responsible for 7.2% of the reported outbreaks of staphylococcal intoxication in the United States. The vehicle for most of these outbreaks were tuna, shrimps and other unspecified salads. One case of staphylococcal food poisoning because of the consumption of cooked peeled frozen shrimps in Australia has been reported by Sutton (1973). No staphylococcal food borne outbreak due to consumption of fish has been reported in the U.S. in 1980 (CDC, 1983).
Fish caught from unpolluted waters does not contain \textit{Staph. aureus} (Spencer and Georgala, 1958; Ridley and Slaby, 1978). However, contamination with this organism takes place during handling of fish by workers engaged for processing (Raj and Liston, 1963; Phillips and Peeler, 1972; Bryan, 1973; 1974; Lee and Pfeifer, 1975; Liston, 1980; Sumner et al., 1982; Hobbs, 1982). Further, it is also known that \textit{Staph. aureus} can grow vigorously in fish if conditions are suitable (Matches and Liston, 1968; Bryan, 1973; Liston, 1980).

Sufficient information on the incidence and sources of contamination of \textit{Staph. aureus} in fish has been collected in cold countries. Unfortunately, there is a lack of similar information from tropical countries where the high ambient temperature usually favours multiplication of \textit{Staph. aureus}. Particularly no such published information, based on the work carried out in India, is available even though the country ranks now the 8th largest fish producing country of the world.

Work carried out by various authors on the incidence of coagulase-positive staphylococci in fish
and fishery products up to 1962 has been covered in the review of Shewan (Shewan, 1962). In this review, Shewan has stated that "10-30% of fish handled onboard ship, filleted onshore or purchased over counter in the fresh or frozen state contained coagulase-positive staphylococci". Further, he has also reported the incidence of the organism in Atlantic cod and North sea fish caught during summer and expressed the opinion that the incidence of \textit{Staph. aureus} in freshly caught fish was less significant compared to the subsequent contamination during handling and processing onboard vessels and onshore. Studies carried out in subsequent years have further substantiated Shewan's above observation. For example, Lovell and Barkaté (1969), in their studies on freshwater cray fish from 22 sources representing the major commercial cray fishing areas in Louisiana, could isolate \textit{Staph. aureus} only from 3% of the samples. Similarly, the recent work of Ridley and Slabyj (1978) on shrimps (\textit{Pandalus borealis}) in Maine has reported the absence of this organism in the sample collected onboard but it was present, to the extent of 4 organisms per gram, in the material handled by workers.
The incidence of *Staph. aureus* has been found to be comparatively higher in cooked and prepared fishery products evidently due to the additional human handling after cooking and the inherent behaviour of *Staph. aureus* to grow competitively in substrates containing minimum number of competing microorganisms. Thus, 40% of the frozen fish cakes and 60% of smoked fish tested by Appleman *et al.* (1964) were found to contain *Staph. aureus*. Studies by Surkiewicz *et al.* (1967) have isolated the organism from 20% of the samples of frozen raw-breaded fish processed under "good" and "poor" conditions of sanitation. Carroll *et al.* (1968a) isolated coagulase-positive staphylococci from 87% of the samples of frozen raw breaded shrimps examined by them and the average count recorded was $1.9 \times 10^4$ per gram. About 70% of the cooked frozen shrimps imported by Canada before 1968 carried more than 1000 coagulase-positive staphylococci per gram whereas the organism exceeded 100 per gram, in only 16.7% of the breaded raw shrimps (Neufeld, 1971). Virgilio *et al.* (1970b) have reported that 70 out of 392 samples of pre-cooked frozen shrimps from two Chilean manufacturers contained more than 100 cells of *Staph. aureus* per
gram. A few studies have indicated that, in picked crab meat, the *Staph. aureus* count is related to the hygienic conditions maintained in the processing units. Thus, all the samples of cooked and picked crab meat collected from factories along the Atlantic and Gulf coasts showed incidence of *Staph. aureus*: in "good" factories, the count was between 3 and 37 per gram while in poorly maintained factories, the count varied between 3 and 1070 per gram (Phillips and Peeler, 1972). In a study conducted by Olson and Shelton (1973) in 46 crab meat processing factories, the log. average MPN coagulase-positive staphylococci per gram of the "lump" and "special" crab meat was 38 and 29 respectively in the processing factories having "good" hygienic status. On the contrary, the corresponding figures in respect of the processing plants with "poor" hygiene were 70 and 450 respectively. Almost 50% of the frozen breaded raw shrimps of Gulf coast was also found to contain coagulase-positive staphylococci (Vanderzant *et al.*, 1973). In the U.K., Gilbert (1974) isolated coagulase-positive staphylococci from 110 of the 3023 samples of imported cooked peeled and frozen shrimps: in each of the positive samples, the organism was more than 100 per gram.
According to Cann (1977), pre-cooked frozen shrimps imported to the United Kingdom contained 50 to $1.9 \times 10^4$ coagulase-positive staphylococci per gram.

No general conclusion can be drawn on the occurrence of Staph. aureus in shrimps processed in tropical countries. Shrimps processed in Thailand were free from Staph. aureus (Cann, 1974) and only 1% of the cooked frozen shrimps processed and exported from Malaysia had Staph. aureus count more than 1000 per gram (Hobbs, 1976). According to Beckers et al. (1981), 34% of the frozen pre-cooked and peeled shrimps from the South East Asian countries had Staph. aureus exceeding $2.0 \times 10^3$ per gram. In a recent study of process hygiene in Sri Lanka Prawn industry, the Staph. aureus count of all the samples of fresh shrimps tested were in excess of 100 per gram whereas in frozen shrimps only 32% of the HL, 35% of PD and 37% of the cooked shrimps carried this much of Staph. aureus (Sumner et al., 1982). Such differences in results are not surprising as the levels of hygiene maintained in the different tropical countries are not uniform.
Recent reports have indicated that the
Staph. aureus counts in fishery products processed
in the developed countries have come down considerably.
For example, in the U.S., 100% of fish sticks and
99.7% of fish cakes and breaded raw shrimps had
Staph. aureus less than 100 per gram (Baer et al. 1976; Liston, 1980). Licciardello and Hill (1978)
of the United States Department of Commerce have
reported that Staph. aureus counts in the frozen
minced fish blocks imported from Japan, Canada,
Denmark, Greenland, Norway and Poland did not exceed
24 per gram. Again, studies conducted by Van den
Broek at Netherlands indicate that 77% of frozen fish
fillets collected from the retail market carried less
than 100 Staph. aureus per gram (Van den Broek, 1982).

Though a virulent organism capable of producing
toxin, Staph. aureus cannot compete with saprophytes
and many other bacteria during phases of active growth
and are outnumbered by many competitors (Straka and
Combs, 1952; Miller, 1955; Peterson et al., 1962;
Digiacinto and Frazier, 1966; Haines and Harmon, 1973;
Kraft et al., 1976; Mossel, 1982). It has been
reported that the natural bacterial flora present in
pot pies (Dack and Lippitz, 1962), chicken pot pies (Peterson et al., 1962) and turkey and beef pies (Bryan, 1968) exert antagonistic influences on Staph. aureus. The ability of E. coli, Str. faecalis, Bacillus cereus, Lactobacillus, Achromobacter and Pseudomonas to suppress the growth of Staph. aureus has also been documented (Rosebury et al., 1954; Oberhofer and Frazier, 1961; Dack and Lippitz, 1962; Digiacinto and Frazier, 1966; Kraft et al., 1976; Mossel, 1982). However, in cooked foods, Staph. aureus is known to compete with other bacteria present in the product (Hodge, 1960; Bryan, 1973; Mossel, 1982). Available information regarding the interaction between different species of bacteria pertains only to few food items only. Apparently, we have no knowledge of this important aspect relating to fish and fishery products.

