Microbial Diseases
CHAPTER 5

MICROBIAL DISEASES

5.1. INTRODUCTION

All living beings, in their natural surroundings, become susceptible to disease at
one time or the other in their life time. Fishes and shell-fishes are also no exception.
Occurrence of disease in these organisms either in their natural environment or in the
culture system adversely affect the population. So, a thorough study of the disease is
essential, especially in the context of global awareness to enhance food production
through aquaculture in order to meet the requirements of animal protein to the increasing
population (Thankappan Pillai, 1981).

Bacteria are ubiquitous in the marine environment. Some of them are facultative
pathogens to weaker animals and a few may be severe primary pathogens. Massive
populations can develop rapidly in infected hosts as well as in the host’s environment.
Considerable post capture mortalities in holding tank conditions have been reported from
decapods following epizootic infection by Bacteria (Stewart, 1980; Cawthorn, 1997;
Cheng and Chen, 1998). Sindermann(1990) stated that the most commonly encountered
bacterial diseases in crustaceans are shell disease and vibriosis due to interaction
between bacterial pathogens and environmental stress. The causative agents of these diseases are opportunistic in nature and proliferate rapidly under environmental stress conditions. Under these conditions the host also loses the resistance and this results in the outbreak of disease. Thus, there exists a delicate balance between the host, pathogen and the environment. These diseases manifest themselves by progressive chitinolysis and necrosis of the exoskeleton of aquatic crustaceans.

Shell disease is the most common bacterial disease affecting crustaceans (Sindermann, 1990). Shell disease causes various kinds of exoskeletal lesions due to the action of chitin-destroying bacteria (Benton, 1935; Zobell and Rittenberg, 1938; Hock, 1940; Hock 1941; Lear, 1963; Hood and Meyers, 1974). Shell disease caused by chitin-destroying micro-organisms in crabs were reported by Hess (1937), Mann (1940), Schafer (1954), Dogel and Petrushevski (1957), Gordon (1966), Rosen (1967), Iversen and Beardsley (1976) and Overstreet (1978). Shell disease cause exoskeletal lesions in tanner crabs (Baross et al. 1978). The exoskeletal lesions under the name of brown spot disease was reported in European freshwater river crabs (Mann and Pipelow, 1938 and Mann, 1939) and in the European edible crab Cancer pagurus (Schafer, 1954 and Gordon, 1966). Rosen (1967) reported the presence of an exoskeletal shell disease in blue crabs from Chesapeake Bay and isolated chitinoclastic bacteria from damaged
areas of the exoskeleton. This disease was also reported from blue crabs of the South Carolina coast (Sandifer and Eldridge, 1974), Biscayne Bay Florida (Iversen and Beardsley, 1976), Mississippi coast (Overstreet, 1978). Shell erosion seems to involve the activities of chitinoclastic micro-organisms, with subsequent secondary infection of the underlying tissue by facultative pathogens. Initial preparation of the exoskeletal substrate by mechanical, chemical and microbial action, contaminated chemicals, detrital and epibiotic fouling of the gills combine to make shell disease in Pamlico river North Carolina (Engel and Noga, 1989). *Vibrio* spp. are aetiological agents of shell disease [Taylor (1948), Sawyer and Taylor (1949), Taylor (1949), Young and Pearce (1975), Fisher *et al.* (1976), Fisher (1977), Malloy (1978), Roald *et al.* (1981)]. The occurrence of shell disease was also reported in fresh water prawns by Anderson and Conroy (1968), Rosen (1970), Cook and Lofton (1973), Dugan and Frakes (1973), Delves Broughton and Puopard (1976), Johnson (1977), Cipriani *et al.* (1980), Brock (1983), Getchell (1989), Paynter (1989), Tonguthai (1993). Lombardi and Labao (1991) further reported that the bacterial diseases are caused by chitinoclastic bacteria *Pseudomonas, Vibrio, Beneckea* and *Aeromonas*. Studies on bacterial diseases in freshwater shell fishes were also carried out by Natarajan *et al.* (1982), Sankoli *et al.* (1982), Sebastian (1990), Mukherjee and Chandra (1991); Jayasree (1998). Diagnosis is based on the demonstration of brown to black cuticular lesions and/or the loss of appendage segments (Paynter, 1989).
Wounds, abrasions or chemical degradation of cuticle are required to initiate infection (Brock and Lightner, 1990). Poor culture conditions such as crowding, poor water exchange, elevated temperature and poor diet result in an increase in the incidence of shell disease (Brock and Lightner, 1990). Physical surrounding is required for infection by chitinoclastic bacteria (Cook and Lofton, 1973). Shell disease may be contagious under poor environmental condition (Getchell, 1989).

