ON THE OCCURRENCE OF ECTOCOMMENSAL CILIATES ON *Metapenaeus dobsoni* (MIERS) IN RELATION TO WATER QUALITY PARAMETERS IN POND ECOSYSTEM AT VYPEEN

DISSERTATION SUBMITTED BY

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Certificate

Certified that the dissertation entitled **'ON THE OCCURRENCE OF ECTO COMMENSAL CILIATES ON *Metapenaeus dobsoni* (MIERS) IN RELATION TO WATER QUALITY PARAMETERS IN POND ECOSYSTEM AT VYPEEN'** is a bonafide record of work done by Miss **Deepa K.G.** under our guidance at the Central Marine Fisheries Research Institute during the tenure of her M.F.Sc (Mariculture) Programme of 1995 - 1997 and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.

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Declaration

I hereby declare that this thesis entitled **'ON THE OCCURRENCE OF ECTOCOMMENSAL CILIATES ON *Metapenaeus dobsoni* (MIERS) IN RELATION TO WATER QUALITY PARAMETERS IN POND ECOSYSTEM AT VYPEEN'** is based on my own research work and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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DEEPA K.G.

सारांश

वाइपीन के एक ताल से प्राप्त ज्वारनदमुखी झींगा मेटापेनिअय डोबसोनी में दिखाए जाने वाले प्रोटोजांअन सहयोगियों पर अध्ययन चलाया गया । शरीर के विभिन्न भागों में पक्षमाभों की उपस्थिति और क्लोम में इनकी सान्द्रता का आकलन किया गया । पक्षमाभों का सुक्ष्मदर्शीय निरीक्षण करने पर इनमें परिपक्षमाभी पक्षमाभ जूताम्नियम जाति, वेर्टिसेल्ला जाति, एपिस्टाइलिस जाति और सक्टोरियन असिनेटा जाति दिखाई पडी । परीक्षण की गई 90 % जातियों में जूताम्नियम जाति की प्रमुखाता थी । पक्षमाभ की उपस्थिति का सह संबंध देखने के लिए पानी की गुणता के विभिन्न परारों का विश्लेषण भी किया गया । इस से यह व्यक्त हो गया कि झींगों में ग्रसन करने के लिए प्रोटोजोअनों को प्लवकों की पुल्लिका, निर्लबित ठोस पदार्थ और ऑक्सिजन का अपक्षय अनुकूल बनाते हैं । पक्षाभों में लवणता का सहन और रोग ग्रसन में पानी की गुणता का प्रभाव जानने के लिए भी परीक्षण किए गए । इस से यह मालूम पडा कि 30 पी पी टी लवणता में रोग ग्रसन अधिक था और स्वाभाविक स्थिति की अपेक्षा प्रग्रहण की स्थिति में ग्रसन अधिक था । एम. डोबसेनी के विभिन्न आकार के नमूनों में पक्षाभों के ग्रसन का विश्लेषण करने पर 51 - 60 मि मी आकार वाले झींगों में ग्रसन की प्रवणता अधिक देखी गई । यह भी देखा गया कि झींगों के मध्य और पार्श्व क्लोमों की अपेक्षा पश्च क्लोम में पक्षाभों का समाहरण अधिक था । कुल निर्लंबित ठोस पदार्थों में पक्षाभों की प्रचुरता से यह व्यक्त होता जाता है कि झींगों में पक्ष्माभों का ग्रसन पानी की कम गुणता की ओर इशारा करता है ।

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PREFACE

Fishery sector has a place of pride in the national economy of India. Shrimps are the single item in the basket of marine products with universal taste-remain the backbone of Indian marine product export. Due to the declining trend of prawn catches from the sea, brackish water and fresh water prawn farming has immense scope to fulfill the growing demands of the population. The culture practices are mostly restricted to *Penaeus* sp. obviously because of certain special qualities attributed to it. However, in traditional prawn culture, practised in coastal waters, prawns other than *Penaeus* sp. also enter the culture system and grow. Thus about 60-80% of the catch is contributed by *Metapenaeus dobsoni* and smaller sizes of *Penaeus indicus. M. dobsoni* forms a major part of the all India landing of prawn followed by *P. indicus* and *P. monodon.*