5. Salmonella

Among Gram-negative rod-shaped bacteria causing foodborne gastroenteritis, the most important are members of the genus Salmonella. Salmonellae are enteric organisms belonging to the family
Enterobacteriaceae. They are Gram-negative, asporogenous, motile, rod shaped bacteria with peritrichous flagella. The alimentary tract of man and animals is considered to be the primary habitat of salmonellae. From the alimentary tract, these organisms are excreted through faeces and are transmitted by insects and other living creatures to a number of places, thus gaining entry to water and food materials (Hobbs, 1974; Jay, 1978). When such contaminated waters and foods are consumed by man or animals, these organisms are once again shed through faecal matter. The capacity of salmonellae to survive in rodent faeces, poultry faeces, dust, clothing and frozen foods has been elaborately reviewed by Bryan (1968). Salmonella serotypes are identified by their antigenic structure usually following the "Kauffman-White scheme", according to their somatic and flagellar antigens (ICMSF, 1978; Saxena et al., 1984). Each year new serotypes are added to the list and, at present, over 2000 serotypes are known to exist (Saxena et al., 1984).

Ingestion of Salmonella by man or animals does not often result in sickness. In certain cases, man
or animals may become carriers without exhibiting symptoms of the disease. The absolute number of the cells required to trigger off foodborne illness, depends upon the *Salmonella* serotype in question, the conditions and age of the person consuming the food and whether or not the contamination food is ingested in an empty stomach (Bryan, 1968; Mossel, 1982). Infants, the elderly, undernourished and debilitated are, thus, more susceptible to the disease and salmonellosis is known to occur in such individuals even on ingestion of a small dose such as 0.6 cells per gram (ICMSF, 1978). *Salmonella* produces mainly three types of clinical manifestations: i) the typhoid and paratyphoid fever due to *S. typhi* and *S. paratyphi* A, B and C. ii) the septicemia due to *S. choleraesuis* and iii) the gastroenteritis due to species other than *S. typhi* and *S. paratyphi*.

*Salmonella* food poisoning symptoms are presumed to be due to the liberation of endotoxin from the cells by the action of the low pH in the stomach (Jay, 1978). The onset of symptoms is usually within 12 to 24 hours after consuming the infected food. The usual symptoms are nausea, vomiting, abdominal pain, headache, diarrhoea and fever accompanied by
prostration, muscular pain, restlessness and drowsiness which last for 2 to 3 days (Bryan, 1968; Hobbs, 1974; Jay, 1978; Centres for Disease Control, 1983). Among the different species of Salmonella, S. choleraesuis has been reported to cause the highest mortality rate of 21% (Jay, 1978).

In the case of the Salmonella species that produce enteric fever, the symptoms are more severe. Infection is by ingestion of the organism which pass from the small intestine to mesentericus and after a period of multiplication, they invade the blood stream and then come back to the gut. Liver, gall bladder, spleen, kidneys and bone marrow are affected. Fever, diarrhoea, restlessness, weakness, abdominal pain, severe headache and chills are the usual symptoms. Complete recovery is possible only in about 20 days. At this stage, while these organisms generally disappear from the intestinal tract of most of the patients, up to 5% of the victims may become carriers of the organism for about 3 to 4 months. These symptoms and features are elaborately discussed by Dey (1964). In fact "Carriers" are known to be the main source of Salmonella contamination in many types
of food materials (Black et al., 1960; Hobbs, 1974). It is generally accepted that the presence of any serotype of Salmonella at any level in a food material has to be regarded as potential hazard (Bryan, 1968; Jay, 1978; ICMSF, 1978; Mossel, 1982).

In a comprehensive review on the food poisoning hazards on consumption of fishery products, Shewan (1970) stated, with certain reservations, that they are still amongst the safest articles of food. But this should not be regarded as a clean certificate given to fishery products as contamination of fish with Salmonella can take place at any stage during harvest, processing or retail distribution (Morris et al., 1970; Wyatt et al., 1979; Hobbs, 1983). Further, it is also known that Salmonella will grow readily in fish if conditions are suitable (Matches and Liston, 1968; Liston, 1980).

There are several reports on the incidence of Salmonella in freshwater fish. About 20% of the fish from river Plate, 11% from the river Nile, 6% of the freshwater cray fish from Polish rivers and lakes and 2% of fish from the lakes of Central Africa contain Salmonella (Shewan, 1962; 1977). Incidence
Of Salmonella in 3% of the commercial freshwater crayfish of Louisiana has been reported by Lovell and Barkate (1969). Presence of this organism in freshwater crayfish is not unexpected as this fish is a bottom-feeder and burrows into mud. Estuarine waters and the shellfish grown in such waters have been known to inhabit Salmonella (Metcalf et al., 1973). Salmonella may also persist in fish for long periods. Studies by Lewis (1975) have indicated that S. typhimurium persisted in freshwater fish for 30 days. Andrews and his colleagues (1977) have reported isolation of salmonellae from 4.5% of fresh and 1.5% of frozen channel cat fish from retail markets in the United States. Wyatt et al. (1979) have also noted the incidence of Salmonella in frozen freshwater cat fish to the extent of 48.6% in the finished product and 8.4% in the retail samples. Apparently, no work seems to have been reported on the incidence of Salmonella in freshwater fishes of Asian countries.

Sea water, far away from the shore, has been reported to be free of Salmonella (Shewan, 1962; Buttiaux, 1962). However, contamination of sea water with Salmonella may take place occasionally due to
faecal contamination from ships or birds. For all practical purposes, birds constitute a negligible source of contamination of sea water but ships contaminate sea water heavily (Butiaux, 1962). The sewage discharge from the land may also contaminate sea water. Thus, waters of port Husam (Germany), port of Barcelona and port Santa Monica were found to contain Salmonella (Butiaux, 1962). Therefore, it is not exceptional to find viable salmonellae in sea water contaminated with sewage or faecal matter.

After examining a considerable amount of data from the published work in the U.S.A. and Europe, Shewan (1970) came to the conclusion that freshly caught marine fish, collected from the open sea, is free from Salmonella. Further work in the United Kingdom and the U.S.A. have supported the above observation of Shewan (Bryan, 1973; Cann, 1977; Hobbs, 1982; Hobbs and Hodgkiss, 1982). However, fish from polluted coastal waters are usually infected with salmonellae. Thus, the fish from Colombo market frequently carry Salmonella (Gulasekharam et al., 1956). While some of this infection is undoubtedly due to unhygienic handling onboard vessels, part of
it is believed to be the result of fishing in polluted coastal waters and lagoons (Shewan, 1962). One benchmark survey of the menhaden industry has shown that raw fish has been contaminated with *Salmonella* in fishing boats and the holding areas of the plant (Morris *et al.*, 1970). In his review on salmonellosis on consumption of fish and shellfish, Bryan (1973) has remarked that fish caught from sewage-polluted waters will contain *Salmonella* and has quoted examples from the literature to substantiate this point. Again, flat fish from German coastal waters has been reported to carry salmonellae (Cann, 1977). In Karachi, the practice of washing the catch with coastal sea water has resulted in the contamination of fish with these organisms (Zuberi and Qadri, 1981). These authors have reported that 5% of the fish from trawlers and 14% of the samples from harbour are contaminated with salmonellae.