Shell disease causes visible lesions on the cuticle, appendages or gills of affected crustaceans (Sindermann, 1990). Lesions are soft, often melanised and may progressively enhanced to cover large areas of cuticle (Getchell, 1989). Segments of the affected appendages may lost. Lesions are typically lost when the animal moults (Gopalana and Young, 1975). Death may occur at the time of ecdysis when the old and new exoskeleton fail to separate or may occur as a result of secondary infection (Fisher et al., 1976; Lightner, 1983). Rongxing et al. (2000) reported mud crabs from Port curtis, Central Queensland also showed shell lesions on the carapace. Females are highly affected by this disease.

Black gill disease was reported earlier as being of common occurrence in cultured crustaceans (Egusa and Veda, 1972; Hatai et al. 1974; Johnson, 1974; Hatai et al. 1978, Lightner, 1978; Burns et al. 1979 and Ruangpan, 1982). Jayasree (1998) also reported black gill disease in fresh water shell fishes (crustaceans) was due to the environmental
stress and facultative micro-organisms. Loci of black discolouration were found to be in areas where several adjacent lamellae were dead and necrotic, with accompanying melanization and necrosis of the gill tissue (Sawyer et al., 1983).

The presence of black gill disease in lobsters of Massachusetts waters was observed by Sawyer (1982), Sawyer et al. (1983) and Estrella (1984). Sawyer et al. (1984) reported the presence of black gill disease with highest prevalences in samples from the most polluted sites of Atlantic coasts in North America. 30% prevalence of black gill disease was observed from New York Bright Apex.

Vibriosis has been identified as one of the serious diseases of wild and cultured fish and shell fishes in many regions (Egidius, 1987; Austin and Austin, 1989 and Sindermann, 1990). Vibriosis is associated with deteriorating environmental conditions caused by high stocking density and increase inputs as evident through the accumulation of organic matter in the bottom. These conditions cause severe stress and injury to the animals thus rendering their susceptibility to bacterial role in the disease process. The shell fish either lowers the resistance of the individuals or enhances the population density and pathogenicity of the pathogen (Liu, 1990). Lightner (1988) suggested that minimizing stress may act as a prophylatic measure preventing the outbreaks of vibriosis in the shell fishes.
Mortalities of several species of crabs were also studied when the animals were held in captivity. Colwell et al. (1975), Johnson, (1976) observed vibriosis in wild caught blue crabs from the middle Atlantic coast. The infected crabs showed severe effects within 10 days after capture and this proved that most of the bacterial infection were acquired during capture and transportation from facultative pathogens to the water in adherent to the body surface. Heavy infection in the blue crabs by vibriosis was noticed in summer (Johnson, 1976). Newman and Ferg (1982) reported the prevalence of vibriosis in 12% rock crab, Carcinus irroratus of New England waters. Spindler - Barth (1976) reported the presence of presumptive vibriosis in the shore crab Carcinus maenus from Northern European coast.

Vibriosis usually occurs in warm weathers, particularly when stocking density, salinity and organic load are high. The occurrence of other stressors such as parasites, bacterial load and handling crustaceans may well precipitate the situation.