One of the major problems faced by prawn farmers is the vulnerability of these animals to contract diseases. The most important requirement of any type of culture system is clean water. Environment and water quality parameters may be direct or accessory aetiological factors (Stressors) of prawn diseases. General environmental conditions, substrate characteristics and physical, chemical and biological components of the culture medium are included in this broad category. When populations of stressed animals are dense, even low levels of stress may give pathogen an opportunity to enter upon the exponential segment of their growth curves and cause devastating epizootics. A number of diseases are of undetermined aetiology. Even in cases where etiology is known, our understanding of the host-agent-environment interaction is incomplete.

The present work is an attempt to find out the relationship between ectocommensal ciliates in *Metapenaeus dobsoni* and water quality parameters.

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INTRODUCTION

Among the more serious diseases of shrimps are those caused by non-infectious epicommensal organisms. Almost all aquatic habitats are used or exploited by some species of peritrichous ciliates. Both sessile and mobile forms of peritrich make extensive use of the tegument of both invertebrates and vertebrates as substrates upon which to live and reproduce. Many peritrichs are epibionts (epizoonts, ectocommensals or ectoparasites) (Couch, 1983). Crustaceans are known to harbour several species of ectocommensalic ciliates especially peritrichs (Kidder and Summers, 1935; Stiller, 1953; Kane, 1965; Couch, 1971). An encysted form of an unidentified apostome ciliate, associated with black gill disease in *Penaeus duorarum* has been described by Couch (1978). Another ciliate *Parauronema* sp. has been observed by Couch (1978) in the haemocoel of protozoea, mysis and juvenile stages of living, moribund and dead *Penaeus aztecus*. Suctorians such as *Acineta* sp. (Johnson, 1978), *Ephilota* sp. (Couch, 1978) and *E. gemnipara* (Gucatan *et al.*, 1979) have also occasionally been encountered on the body and gills of penaeid prawns. Protozoan associates found in the estuarine shrimp *Metapenaeus monoceros* (Fabricus) and the tenaidacean *Apseudes chilkensis* (Chilton) from Cochin backwaters have been described by Santhakumari and Gopalan (1989). A peritrich ciliate *Zoothamnium rigidium* (Precht), and a heterotroch *Stentor coeruleus* (Ehrenberg) were reported in both the hosts. Another ciliate *Lagenophrys cochinensis* was observed on the tenaidacean (Santhakumari and Gopalan, 1980). A variety of ectocommensal protozoans including *Epistylis* sp., *Cothurnia* sp., *Acineta* sp., *Zoothamnium, Lagenophrys* have been observed on the carapace of wild crawfish and rarely for the gill chambers (Scott and Thune, 1986).

With the exception of Shomey's (1955) Couch's (1971) and Overstreet's (1973) works, no studies concerning epizootics of commensal peritrichs on natural population of crustaceans have been reported. (1) seasonal prevalence of ciliate Lagenophrys callinectes in the natural population of blue crab; and (2) seasonal intensity of infestation of individual hosts; both in relation to seasonal behaviour of the host was studied by Couch (1966, 1983). Relatively high rate of infestation with more dense growth of larger colonies observed on the reared individuals than those collected from the nature, which showed the probable conduciveness of stagnant water and overcrowding for infestation as has been observed by Overstreet (1973). Species of *Zoothamnium* infestation is rare to moderate in cultured populations and rare in individuals of wild populations (Overstreet, 1970; Santhakumari & Gopalan, 1980; Soni, 1986).