Most of the work on the incidence of salmonellae in marine fish and shellfish is from western countries and definitely, there is a serious lack of information on the incidence of these organisms in fishes caught from Indian waters. The information available on the incidence of *Salmonella* in marine fish and
salmonellosis on consumption of fish and fishery products, up to 1962, have been included in the reviews of Shewan (1962) and Buttiaux (1962). These reviews report the isolation of salmonellae from eel, tuna, smoked fish, mussels and the marine fishes of Colombo market. Later studies have indicated presence of *Salmonella* in fish cake (Appleman *et al.*, 1964), raw shrimps, cooked shrimps and picked crab meat (Anderson *et al.*, 1971). Another review, on outbreaks of salmonellosis from fish and fishery products, has reported isolation of *S. oranienburg* from fish cakes, *S. typhi* from raw oysters, *S. weltevreden* from canned salmon, *S. jawa* from smoked white fish and *S. enteritidis* from the skin and blubber of whale (Bryan, 1973).

Many of the recent studies particularly those from developed countries, have indicated a better situation with regard to the incidence of *Salmonella* in fish, shellfish and fish products. For example, Lerke and Farber (1971) from the United States have reported the absence of *Salmonella* in 26 samples of Pacific coast shrimps and 74 samples of Dungeness crab. Vanderzant *et al.* (1973) from Texas could not
isolate the organism from any of the 89 samples of frozen breaded raw shrimps. Again, the organism was absent in all the 208 samples of frozen minced fish blocks imported to the U.S. from Japan, Canada, Denmark, Greenland, Norway and Poland (Licciardello and Hill, 1978). While 3% of the cooked and peeled shrimps from South East Asian countries were contaminated with Salmonella, none from North Sea had this pathogen (Beckers et al., 1981). Salmonella was absent in all the 217 fresh fish fillets collected from the retail shops in The Netherlands (Van den Broek & Mol, 1982). Obviously, the necessary care and precautions taken in these countries to avoid bacterial contamination of fish are reflected in these results.

Fish meal, among other feed components, has been widely blamed for the apparent dissimination of Salmonella throughout the world (Liston, 1980). In fact the concern as to the prevalence of Salmonella in fish meal became serious only from 1960's: several research workers have indicated that their incorporation into animal feeds could transmit the organism to livestock and poultry (Garrett, 1973; Lee, 1973; Harvey, 1973). Salmonella problem in fish meal has been reported by Jacob et al. (1963); Morris et al.
Morris et al. (1970) have reported some cases where contamination of fish meal with Salmonella took place when the feed was kept unprotected in the air. It was further observed by these workers that only the first 30 to 45 minutes of each day's production yielded Salmonella and this was pointed out to be due to the growth of the organism in the moist fish material, which was left in presses and conveyors overnight when the plant was shut down. Once the equipments in the processing factory were fully heated up, salmonellae were destroyed and did not appear in the final product. Sanitary guidelines for the control of Salmonella in the production of fish meal have been outlined by Carroll and Ward (1967), Morris et al. (1970), Garrett and Hamilton (1971) and Garrett (1973).

Presence of Salmonella in froglegs has been documented by research workers from various parts of the world. The problem of Salmonella in frozen froglegs has become more apparent by the end of 1960's when the World Health News Service (1968)
reported that froglegs were usually the source of Salmonella in Belgium, France and Canada. Similarly, the Canadian Food and Drug Directorate and the U.S. Food and Drug Administration have reported frequent detection of Salmonella in froglegs from India, Japan, Pakistan, Mexico, Cuba, Canada and the U.S. (USDHEW, 1968). The incidence of Salmonella in raw and frozen froglegs has also been reported by Ang et al. (1973), Fantasia (1974), Shrivastava (1974), Iyer et al. (1975), Nickelson et al. (1975), Rao and Nandy (1976), Shrivastava (1978) and Rajagopalan (1978). While Ang et al. (1973) from Istanbul could isolate Salmonella from 0.7% of the samples only, the froglegs processed in Greece, Indonesia, India and the U.S. seem to have a higher percentage of incidence of this organism. For example, Trichoupoulos et al. (1971) found Salmonella in 42.3% of froglegs prepared in Greece. Nickelson et al. (1975) have reported that 35% of frozen froglegs prepared in the U.S. by the "traditional methods" contained Salmonella. Frozen froglegs prepared in Indonesia were contaminated with Salmonella to the extent of 3 to 14%. In India, Shrivastava (1978) noted the incidence of salmonellae in frozen froglegs to the extent of 40% and 36% in two
separate surveys. Similarly, Rajagopalan (1978) isolated Salmonella from 33.75% of the frozen froglegs studied by him. However, the examination of aseptically cut legs were found to be free of Salmonella (Trichoupoulos et al., 1971; Nickelson et al., 1975). The intestinal tract and other internal organs of frogs have been known to be the source of Salmonella in froglegs. Thus, Salmonella has been isolated from the intestinal contents and liver of frogs (Ang et al., 1973; Nickelson et al., 1975; Rao and Nandy, 1976). Occurrence of Salmonella in the saddle portion and bone-marrow of froglegs has been reported by Shrivastava (1974). Iyer et al. (1975) have reported the presence of this organism on the skin and in the intestine and cloacal portion of frogs while it was absent in the blood of the animal.

Even though Salmonella has been isolated from fish and fishery products by many workers, very little attempt has been made to locate the sources of contamination at different stages of handling and processing. However, some information is available on the incidence of Salmonella during processing of
food products. Poor sanitary conditions in and around the processing unit were found to be responsible for the contamination of tuna with *Salmonella* (Badiali *et al.*, 1957). Food handlers are known to be the main source of contamination of food products with *Salmonella* (Olitzsky *et al.*, 1956; Anderson *et al.*, 1971; Lee, 1973; Bryan, 1973; Basu *et al.*, 1973; Hobbs, 1974). According to Pether and Gilbert (1971), *Salmonella* can be recovered from fingertips of food handlers even after 3 hours of contamination. The utensils used for processing have, in certain cases, resulted in the contamination of the processed products with this pathogen (Hobbs and Gilbert, 1970; Anderson *et al.*, 1971). Salmonellae have also been isolated from fishing boat and "pump" water used onboard (Morris *et al.*, 1970). In Karachi, the practice of washing the fish catch with coastal sea water has resulted in the contamination of the landed material with salmonellae (Zuberi and Qadri, 1981).

Available information on the viability of salmonellae in fishery products during freezing and subsequent frozen storage is meagre and, from the
existing literature, no definite conclusion can be drawn regarding the behaviour of these organisms during freezing and subsequent cold storage. Raj and Liston (1961), on the basis of their studies on the viability of salmonellae in fish, reported that these organisms can survive -17.8°C for more than an year. Digirolamo et al. (1970), on the other hand, recorded 95% reduction in the number of salmonellae in oysters during freezing and subsequent storage for 24 hours at -17.8°C and the reduction in the count at the end of one week at the same storage temperature was 99.9%. Further work is, therefore, needed to have a correct assessment of the behaviour of *Salmonella* at sub-zero temperatures.