In India reports on microbial diseases of prawns and vibrio infections in penaeids were made by Subramanyam (1986), Rosily et al. (1987), Nanjaiyan (1992), Prasad and Rao (1994), Nayyarahameed and Karunasagar (1995) ; Anand et al (1996). In Tuticorin coastal waters no work has been carried out on the microbial diseases of S. serrata for a prolonged period and hence the present study has been carried out.
5.2. MATERIALS AND METHODS

The crabs for bacteriological analysis were collected from the Gulf of Mannar of Tuticorin coastal waters. The crabs showing the symptoms of disease were sacrificed randomly to examine the presence of bacteria in gills, muscles and shell. The affected tissues were placed in 5 ml of nutrient broth and macerated with a glass rod. Ten fold dilutions were done to avoid the overgrowth of the bacteria (Bullock, 1971). The diluted samples were inoculated on nutrient agar by pour plate technique. After obtaining the pure cultures by repeated streaking of colonies, they were stored in bottles at room temperature (20 - 25°C) covering the culture with wax in order to avoid moisture. These pure cultures were maintained to conduct bio-chemical characterization tests for identifying the bacteria. All the taxonomical tests conducted to identify the bacteria were followed from Bergey’s manual of systematic bacteriology.

Identification of bacterial isolates

For isolation of bacteria from diseased crab, the infected portions were cleaned with spirit, cut with sterile blade into small pieces and placed in 2 ml of nutrient broth, macerated with a glass rod. Ten fold dilutions were made to avoid overgrowth of bacteria as described by Bullock, (1971). The diluted samples were incubated on nutrient agar and tryptone soya agar (TSA) by spread plate or pour plate technique. The inoculated plates were incubated at 28°C for 24 to 28 hrs. Morphologically similar, and dominant bacterial colonies were selected and streaked on nutrient agar plates to obtain
pure culture. Bacterial isolates were identified according to the taxonomic schemes in Bergey's manual of systematic bacteriology (Baumann and Schubert, 1984); Cowan and Steel's manual for medical bacteria (Barrow and Feltham, 1993). After attaining the pure culture the bacteria were smeared on a slide and stained by Gram's method. Based on the staining reaction, they were categorized as gram positive or negative. Tests such as motility, oxidase, catalase and acid production from glucose tests were employed to identify up to generic level. A series of tests such as acid production from carbohydrates like glucose, adonitol, arabinose, cellobiose, dulcitol, fructose, inositol, lactose, maltose, mannitol, sorbitol, rhamnose, sucrose, trehalose and xylose, phenylalanine deaminase test, nitrate reduction, citrate utilization, urease production, starch hydrolysis, $\beta$-galactosidase, H$_2$S production, growth on KCN medium, decarboxylase (Arginine, Lysine, Ornithine) sensitivity to O/129, MRVP reaction, indole production and NaCl tolerance (0 - 8%) were used for identification of species. All these tests were conducted following the methods of Barrow and Feltham, (1993).

**Gram's staining of bacteria**

Gram stain is a very useful stain for classifying the bacteria into two major groups, the gram positive and the gram negative. In this staining, the bacterial smear is subjected to four different reagents i.e., crystal violet (primary stain), iodine solution (mordant), alcohol (decolorizing agent) and safranin (counter stain). The bacteria which retain the primary stain (appear dark blue or violet) are called gram positive and those that are stained by safranin (appear red) are referred to as gram negative.
Thin smears of bacterial isolates were prepared, air dried and fixed by gently warming the slide. The bacterial smear was flooded with crystal violet solution and left for one minute. The slides were washed for few seconds and covered with Gram's iodine for 30 seconds. The slides were washed for 15 seconds, decolorized with 95% ethyl alcohol incorporating 5% acetone, washed again and counterstained with safranine for ten seconds. The stained and dried slides were examined under oil immersion lens.

Motility

Hanging drop preparation is useful to observe the motility of the bacteria. Young broth culture of the organism, incubated at or below the optimum growth temperature was examined in hanging drop preparation using a high power dry objective and reduced illumination.