Heavy infestation of an encysted form (pleuront) of an un-identified apostome ciliate, associated with blue gill disease in *Penaeus duorarum* occurs on the gills of prawn during periods of warm to moderately cool weather (Couch, 1978). Seasonally infestation is moderate during pre-monsoon (April to June) and rare during other months (Soni, 1986).

In a healthy environment penaeids can tolerate a large number of these epicommensals with no apparent detrimental effects. The protozoan epibiont *Zoothamnium* sp., has been reported to preferentially locate on the gill lamellae of *Macrcbrachium* while other protozoan epibionts do not show site specificity (Hall, unpublished data). Shomey (1955) and Couch (1973) have examined the physical relationship of the lorica and trophonts of *Legenophrys* sp. to the tissue of their host. Heavy infestation of a cuticle covering ecto commensal protozoen could interfere with proper respiratory or excretory functions of host gill tissue (Couch, 1967, 1978; Overstreet, 1973). Johnson (1972) has attributed instances of mortality of pond-reared shrimps to infestation of species of *Zoothamnium* sp. Stalked peritrich such as *Vorticella* sp., *Zoothamnium* sp., *Epistylis* sp. and *Lagenophrys lunatus* are generally found attached to gills, appendages and body surfaces of larval, post-larval, juvenile and adult penaeids attachment on the gill can cause hypoxia and death (Overstreet, 1973, 1978). Couch (1978) has pointed out that certain species of the genus *Ephilota* may act as stressors in infected prawn while *E. gemnicola* infestation on *P. monodon* larvae in rearing tank has been implicated for weakening and mortality of the populations (Gucaten *et al.*, 1979). The deterioration of exoskeleton due to overcrowding of this ciliate might serve as portals for other harmful organisms such as bacteria and fungi, capable of producing serious shell diseases (Rosen, 1970; Gopalan and Young, 1975). This can lead to mortality of the host (Overstreet, 1973). However epizootics of *Epistylis* sp. fouling on otherwise healthy appearing sub-adults and adult prawns in which upto 40% of the pond population were affected have infrequently occurred in Hawaiian prawn ponds (Brock, J.A; unpublished data, 1981).

However heavy infestation of *Zoothamnium* sp. in the gills of crowded pond reared shrimps in conjunction with low dissolved oxygen levels have been implicated in mass mortalities of cultured stock in Texas & Louisiana (Johnson *et al.*, 1973; Overstreet, 1973). Experimental infestation of *Z. rigidium* in shrimp and the effect of stress produced by high turbidity and low oxygen tension in the infested prawns were carried out by Overstreet (1973) and in tenaidecean by Santhakumari and Gopalan(1980). Lightner (1977) and Fisher (1977) have indicated that epibionts may restrict gas exchange by accumulating on eggs and gill surfaces in susceptible crustaceans, thus causing mortality through asphyxiation.

Johnson *et al.*, (1973) reported the loss of an estimated 2000 pond held prawn the white shrimp in a single day, due to the presence of large numbers of *Zoothamnium* sp. on the gills and a reduction in dissolved oxygen levels. Mortality was attributed to anoxia as the mortalities occurred when the infestation of ciliates became heavy enough to restrict oxygen exchange and when the dissolved oxygen levels in the pond dropped below 3 ppm to a low level of 2.6 ppm. Death occurs when the effective respiratory surface of the gill is reduced by presence of numerous colonies of *Zoothamnium* species and subsequently the suffocation of the animal (Lightner, 1975). Death usually coincides with periods of low concentration of dissolved oxygen in the water, a common condition following several warm overcast days or following decomposition of large algal blooms (Overstreet, 1975). Lightner (1975) pointed out that in normal conditions, when *Zoothamnium* sp. was absent, dissolved oxygen levels of 2.6 ppm was not lethal, as good survival was experienced with *Penaeus aztecus* in culture ponds, even when the dissolved oxygen levels fell to 1 ppm. Rajendran *et al.* (1982) reported that protozoans are found to affect the juvenile prawns in the culture ponds where the dissolved oxygen levels in pond water decreased to 1 ppm due to non-flushing of pond water with tidal water.