The survey of literature has further revealed that no detailed study has, so far, been carried out in any country to study the pattern of *Salmonella* serotypes in fish and fishery products.

6. *Vibrio parahaemolyticus*

*V. parahaemolyticus* is a truely marine organism and is a part of the natural flora of marine environment and fish especially from warmer waters
The bacterium is a Gram-negative, non-spore forming, facultatively anaerobic rod. The organism was first isolated and identified as a cause of food poisoning in Japan in 1951 by Fujino and his colleagues (Fujino et al., 1953). It was first thought to be confined to Japan and the Far East but its isolation from many species of fish, shellfish and marine environments such as bottom sediments and plankton from many countries such as the U.S.A. (Liston, 1973), the U.K. (Barrow, 1974), Philippines, Taiwan, Hong Kong, Singapore (Sakazaki, 1969), Panama, West Africa, Indonesia (Beuchat, 1977), India (Chatterjee et al., 1970; Nair et al., 1975; Natarajan et al., 1980) and Malaysia (Cann et al., 1981) suggests that the organism has a world wide distribution (Liston, 1973; Barrow and Miller, 1976; ICMSF, 1978; Liston, 1980; Beuchat, 1982). The earlier work on *V. parahaemolyticus* has been extensively reviewed (Sakazaki, 1969; Lee, 1973; Liston, 1973; Sakazaki, 1973,b; Barrow, 1974) and in 1973, an international conference was held in Tokyo on *V. parahaemolyticus* (Anon, 1974).
V. parahaemolyticus is widely distributed in inshore marine areas and it can be readily isolated from marine animals in warm water areas throughout the year (Liston, 1976). But, its prevalence in temperate regions appears to be seasonal and restricted to warmer inshore waters—particularly where organic content is high (Baross and Liston, 1970; Cann, 1977; Liston, 1980). It rarely occurs in the North sea although it has been found in the sea mud, crabs and shellfishes off South West of England (Cann, 1977). Shewan (1977) has reported the incidence of V. parahaemolyticus in the Baltic, Adriatic, Mediterranean and Indian Oceans and off the East and West coasts of North America.

The illness resulting from the consumption of food, contaminated with V. parahaemolyticus, has been recognized in many countries of the world (Beuchat, 1982). This organism has been regarded as a common cause of food poisoning, particularly in Japan, where it is responsible for more than 52% of the total food poisoning outbreaks (Todd, 1978). Such an unusually high figure is indeed explainable as eating raw fish is a tradition in Japan (Sakazaki, 1969). In fact, in Japan, most of the food poisoning
outbreaks originate from the consumption of raw fish (Okabe, 1974; Barrow and Miller, 1976). But, in other countries such outbreaks originate from the consumption of shrimps and crab meat recontaminated after cooking and held at higher temperatures permitting rapid growth (Barker et al., 1974). Thus, almost without exception, V. parahaemolyticus food poisoning is associated with the consumption of fish (Baross and Liston, 1970; Liston, 1973; Beuchat, 1982).

V. parahaemolyticus is very sensitive to heat above 48°C and the optimum temperature for growth is between 35 and 37°C (Sakazaki, 1973b; Beuchat, 1975; Liston, 1980; Beuchat, 1982). Most of the strains do not grow at temperatures below 5°C, although the organisms may survive for long periods at these temperatures (Beuchat, 1975). V. parahaemolyticus is more sensitive to chilling than to freezing (Johnson and Liston, 1973; Liston, 1980; Beuchat, 1982). Therefore, a thorough washing of the fish quickly after catch and immediate icing after washing is the best method to control the incidence of the organism. Heating above 60°C for 15 minutes kills the organism and, therefore, if seafoods are heated
to 100°C just before consumption, food poisoning due to *V. parahaemolyticus* would rarely occur (Sakazaki, 1973,b). Under suitable conditions of culturing, the generation time for *V. parahaemolyticus* is exceptionally short, usually 12 to 15 minutes (Sakazaki, 1973,b; Liston, 1973; Beuchat, 1982). Katoh (1965) has reported a generation time of 8 to 9 minutes, thus perhaps establishing a record in the case of *Eubacteria*. When *V. parahaemolyticus* is ingested at a level of $10^6$ cells or more, it frequently causes a characteristic food poisoning syndrome (Sakazaki et al., 1968; Liston, 1980). The illness caused by *V. parahaemolyticus* varies from an acute cholera-like gastroenteritis to mild diarrhoea, but in some cases, with blood and mucus in stools. The average incubation period is between 12 and 24 hours, presumably because of the size of the infective dose, acidity of the stomach and the nature of the food (Barrow, 1973). As the organism can multiply quickly compared to other pathogens, under favourable conditions, it can multiply to a dangerous level even from a low level (Smith, 1971; Barrow and Miller, 1976; Liston, 1980). During illness, *V. parahaemolyticus* is excreted in large numbers but
the numbers diminish rapidly with recovery. The recovery usually takes place within a few days of the onset of illness and mortality rate is less than 10% (Liston, 1973).

*V. parahaemolyticus* produces endotoxins similar to those produced by the members of the family *Enterobacteriaceae* which may also play a part in its pathogenicity (Sochard and Colwell, 1976). Like *Salmonella* spp., strains of *V. parahaemolyticus* may be differentiated into 54 serological types by agglutination tests using specific 'O' and 'K' antisera (Barrow and Miller, 1976).

Most of the published literature on the incidence of *V. parahaemolyticus* deal with the presence of the organism in the freshly landed fish and shellfish. Only a limited amount of information is available on the incidence of the organism in fish products involved in the export trade. Even though India exports a considerable quantity of frozen shrimps to Japan and the U.S.A., there is no information on the incidence of *V. parahaemolyticus* in shrimps processed and exported from this country. Liston (1973) reported isolation of *V. parahaemolyticus*
from frozen breaded shrimps, breaded oysters, crab meat, clams and sucked oysters. However, Vanderzant et al. (1973) could not isolate this organism from frozen breaded shrimps collected from processing units. The incidence of *V. parahaemolyticus* has been reported in 15% of clams 19% of mussels and 38% of oysters from eight sampling areas in the Canadian Maritime Provinces (Thomson and Thacker, 1972). Several surveys have shown that this organism is frequently present in normal shellfish caught from Canadian and the U.S. waters, although the number of cells in the fresh material is low, i.e. 100 or less per gram (Thomson and Pivnick, 1972). In The Netherlands, 2.4% of mussels are known to carry the organism (Kampelmacher et al., 1972). Wide variations in the incidence of the organism have been reported in the market samples: the incidence being 6% in Korea and 13% in Japan during summer (Liston, 1973). In India, *V. parahaemolyticus* has been isolated by Nair et al. (1975) from 24% of fresh fish analysed by them. In a detailed study, Natarajan et al. (1980) have isolated the organism from 40.4% of brackish water fish and 48.6% of crustaceans. Although Cann (1977) could not isolate
V. parahaemolyticus from Thai fishing grounds, he could isolate this organism from 8 out of the 12 samples of fish collected from the different landing places in Malaysia. Moreover, an intensive examination of 140 crabs commercially caught off the Devon and Cornish coasts of England, yielded only one positive isolate despite the presence of the organism in 10 out of the 47 samples of sand and sea water from the same area (Cann, 1977). In their studies, Lall et al. (1979) have isolated V. parahaemolyticus from 79.3% of crustaceans and 37.5% of sea fish. About 8% of the oysters collected from purification areas in The Netherlands have been reported to contain V. parahaemolyticus (Van den Broek et al., 1979).