Hanging drop slide (cavity slide) was cleaned and a little vaseline or petroleum jelly was spread around the cavity of the slide. A clean cover slip was taken and vaseline applied on each of the corners of the cover slip. A drop of the culture was placed at the center of cover slip and cavity slide was placed on the cover slip, with the cavity facing down so that the depression covers the suspension. The slide was pressed gently so as to form a seal between the cover slip and the slide and this hang drop preparation was turned quickly right side up so that the drop was suspended. The depression slide was
inverted over the cover slip in such a way that the suspension should not touch the surface of the concavity at any point. The prepared slide was examined under high power objective with reduced light.

Catalase test

Many microorganisms produce catalase in order to counteract the effects of toxic hydrogen peroxide that is produced in large quantities under aerobic conditions. Therefore, presence or absence of catalase is used to differentiate aerobic and anaerobic forms.

Catalase

\[
2\text{H}_2\text{O}_2 \rightarrow \text{2H}_2\text{O} + \text{O}_2
\]

A loopful of culture grown on nutrient agar plate for 24 - 48 hrs was taken and placed in 1% H$_2$O$_2$ on a glass slide. The slide was observed for the production of bubbles, which indicates the presence of catalase.

Oxidase activity

18 - 24 hrs, culture grown on nutrient agar was taken and placed on wet oxidase disc (commercially available: Himedia) and the appearance of a dark purple colour on the disc within 30 seconds indicates a positive reaction.
Oxidation and fermentation Test

Organisms use carbohydrate differently depending upon their enzyme complement. The pattern of fermentation is characteristic of certain species, genera or groups of organisms and for this reason this property has been extensively used as a method for biochemical differentiation of microbes. Glucose after entering a cell can be catabolized either aerobically (oxidative metabolism) or anaerobically (fermentative metabolism) or both. Some organisms lack the ability to oxidize glucose by either. The metabolic products of carbohydrate fermentation can be either organic acids (e.g. Lactic or formic or acetic) or organic acid and gas.

Whether the organism is oxidative or fermentative can be determined using Hugh and Leifson’s medium (OF medium), which contains desired carbohydrate (1% glucose), tryptone and bromothymol blue (an indicator). Two tubes were used, one of them open with free access to air and the other sealed with paraffin (to keep air out). Growth of the microorganisms in this medium is either by utilizing the tryptone, which results in an alkaline reaction (dark blue colour) or by utilizing glucose, which results in the production of acid (turning bromothymol blue to yellow). Those microorganisms, which produce acid in both closed and open tubes are described as fermentative while those which produce acid only in the open tube are called oxidative.
Preparation of OF glucose agar medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Peptone</td>
<td>2 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Bromothymol blue solution</td>
<td>15 ml</td>
</tr>
<tr>
<td>Agar</td>
<td>3 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
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The weighed constituents were dissolved in distilled water and bromothymol blue solution was added. The basal medium was poured into tubes and sterilized at 121°C for 15 minutes. 1 ml of sterile glucose solution (10%) was added to the molten base to produce a final concentration of 1%. Then the tubes were allowed to cool. Two tubes containing OF-glucose medium were inoculated with the culture. In one of them, paraffin was poured over the medium. Two uninoculated tubes were used as controls. The tubes were incubated at 35°C for 24 - 48 hrs. and observed for the presence of bacterial growth and colour development.

Nitrate reduction

The bacterial culture was inoculated lightly on nitrate broth and incubated for up to 5 days. Then 1 ml of nitrite reagent A and 12 ml of nitrite reagent B were added to 5 ml of the medium. A deep red colour indicated the presence of nitrate and thus showed that a nitrate had been reduced which in turn indicated a positive reaction.
Preparation of nitrate broth

1 gm of KNO₃ dissolved in 1000 ml nutrient broth was distributed into tubes containing inverted Durham’s tubes and sterilized at 115°C for 20 minutes.

Nitrite test reagents

Solution A

0.8% sulphanilic acid was dissolved in 5N acetic acid by gentle heating.

Solution B

0.5% of naphthylamine was dissolved in 5N acetic acid by gentle heating.