This organism is known to occur frequently in mussels obtained from the Norwegian coastal environment during July and August but none could be detected during the cold season from the same environment (Gjerde and Boe, 1981). According to Cann et al. (1981), almost 70% of the shrimps collected from the commercial fishing vessels off the island of Penang and 67% of the samples taken from the different landing places of Malaysia were found to carry V. parahaemolyticus. These authors have also reported the incidence of
the organism in 56% of the samples of shrimps collected from the production line in three processing plants in Malaysia. However, the organism was not isolated from a total of 50 packages from the 2 consignments of frozen Malaysian shrimps either before or after shipment to the U.K.

A considerable amount of work has been done on the effect of refrigerator and deep-freeze temperatures on the viability of *V. parahaemolyticus*. This organism does not grow below 5°C and the organism is more sensitive to chilling than to freezing (Liston et al., 1971; Sakazaki, 1973, b; Thomson and Thacker, 1973; Beuchat, 1975; 1982). But, there is contradiction with respect to the behaviour of the organism to sub-zero temperatures. For example, Thomson and Trenholm (1971) reported that specimens of shellfish held in deep-freeze at -20°C for more than 2 weeks seldom contained viable *V. parahaemolyticus* and that fish homogenates inoculated with this bacterium indicated log. reduction of values of 2.2 to 6.2 at -18°C in 12 to 19 days whereas the same reduction value was noted in less than 12 days at -34°C (Matches et al., 1971). According to Thomson and Thacker (1973), *V. parahaemolyticus* survived only
for 1 to 3 weeks in oysters at -20°C depending upon the initial concentration of the organism. Vanderzant and Nickelson (1972) observed substantial destruction of the organism when inoculated to whole and homogenized shrimps held at -18°C but they could still isolate the organism even after 8 days of storage. Isolation of this organism from frozen crab meat and fish fillets stored at -15°C and -30°C after 30 days and 60 days respectively has been reported by Johnson and Liston (1973). These workers could also isolate *V. parahaemolyticus* from inoculated oysters after 130 days of storage at -15°C and -30°C. When lobster tails were heavily contaminated with *V. parahaemolyticus* at a concentration of $10^4$ to $10^6$ organisms per ml., it was possible to recover the organism upto at least 3 months at -18°C whereas if the inoculum contained $10^2$ to $10^3$ organisms per ml., the viability of the organism was for one month only when the material was stored at the same temperature (Lamprechet, 1980). Further, this organism could not be detected after one week at -18°C if the initial inoculum contained less than 100 cells per ml.

Despite its pathogenicity, the public health significance of *V. parahaemolyticus* when isolated from
seafood during routine surveillance is debatable (Cann et al., 1981). Earlier, it was widely accepted that all strains of V. parahaemolyticus, irrespective of their source, were enterotoxigenic to man. Later, Kato (1965) found that the strains of this species of Vibrio isolated from diarrhoeal stools gave a haemolytic reaction in a specified media while those isolated from marine sources failed to do so. The medium was later modified by Wagatsuma (1968) to get a more clear cut reaction and the test was named as "Kanagawa reaction". Sakazaki et al. (1968) carried out a detailed study of Kanagawa reaction with 3370 cultures and observed that 96.5% of human cultures were Kanagawa-positive while only 1% of the marine cultures gave a similar reaction. Later, it was confirmed by Sakazaki (1973,b) that only those strains which were isolated from human sources gave characteristic illness on feeding tests while the strains from marine sources failed to produce illness in human volunteers. Even in the outbreaks of food poisoning, the isolation of Kanagawa-positive strains from the causative foods in very rare, although all isolates from faecal specimens of the victims are Kanagawa-positive (Sakazaki, 1973,a). The inability of marine cultures to give a positive Kanagawa reaction has also been noted by
Miyamoto and his colleagues (1969), Barrow and Miller (1976), Cann et al. (1981) and Beuchat (1982). It appears, therefore, that a positive Kanagawa reaction may be closely related to human pathogenicity (Sakazaki, 1973a; Beuchat, 1982). However, Twedt et al. (1970) found a much higher incidence of Kanagawa-positive cultures from estuarine sources of the United States (55 to 90%). A few outbreaks due to Kanagawa-negative strains have been reported by Zen-Yoji et al. (1970). Apparently, there is only one report of the isolation of a Kanagawa-positive strain of V. parahaemolyticus from a food implicated in a case of acute gastroenteritis in the U.S. (Spite et al., 1978; Beuchat, 1982) Kanagawa-positive strains have also been isolated from fishes and crustacean shellfishes in India (Lall et al., 1979; Natarajan et al., 1980) and the republic of Korea (Chun et al., 1974).

Several explanations have been advanced to account for the abundance of Kanagawa-positive strains in the stools of patients and the rarity of these pathogenic strains in seafoods. These include the possibility of transformation of negative strains to
positive ones during transit in the intestinal tract, comparative advantage of Kanagawa-positive strains to be able to proliferate more rapidly in the intestine and the inadequacy of isolation and identification procedures used to detect Kanagawa-positive strains in presence of competing negative strains in seafoods (Beuchat, 1982). In spite of these explanations the question is yet to be resolved and it still continues to attract considerable attention of researchers.

7. Culture media and methods for the determination of bacterial count

a. Total bacterial count. Angelotti (1964) made a detailed review of the media and methods available for the determination of total bacterial count. According to this review, the common media used and recommended were Standard methods agar, Tryptone glucose agar and Plate count agar. The incubation temperature varied from 32 to 37°C for 48 to 72 hours. Angelotti had also made the observation that the papers published in the U.S. around 1960 revealed an unusual unanimity in the choice of Tryptone glucose agar for the determination of total bacterial count.
The standard methods currently available for the determination of total bacterial count are those recommended by the Association of Official Analytical Chemists (AOAC, 1975), the American Public Health Association (APHA, 1975; Speck, 1976) the U.S. Food and Drug Administration (FDA, 1978), and the International Commission on Microbiological Specifications for Foods (ICMSF, 1978). The agar medium recommended in these methods is either Plate count agar or Tryptone glucose agar. For example, the AOAC has recommended the use of Plate count agar at 35°C for 48 hours. The Joint FAO/WHO Expert Consultation held in Geneva (Anon, 1975) has suggested Plate count agar at 30°C for 72 ± 3 hours. The recommendation of APHA is to use either Tryptone glucose extract agar or Plate count agar for the determination of total bacterial counts in foods and water supplies (Incubation temperature is 35°C for 48 hours). The U.S. Food and Drug Administration and the ICMSF have also recommended Plate count agar: but the incubation temperature is 35°C for 48 ± 2 hours in the former, but 29 to 31°C for 48 ± 3 hours in the latter.
Tryptone glucose extract agar is the medium recommended by the Indian Standards Specifications for the determination of total bacterial count in fish and fishery products (Joseph, 1979). The incubation temperature recommended in these standards is 37°C for 48 hours. The Export Inspection Council (EIC) in India has also made similar recommendations (Anon, 1982) and every consignment of fish exported from this country is tested according to the procedure outlined by the EIC.

b. E. coli and other coliforms. E. coli and other coliforms present in water and foods are generally enumerated by two different techniques.

1. Direct plating on differential media.

2. Most Probable Number (MPN) method.

In the case of water, a membrane filter technique is also recommended (Delaney et al., 1962; APHA, 1975). Tergitol-7 agar (Chapman, 1951), Desoxycholate agar (APHA, 1967), MacConkey agar (Hall, 1964,a) and Violet red bile agar (Hall, 1964,a; APHA, 1967; ICMSF, 1978) are the common solid agar media for the enumeration of coliforms. Out of these, Tergitol-7
agar is highly selective for *E. coli* and will differentiate the organism from other coliforms within 24 hours (Chapman, 1951). Desoxycholate agar, Violet red bile agar and MacConkey agar can give only the total coliform count from which *E. coli* has to be determined by the Eijkman test (Hajna and Perry, 1943). The methods for the isolation and enumeration of coliform organisms have been reviewed in detail by Hall (1964, a). The work of many investigators covered in this review has indicated that Desoxycholate agar and Violet red bile agar can be successfully used to isolate coliform organisms from foods. Silverman *et al.* (1961) and Nickerson *et al.* (1962) found that Desoxycholate agar produced excellent results in the examination of frozen raw and cooked shrimps and fish sticks for coliforms. Similarly, Violet red bile agar has been reported to give good results for the isolation of coliforms from cooked seafoods (Gjerde and Boe, 1980). The Indian Standards Specifications (IS:5887, 1976) on methods for the detection of bacteria responsible for food poisoning have recommended Tergitol-7 agar as one of the agar medium for the detection and enumeration of *E. coli*. 
Mossel (1967) was of the opinion that the determination of coliform organisms alone in foods is of limited significance and, therefore, he developed the Crystal violet-neutral red-bile-glucose agar in which all the members of the Enterobacteriaceae family will grow.

For the enumeration of coliforms by the MPN technique, MacConkey broth (WHO, 1963; PHLSWC, 1969; ICMSF, 1978), Lauryl sulphate tryptose broth (APHA, 1966; 1975; AOAC, 1975; Fishbein et al., 1976; FDA, 1978; ICMSF, 1978) and Brilliant green lactose bile broth (AOAC, 1975; ICMSF, 1978) are recommended. Each of the tube showing positive reaction are inoculated to EC media, MacConkey broth, Brilliant green lactose bile broth or Lauryl sulphate tryptose broth and incubated at 44.5±0.5°C for 24 to 48 hours for the enumeration of E. coli. Some of the other liquid media found suitable for the enumeration of coliforms are Improved formate lactose glutamate medium (Gray, 1964), Minerals - modified glutamate medium (Anon, 1969) and Lactose broth (APHA, 1975).

The review of literature has indicated that Tergitol-7 agar is highly selective for the
enumeration of *E. coli* within 24 hours. Because of this reason, the medium appears to be useful for the routine quality control laboratories. But, its suitability to isolate *E. coli* from fishery products does not seem to have been established. Further, desoxycholate agar and violet red bile agar are known to give good results in the isolation of coliforms from fishery products and there are easy techniques to determine the *E. coli* count from the total number of coliforms. A comparative study of Tergitol-7 agar, desoxycholate agar and violet red bile agar to select the most suitable one for fishery products is found to be quite useful. Further work is needed on this aspect.

c. Faecal streptococci. Many of the media used for isolating and enumerating faecal streptococci use either sodium azide or thallus acetate as inhibitory agent. To enhance the selectivity, either of these substances are used in conjunction with crystal violet. Barnes (1959) discussed many media containing these inhibitory substances and remarked that neither sodium azide nor thallus acetate media is completely selective for faecal streptococci. The methods for
the isolation and enumeration of enterococci have been reviewed by Hall (1964,b). Hartman et al. (1966) made a very detailed review of 43 selective agar media and 33 selective broth media developed by various investigators from 1918 to 1965 using various selective agents like crystal violet, sodium azide, thallus sulphate, thallus acetate, sodium selenite, aniline blue, potassium tellurite, neomycin sulphate etc. Citrate azide agar, M-enterococcus agar, Ethyl violet azide agar, KF agar, MN trypticase soy agar, Azide blood agar, Tween carbonate agar, Mitis salivarius agar, Esculin lactose azide agar, Streptocel agar and many others were covered in this review. Isenberg et al. (1970) developed the Pfizer selective enterococcus medium containing bile, esculin and sodium azide for the enumeration of faecal streptococci. On comparing with two other media, Sabbaj et al. (1971) found the Pfizer selective enterococcus agar to be better for the detection and enumeration of Group D streptococci. For the selective enumeration of Group D streptococci in foods and water, Mossel et al. (1977) developed the Kenamycin esculin azide agar.
KF agar appears to be the agar medium of preference of many research workers at the moment (Licciaardello and Hill, 1978; Gore et al., 1979). It has been recommended by the American Public Health Association (Speck, 1976) for the enumeration of faecal streptococci. The International Commission on Microbiological specifications for Foods (ICMSF, 1978) has recommended Packer's crystal violet azide blood agar and KF agar and has remarked that KF agar appears to be more differential than Packers medium. However, no comparative study does not seem to have been made so far to establish the suitability of KF agar for the detection and enumeration of faecal streptococci present in fresh and frozen fishery products. Further work is needed on this aspect.

d. Staph. aureus. A number of media have been formulated for the detection and enumeration of Staph. aureus. They differ mainly in the nature of the selective agents used: chief among them are potassium tellurite, lithium chloride, sodium azide and polymyxin B. Crisley (1964) made a detailed review of the methods for the isolation of Staph. aureus. A great majority of selective media
appear to have been designed for use in the hospital laboratory. The media suggested for the examination of food for *Staph. aureus* indicate extreme variation in their efficiency. Much of this variation is due to the difference in the subjective judgements as to what constitutes a staphylococcal colony. The problem is less acute with tellurite-containing media.

The most important media in use for the enumeration of *Staph. aureus* are the following (Gilbert *et al.*, 1969).