Decarboxylase reactions or Decarboxylation of amino acids

Moller’s decarboxylase broth base (commercially available) was prepared and divided into four aliquots. To three of the aliquots 10 ml of 10% solution of the appropriate amino acid (L-arginine, L-lysine or L-ornithine) was added per 100 ml of the medium. The fourth aliquot was used as a control with no amino acid added to it. All the aliquots were dispensed into tubes and autoclaved at 15 lb for 15 min. After cooling they were inoculated heavily into TSA with 2.5% NaCl. Then paraffin oil was poured on the inoculated aliquots with 2 - 3 mm thickness. They were all incubated for up to 14 days which was checked daily. Tubes of the organisms producing carboxylase become purple, though some turned yellow at first they gradually reverted to purple in 48 hrs and these were recorded as positive reactions. Tubes of organisms not producing decarboxylase turned yellow and remained so, these were seen as the negative reactions, though the control remained yellow.
**O/129 Vibriostatic compound sensitivity**

O/129 discs (commercially available) (10 µg and 150 µg) were placed on the surface of Muller Hinton Agar medium which was previously inoculated with the test organism. The plate was incubated overnight and observed for a clear zone of inhibition around the disc. The resistance versus sensitivity to O/129 was determined by the presence or absence of the bacterial growth.

**NaCl tolerance**

24 hr. culture inoculated into tryptone broth containing varying concentrations of NaCl (0% to 8%) and was observed for growth within 48 hrs.

**Indole production**

This test demonstrated the ability of certain bacteria to decompose the amino acid tryptophan into indole, which accumulates in the medium. The test organism was inoculated into tryptone broth and Kovac’s reagent strip was hung on the edge of the test tube containing inoculated tryptone broth. Appearance of purple colour on the Kovac’s reagent strip indicated the production of indole.

**β-galactosidase**

TSA was prepared and the test organism was streaked on the agar. After 25 hr growth, ONPG differentiation disc was placed on the culture. An yellow colour change of the disc indicated the reaction (or) 0.2 - 0.5 ml of 2.5% saline was dispensed in a small test tube and a small amount of culture material was suspended aseptically in the saline.
ONPG differentiation disc was placed in the tube and incubated for 20 - 30 minutes at 25 - 30°C and observed for colour change for 4 hrs. A yellow colour indicated a positive reaction.

**Methyl red and Voges - Proskaur test**

The MRVP test was used to distinguish between the bacteria that produced large amounts of acid and those that produce the neutral product acetone as end product.

5 ml MRVP broth (commercially available) was poured into the test tubes that were sterilized by autoclaving at 15 lb pressure for 15 minutes. The tubes were then inoculated with the test organisms maintaining suitable controls and incubated at 35°C for 48 hrs. Then 5 drops of methyl red indicator was added. The development of yellow colour indicated negative reaction, while no change in colour indicated a positive reaction.

**VP test**

The MRVP broth was inoculated with the bacterial isolates and 12 drops of VP reagent I and 2 - 3 drops of VP reagent II were added to the broth. The tubes were shaken gently and the reaction was allowed to complete for 15 - 30 minutes. The development of a crimson to ruby pink (red) colour was indicative of positive reaction and no change in colouration a negative test.
**Starch hydrolysis**

The ability to degrade starch is used as the criterion for determination of amylase production by a microbe. In the laboratory the absence or presence of starch in the medium was tested by using iodine solution as an indicator. Starch in the presence of iodine produces a dark blue colour or the blue medium and it is an indicative of amylolytic activity.

Starch agar medium was prepared, cooled to 45°C and poured into sterile petridishes. A single streak inoculation was made at the center of the plates and incubated for 48 hrs. at 37°C. The surface of the plates was flooded with iodine solution with a dropper for 30 seconds. A typical positive starch hydrolysis reaction was noticed by the formation of a clear zone surrounding the microbial colonies. A negative reaction was indicated by dark blue colouration of the medium.