<table>
<thead>
<tr>
<th>No.</th>
<th>Medium</th>
<th>Selective agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vogel and Johnson agar (U.S.A.)</td>
<td>Pot. tellurite</td>
</tr>
<tr>
<td>2.</td>
<td>Egg-yolk sodium azide agar (Sweden)</td>
<td>Sod. azide</td>
</tr>
<tr>
<td>3.</td>
<td>Baird-Parker medium (U.K.)</td>
<td>Pot. tellurite</td>
</tr>
<tr>
<td>4.</td>
<td>Milk salt agar (Russian)</td>
<td>Sod. chloride</td>
</tr>
<tr>
<td>5.</td>
<td>Milk salt agar (English)</td>
<td>Sod. chloride</td>
</tr>
<tr>
<td>6.</td>
<td>Staphylococcus medium No.110 (U.S.A.)</td>
<td>Sod. chloride</td>
</tr>
<tr>
<td>7.</td>
<td>Tellurite polymyxin egg-yolk agar (U.S.A.)</td>
<td>Pot. tellurite</td>
</tr>
<tr>
<td>8.</td>
<td>Phenolphthalene diphosphate agar with polymyxin (U.K.)</td>
<td>Polymyxin B</td>
</tr>
</tbody>
</table>

Polymyxin B sulphate
On comparison of six different media, Neufeld and Garm (1963) found that Tellurite glycine agar with added egg-yolk is the most promising. Raj (1966) has developed a two-step procedure comprising Mannitol-salt-sorbic acid broth for presumptive test and Staphylococcus medium No.110 (fortified with egg-yolk) incubated at 45°C for a confirmatory test for the detection and enumeration of \textit{Staph. aureus} in seafoods. The KRANEP agar of Sinell and Baumgart (1967) has been used extensively in Germany for the detection of \textit{Staph. aureus}. A technique comprising non-selective enrichment in Trypticase soy bean broth for 24 hours (35°C) followed by streaking 0.1 ml aliquots onto Baird-Parker medium, Vogel and Johnson agar and Staphylococcus medium No.110 has been suggested by Baer \textit{et al.} (1975). The second Joint FAO/WHO Expert Consultation (Anon, 1977) has recommended Baird-Parker medium for the enumeration of \textit{Staph. aureus}. The International Commission on Microbiological Specifications for Foods (ICMSF, 1978) has described five methods for the detection and enumeration of the organism. Among these five methods, four are direct streaking techniques on Baird-Parker medium, Tellurite Polymyxin egg-yolk
agar, KRANEagar and Phenolphthalene diphosphate agar with polymyxin. The fifth method is an enrichment procedure using Tellurite-mannitol-glycine broth followed by streaking on Milk salt agar. The Bacteriological Analytical Manual of the Food and Drug Administration (FDA, 1978) has recommended Baird-Parker medium for the enumeration of Staph. aureus.

In all the methods cited above, the ability of Staph. aureus to produce "coagulase" is tested by the well known "coagulase test".

Recently, an accelerated procedure for the enumeration of food-borne Staph. aureus has been described by Lachica (1980). In this method, Baird-Parker medium is used for the detection of the organism and the isolated strains are subjected to a simplified thermonuclease test.

A number of investigators have reported low recovery of Staph. aureus on media containing high percentage of sodium chloride, especially if the cells have undergone some degree of stress such as freezing, heating or drying (ICMSF, 1978). The
problem with media containing polymyxin is that this chemical inhibits some strains of \textit{Staph. aureus} (Hobbs, 1967). The Baird-Parker medium is now widely used in Europe and North America (ICMSF, 1978). The medium has been approved for use in the AOAC Official First Action Method (Baer et al., 1975). The medium is also recommended by the Indian Standards Specifications for frozen fishery products (Joseph, 1979), the U.S. Food and Drug Administration (FDA, 1978) and also by the International Commission on Microbiological Specifications for Foods, the International Organization for Standardisation and the United States Department of Agriculture (ICMSF, 1978).

According to Mossel (1982), Baird-Parker medium is the most reliable for the isolation and enumeration of \textit{Staph. aureus} present in food products.

From the review of literature, the Baird-Parker medium appears to be the best for the enumeration of \textit{Staph. aureus}. But, the recommendation of the Canadian Fisheries Department is to use Tellurite glycine agar for the enumeration of \textit{Staph. aureus} present in fishery products. A study involving both Baird-Parker medium and Tellurite glycine agar
with added egg-yolk to select the most suitable for fishery products will be quite useful.

e. *Salmonella.* Numerous methods with a variety of media have been suggested by various workers for the detection of *Salmonella.* Although the number of salmonellae in a sample can be estimated by a Most Probable Number method, qualitative methods are usually used for the detection of *Salmonella* in foods (Bryan, 1968). The methods used for the detection of salmonellae have been reviewed by Galton *et al.* (1968). According to this review, the procedure for the isolation and identification of salmonellae in foods consists of a sequence of five basic steps.

1) Non-selective enrichment for rejuvenating salmonellae that are injured by processing or storage conditions. Lactose broth, Mannitol broth, Nutrient broth and Lauryl tryptose broth are some of the non-selective enrichment broths commonly employed by various workers.

2) Selective enrichment which favours the growth of salmonellae in an environment which may contain large numbers of bacteria other than *Salmonella.*
The three selective enrichment media that have been widely used are Selenite-cystine broth, Tetrathionate broth and Tetrathionate brilliant green broth.

3) Plating on selective and differential media which permits the sorting of suspected salmonellae based upon colony characteristics. Brilliant green agar, Bismuth sulphite agar, Salmonella-Shigella agar, Desoxycholate citrate agar, Brilliant green sulphadiazine agar and Xylose lysine brilliant green agar are most commonly used for this purpose.

4) Biochemical screening of the suspected strains which weed-out the non-Salmonella cultures and provide a tentative identification of Salmonella. Reactions of the suspected colonies on Triple sugar iron agar, Lysine iron agar, Urea agar, Tryptone broth, Melonate broth, Potassium cyanide broth, Lactose broth, Sucrose broth, Salicin broth and Dulcitol broth are studied to come to a conclusion.

5) Serological tests using the various 'O' and 'H' antisera which lead to the ultimate identification of the organism to the genus level.
An MPN technique using Dulcitol selenite broth as a primary selective enrichment medium has been suggested by Raj (1966b) for the enumeration of Salmonella present in seafoods. Galton et al. (1968) recommended an MPN technique using Lactose broth for enumerating Salmonella present in foods and feeds. Edel and Kampelmacher (1968) reporting on comparative studies conducted at eight European laboratories found that pre-enrichment gave higher isolation rates than direct enrichment. These authors further observed that the incubation of the selective enrichment broths at 43°C profoundly favoured the rate of isolation of Salmonella. Similar observation was also made by Silliker and Gabis (1974).

Fluorescent antibody techniques are also common for the detection of salmonellae in foods. Techniques like enrichment serology and fluorescent antibody procedures, although more rapid than conventional techniques, require special equipments and antisera (Hoben et al., 1973). These authors have developed a rapid presumptive procedure for the detection of Salmonella in foods using Tetrathionate broth for the first enrichment and a modified Lysine -
iron - cystine - neutral red broth for the second enrichment. Recently, Mossel (1982) suggested Muller - Kauffman's Brilliant green tetrathionate broth and Rapport's Malachite green magnesium chloride broth as enrichment (both at 43°C), modified Brilliant green phenol red lactose sucrose agar and Xylose lysine desoxycholate agar for selective isolation and Klinger glucose lactose agar and Lysine iron agar for biochemical characteristics. Tests for urease and Beta-galatosidase are also carried out in addition to 'O' and 'H' agglutination reactions.