**Urease test**

It is a useful diagnostic test for identifying the presence of bacteria. It is performed by growing the test organisms on urea broth containing the pH indicator phenol red (pH 6-8). During incubation, microorganisms possessing urease will produce ammonia that raises the pH of the medium (to pH 8.4). The colour of phenol (indicator) changes from brownish red to deep pink. Failure of the development of a deep pink colour due to no ammonia production is evidence of a lack of urease production by the microorganisms.
Maslen’s urea broth was prepared and inoculated heavily with bacterial growth from a fresh agar culture not more than 18 hrs. old and incubated at 37\(^0\) C for 5 - 18 hrs. The rapid development of a red colour indicated urease activity.

**Acid production from carbohydrates**

Phenol red broth base was prepared in the test tubes and inoculated with 18 - 24 hr. bacterial culture. Different carbohydrate discs were placed in the test tubes. They were incubated for 24 - 48 hrs. and observed for the production of acid. A change in colour of the broth to yellow was indicative of a positive reaction.

**H\(_2\)S Production**

The test organism was inoculated into a tube of TSA by stabbing the butt and streaking the slope. It was observed daily, up to 7 days for blackening due to H\(_2\)S production.

**Gelatin hydrolysis**

Plates of gelatin agar were inoculated and incubated for 3 days. The surface was flooded with 30% trichloro-acetic acid. Clear zones indicated areas of gelatin hydrolysis.

**Phenylalanine deaminase test**

This test was performed to test the ability of organisms to deaminate phenylalanine to phenyl pyruvic acid. Phenylalanine agar was prepared and poured on slants and the bacterial culture was inoculated and incubated for 18 - 24 hrs. at 37\(^0\) C. After incubation, 4 - 5 drops of fresh 10% ferric chloride solution was flooded over the growth of slant. Green colour indicated a positive reaction.
Citrate utilization test

A single streak was made over the surface of a slope of simmon’s citrate agar and examined daily up to 7 days for growth and colour change. Blue colour of the medium indicated that the citrate was utilized.

Tween 80 hydrolysis

On the surface of Tween 80 nutrient agar, the test culture was inoculated and incubated at optimal temperature. An opaque halo precipitation around the bacterial growth indicated the hydrolysis of tween.

Tween medium

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Peptone</td>
<td>10 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>5 g</td>
</tr>
<tr>
<td>CaCl₂, 2H₂O</td>
<td>0.1 gm</td>
</tr>
<tr>
<td>Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
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</table>

All the ingredients were dissolved and adjusted to pH 7.4. The solution was taken in 500 ml flask and were sterilized at 121°C and 5 ml the medium was added aseptically to each flask to give a final concentration of 1%. The medium was then dispersed into petridishes.
Pigment production

Nutrient agar plates were inoculated with a drop of a high suspension of the organism and incubated for 24 hr at 37\textdegree\ C and then transferred to room temperature and observed for 5 days. Colours were recorded as red, orange, yellow, green, violet, brown or negative i.e that which produced none of these colours. Histopathological changes were also observed by fixing the tissues of diseased crab in Davidson’s fixative and by examining the sections stained with Ehrlick’s Haematoxylin and Eosin as counter stain.

5.3. RESULTS

A total of 738 individuals (345 males and 393 females) in the second year of study were examined for the presence of microbial diseases. Among the 738 individuals examined 386 individuals (157 males and 229 females) showed the occurrence of microbial diseases. Table -19 (page 103)

5.3.1. Shell disease

Among the 158 individuals (67 males and 91 females) showed the symptoms of shell disease. The exoskeleton of the infected organisms showed lesions on the tips of walking legs, ventral sides of chelipods and exoskeletal spines. Gills clogged with detritus, possessed dark brown coating contained localized thickenings and necrosis. Progressive darkening and softening of the exoskeleton particularly on ventral surface. Dark brown to black spots with red margins on the carapace and chelipods. The chitin in the centre of spot become pliable and destroyed exposing the underlying muscle.
The disease was recorded to 28% in males and 30% in females during post-monsoon (January). Minimum occurrence of 15.63% in males was noticed in summer season (June) and 16.66% in females during pre-monsoon season (September) Plate - 2 (page 104)

5.3.2. Black gill disease

Nearly 168 mud crabs (71 males and 97 females) showed the occurrence of black gill disease. The symptoms are black colouration of the gills, presence of slit between lamellae and the presence of epiphytic and epizootic organisms in the gills. Maximum prevalence of 30.77% in males and 40% in females was noticed during post-monsoon (February and January) and the minimum occurrence of 13.79% was noticed in males during monsoon (October) and 16.66% in females during pre-monsoon (August) and monsoon season (October).