The methods widely used at present for the detection of Salmonella in food products are those of the Association of Official Analytical Chemists (AOAC, 1975), American Public Health Association (Speck, 1976), Bacteriological Analytical Manual (FDA, 1978), International Commission on Microbiological Specifications for Foods (ICMSF, 1978) and International Organisation for Standardization - IOS - (Van Leusden et al., 1982). The media used for pre-enrichment, selective enrichment, presumptive isolation and biochemical screening in these methods, are given below.
<table>
<thead>
<tr>
<th>Method</th>
<th>Pre-enrichment</th>
<th>Enrichment</th>
<th>Presumptive identification</th>
<th>Biochemical screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC 1975</td>
<td>Lactose broth</td>
<td>Selenite broth</td>
<td>Brilliant green agar, Salmonella Shigella agar and Bismuth sulphite agar</td>
<td>TSI, agar, LIA agar, urea agar, Tryptone broth, KCN broth, Lactose broth, Sucrose broth, Salicin broth, dulcitol broth*</td>
</tr>
<tr>
<td>APHA 1976</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>EDA 1978</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Hektoen enteric agar, Bismuth sulphite agar, xylose lysine desoxycholate agar</td>
<td>&quot;</td>
</tr>
<tr>
<td>ICMSF 1978</td>
<td>Lactose broth with or without BG. Buffered peptone water. Reconstituted dried milk with B.G. (at 43°C)</td>
<td>&quot;</td>
<td>Brilliant green agar, Bismuth sulphite agar, any one more media</td>
<td>TSI, LIA*</td>
</tr>
<tr>
<td>IOS 1982</td>
<td>Buffered peptone water</td>
<td>Tetrathionate bile BG medium</td>
<td>BG phenol red agar</td>
<td>Urea, TSI, LIA*</td>
</tr>
</tbody>
</table>

*Serological identification is also suggested; IOS = International Organisation for Standardisation (Van Leusden et al., 1982)
The method suggested in the Indian Standards Specifications for the detection of *Salmonella* from fishery products is the same as that suggested by the AOAC (Joseph, 1979).

**f. Vibrio parahaemolyticus.** Two steps are involved in the isolation of *V. parahaemolyticus* from fish samples i.e., enrichment and subculturing. For subculturing, Thiosulphate-citrate-bile-sucrose (TCBS) agar is invariably used (ICMSF, 1978). But, for enrichment, different broths are reported. Polymyxin salt broth, Alkaline peptone water, Glucose-salt-teepol broth (GSTB) and Salt colistin broth are some of the recommended enrichment broths (Sakazaki, 1969; 1973,b). On comparison of 18 agar media for isolation of heat-stressed *V. parahaemolyticus*, Beuchat (1976) noted that Water blue – alizarin yellow agar, Arabinose – ammonium sulphate – cholate agar and Horie's Arabinose–ethyl violet broth gave most promising results. Beuchat (1977) compared three enrichment broths (Glucose-salt-teepol broth, Horie's Arabinose–ethyl violet broth and water blue-alizarin yellow broth) for the isolation of *V. parahaemolyticus* held at -18°C and found all the three broths to be
equally effective. Use of a double strength TCBS broth has been advocated by Cann et al. (1981). An MPN technique using GSTB followed by streaking on TCBS agar has been recommended by Morris et al. (1976) and the FDA Analytical Manual (FDA, 1978). The International Commission on Microbiological Specifications for foods (1978) has recommended a three tube MPN technique using Polymyxin B sulphate broth followed by streaking on TCBS agar. A number of biochemical tests (Gelatin hydrolysis, lysine decarboxylase, arginine decarboxylase, indole production, halophilism test, cytochrom oxidase and fermentation on sucrose, maltose, mannitol, cellobiose and trehalose) are detailed in the reviews of Sakazaki (1973,b) and Barrow & Miller (1976) for identification of the strains of V. parahaemolyticus.

C. Aim and scope of the present work

India has come up as a leading exporter of fishery products. The consumers in the developed and developing nations of the world have started demanding stricter organoleptic and bacteriological standards for this commodity. Thus, the future of the fish
processing industry of India appears to depend largely on the capability of the industry to meet these standards. In order to produce wholesome and bacteriologically sound products, the industry must have a thorough knowledge of the sources and distribution of bacteria, particularly of public health significance, present in fishery products and their behaviour during the different stages of handling, processing, storage and marketing. Moreover, in the field of fisheries, the country's requirement is for research work more of a technological nature rather than purely fundamental.

Although sufficient information is available on the bacterial flora of marine fish and shellfish, only little is known about the sources of bacterial contamination in fishery products - particularly with reference to bacteria of public health significance. Further, only limited amount of work has been done on the sanitary conditions of fishing trawlers and fish processing establishments of tropical countries. In India, the studies undertaken so far, have given more importance to *E. coli* and faecal streptococci with little emphasis on coagulase-positive
staphylococci. Apparently, in this country, the only published work on the occurrence of staphylococci in fishery products is that of Lekshmy and Pillai (1964).

Another deficiency in the studies on the bacteria of sanitary significance so far carried out in this country, is that such studies are mainly on commercially frozen shrimps only, with no work on fish. Though considerable amount of work on the incidence of Salmonella in fishery products has been done in the countries of the west, work done in the South East Asian countries is insignificant and, in India, practically no published reports on the incidence of salmonellae in these products are available. Further, in India, no work has been done on the serological pattern of Salmonella strains isolated from fish and shrimps. Again, there are no studies on the incidence of V. parahaemolyticus in the fishery products meant for export though there are a few detailed investigations on the isolation of this organism from raw fish and shellfish.

Although a few studies on the behaviour of faecal indicator bacteria in fish at sub-zero
temperatures have been reported, similar work on the behaviour of coagulase-positive staphylococci and Salmonella in shrimps is meagre. Moreover, there are contradictions in the results obtained in these studies. So far, no work has been reported from India on the viability of coagulase-positive staphylococci, Salmonella and V. parahaemolyticus in fishery products during freezing and subsequent cold storage.

Though it is well known that, in a mixed bacterial population, some bacteria exert antagonistic influences on other species of bacteria, no systematic study has, so far, been carried out on this important aspect in fishery products. Similarly, no work has apparently been done on the growth rate of bacteria of public health significance in fishery products at different temperatures.

The available information on the source, distribution, growth pattern and behaviour of the organisms of public health significance is not sufficient to the extent it is necessary to enable the processors to ensure consistent production of
bacteriologically sound fishery products. Further, the bulk of the information available in the literature cannot be utilized fully by the fishery industry of India since they are based on the work done in the countries of the west where the climatic conditions, trade practices and the sanitary and hygienic conditions of the processing establishments are quite different from those prevailing in the tropical countries.

Keeping in view of the urgent need of the fishery industry of India as explained above, it was decided to carry out the following studies with particular reference to shrimps, which at present, form the backbone of the seafood export trade of India.

1) Incidence of *E. coli*, faecal streptococci, coagulase-positive staphylococci, salmonellae and *V. parahaemolyticus* in fishery products.

2) Sources of contamination of fishery products with *E. coli*, faecal streptococci, coagulase-positive staphylococci and salmonellae.
3) Viability of *E. coli*, faecal streptococci, coagulase-positive staphylococci, *Salmonella* and *V. parahaemolyticus* in shrimp-homogenate during freezing and subsequent frozen storage.

4) Growth rate of *E. coli*, faecal streptococci and coagulase-positive staphylococci in shrimp-homogenate at 0°C, 7°C, 28°C and 37°C.

5) Intercompetition amongst *E. coli*, faecal streptococci and coagulase-positive staphylococci for growth and survival in shrimp-homogenate at 0°C, 10°C and 28°C.

6) Serotyping of *Salmonella* strains isolated from frozen shrimps, frozen fish, frozen froglegs and from water, ice and utensils used in the processing units.