5.3.3. Vibriosis

A total of 60 crabs, 19 males and 41 females showed the occurrence of vibriosis. The occurrence of the disease is characterized by the loss of appetite, weak response to stimuli and lethargy. Maximum prevalence of 16.60% in males was observed during pre-monsoon season (August) and 14.29% in females during summer season (May). The minimum occurrence of 3.22% (males) in monsoon - November and 6.90% (females) was noticed during pre-monsoon season (July).
The bacterial isolates of diseased crabs formed medium sized, entire, circular, cream coloured, semi transparent colonies on nutrient agar and Zobell’s marine agar. The bacterium was found to be gram negative, rod shaped and motile with a single polar flagellum. It exhibited optimum growth on Nutrient agar, Macconkey agar, Zobell’s, marine agar and TCBS (Thio-sulphate citrate bile sucrose) agar and showed positive reaction to oxidase, catalase, acid production from glucose, nitrate reduction test and glucose fermentation test. It was sensitive to O/129 Vibrio static agent. Based on these characteristics it was identified as the genus Vibrio. The transparent colonies were also found to be gram negative, motile responded positively to oxidase, catalase, tween 20 and tween 80 hydrolysis. The bacteria also produced alkali in the open tube of O/F medium and exhibited optimum growth on the cetrimide agar. Based on all these characteristics the bacteria was identified as the genus Pseudomonas.

5. 4. DISCUSSION

The present study showed the occurrence of shell disease in crabs collected from Gulf of Mannar of Tuticorin coastal waters. The disease is variously referred to as spot disease, brown spot disease, black spot and spotted disease and manifests as black spots and lesions in the exoskeleton. The disease cause various kinds of exoskeletal lesions as a result of the action of chitinoclastic bacteria. Similar finding was reported by
Sindermann (1990) that shell disease cause visible lesions on the body cuticle, appendages or gills of affected crustaceans. Depending upon the severity of infection, the disease has caused morbidity and mortality of various degrees, thus requiring investigations on its aetiology.

In general the maximum prevalence of disease was noticed in post-monsoon season. This season is normally found with rich organic load after the monsoon season and also the recovery period of primary production. This condition could be conducive for the bacterial growth during post-monsoon season as evidenced in the present study. Gopalana and Young (1975) reported that high levels of organic matter provide ideal conditions for the growth of chitinoclastic bacteria.

The diseased crabs in the present study have shown the symptoms of brown spots on the exoskeleton and in the development of various kinds of lesions in the exoskeleton. As a result, the crabs become lethargic. The disease usually disappears after moulting and the newly formed shell was not affected except by reinfection (Mc Leese, 1965). The causative agents of the disease are chitinoclastic bacteria, that have the capacity to produce extra-cellular lipases, proteases and chitinase which are capable of damaging the cuticular layer of the exoskeleton (Lightner, 1983). Rosen (1970) and Fisher et al. (1978) have reported that these bacteria which are the causative agents of the shell disease
originated through the exoskeletal structures due to the wounds formed by injuries and due to mechanical trauma and physiological or notational stress. This they did by isolating chitin degrading bacteria from live lobsters which were able to decompose pure chitin in saline solutions containing no other nitrogen or carbon source. Further studies have revealed that the bacterial agent involved in the production of the disease vary from species to species and within the same species at different regions.

Sindermann (1990) discussed about the bacterial diseases of crustaceans and mentioned the names of over 30 species of chitin destroying bacteria, of which half have been isolated from the shell of crustaceans. Bacteria belonging to the genera Beneckea, Vibrio, Pseudomonas and Aeromonas were identified as associated with the disease. It was already proved beyond doubt that these bacteria were found to be the maximum number in the water, sediment and also on the animal already discussed in the Chapter 4. Getchell (1989) isolated Vibrio sp., Pseudomonas sp., Aeromonas sp. and other gram negative bacteria including Flavobacterium, Spirillum, Photobacterium and Pasturella from shell diseased M. rosenbergii, Peneaus sp. and blue crabs.

The present study revealed the presence of Pseudomonas sp., Vibrio sp. as the causative agents of this disease. In conformation with the present study (Jayasree, 1998) also reported that Pseudomonas sp and vibrio sp form the the causative agents for shell disease in crustaceans. It was possible that the synergetic effects of these species together
with environmental stress had caused the disease. The disease might also be due to the industrial effluents, domestic discharge and the fly ash waste from the thermal power plant. The deteriorated environmental conditions and increased impurity evidenced by increased organic load and hypoxic conditions probably provided ideal conditions for the growth of opportunistic bacteria causing shell disease. The present finding is in agreement with the observation of Jayasree (1998), who reported that fresh water prawns too were subjected to stress due to pollution by domestic sewage and effluent from adjacent industries showed the signs of shell disease. In confirmation with the present study, the females showed high prevalence. Rongxing et al. (2000) also reported a high percentage of females were attacked by this disease showing shell lesions. The high prevalence of this disease in females might be due to the availability of more number of female population in the environment. The reason that could be attributed for the high prevalence of shell disease was that berried females become inactive and dwell at the bottom for a longer time in the sediment than males. Dhevendran (1978) reported a higher percentage of heterotrophic and other marine pathogenic bacteria in the sediment. When the berried females staying a longer periods in the sediment may harbour more pathogens than the males, leading to the disease known as shell lesion.

The present study showed the presence of silts between the gill lamellae, presence of epiphytic and epizootic organisms, black colouration of gills, melanization and
necrosis of the gill tissue. Similar observations, microbial fouling, especially of the gills had shown to be lethal to lobster, shrimp and crab larvac (Nilson et al; 1975 Johnson, 1977). In shrimps black gill disease characterized by necrosis of all types in the dorsal gill filaments was reported by Couch (1977) and Nimmo et al. (1977). The cause of this disease appears to be the high organic load, mainly due to 

\textit{Vibrio} spp and partly by \textit{Pseudomonas} spp (Nimmo et al. 1977).

The present study has observed the presence of vibrosis in the infected crabs of \textit{S. serrata} collected from Tuticorin coastal waters. \textit{Vibrio} spp. are the causative factor of this disease. The symptoms of this disease was more noticeable in the warmer months. The presence of the disease has been identified by the loss of appetite, weak response to stimuli and lethargy. (Krantz et al, 1969; Egidius, 1987; Austin and Austin, 1989; Sindermann, 1990). Blue crabs from middle Atlantic coast also showed similar symptoms (Colwell et al., 1975; Johnson, 1976). Presumptive vibriosis has also been reported in the shore crab, \textit{Carcinus maenas} from northern European coast. Spindler-Barth (1976) also reported the affected haemolymph of the individuals appeared milky because of the presence of numerous bacteria.

The present study showed that the higher prevalence of virbiosis in the summer season might be due to the discharge of effluents from the industries, the domestic
sewage from adjacent household and the waste water from various sources. As mentioned by Sindermann (1990), the maximum prevalence of vibriosis during summer and pre-monsoon season coincided with higher temperature in the study area could be the ideal environmental conditions for the proliferation of *Vibrio* spp. Apart from the above said factors Johnson (1976) also reported maximum temperature in the summer months could be the ideal condition for vibriosis.

Vibriosis is obviously an important problem for crustaceans especially for captive and cultured populations in which environment stressors or injuries facilitate invasion and multiplication of facultative pathogens.
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Table 19

Plate 2

Fig. 1

Vibrio sp.

Fig. 2

Shell disease

Fig. 3

Shell disease