Soni (1986) in his doctoral thesis reported mortality due to heavy infestation of *Zoothamnium* sp. in culture ponds at Valappu (Cochin, Kerala) when the dissolved oxygen level was as low as 2.36 ppm. The gills of the affected prawns were found to be heavily infested with *Zoothamnium*. Sp. Later, Hameed (1989) reported that *Zoothamnium* infestation in protozoea of prawn can cause reduction in its heart beat and further in the infected larvae, the heart beat stopped its continuous beating frequently and started functioning again after an interval of 5 seconds. The virulence was also observed to be rather slow in the infested larvae as compared to that of the normal and healthy ones.

Ectocommensal ciliates utilize crustacean cuticular surface as the substrate for attachment and rely on concentrated bacterial population found in water with high organic load for nourishment. As water quality deteriorates bacterial concentration increases and in such conditions peritrichous ciliates such as *Cothurnia* sp., *Epistylis* sp. and *Zoothamnium* sp.which feed on bacteria flourish. Conversely the presence of the suctorian *Acineta* which is saprotrophic on ciliated protozoans suggests that organisms higner in the food web than bacteria are present (Scott and Thune, 1986). Incidence of peritrichous ciliate *Cothurnia* sp. and *Epistylis* sp. both bacteriovores increase with increased turbidity. Sawyer *et al.* (1976) suggested that suctorian *Ephilota* sp. found on lobster and rock crab may be useful as an indicator species. Brock (1983) reports species of *Epistylis* infestation in *Macrobrachium* hatcheries in association with extremely poor water quality conditions possibly resulting from lack of adequate pond water turn over. Scott and Thune (1986) found significant correlation between the incidence of ectocommensals and primary productivity on freshwater red swamp crawfish *Procambarus clarkii* (Girard). Hudson and Lester (1992) worked on the relationship between water quality parameters and ectocommensal ciliates on prawns (*Penaeus japonicus* Bate) in aquaculture and found out that as the water quality decreased, the number of *Zoothamnium* sp. increased and the number of *Cothurnia* decreased.

The present study was carried out in the prawn culture fields at Vypeen island in Cochin backwaters. Cochin backwaters form an integral part of Vembanad lake-the largest backwater system in Kerala, India. This backwater system is connected to the Arabian sea at two points - one at Cochin and the other at Munambam through which seawater enters into the backwater due to tidal influence.

PLATE - I

Experimental pond at Puthuveyppu

MATERIALS AND METHODS

Site Selection :The present study on the relationship between ciliate infestation on *Metapenaeus dobsoni* and water quality parameters was carried out in a pond ecosystem at Puthuveyppu at Vypeen island.

Area of Study : Site for the study of ciliate infestation on *M.dobsoni* was fixed in a pond at Puthuveyppu. (PLATE-I) The pond was having an area of approximately 0.1ha. The water depth was about 1m. The pond was having a clay bottom. It was connected to the feeder canal through a sluice gate.

Stocking was done in this pond by letting in tidal water into the pond during high tide. The juveniles were trapped as the tidal water recedes at low tide by putting a screen across the sluice gate.

Study Period: The present study was carried out for a period of one month during June 1997. The time of sampling was fixed at 10.30-11.00 A.M.

Identification of the prawn: Body slightly tomentose in patches. Rostrum extending a little beyond the dorsal teeth and having a well marked double curve. Antero-lateral angle of the carapace without spine. The inner antinnular flagellum larger than the outer, extending its peduncle in length. The last pair of thoracic legs do not nearly reach the middle of the antennal scale.

Colouration : The antinnule, antennae and antennal scales are dotted with red. There is a double row of reddish spots on the telson, the margin being greenish.

Method of Study: Weekly samples of *M.dobsoni* and water and sediment samples were taken at the same time. *M.dobsoni* (PLATE-II) being a bottom dweller, it was decided to collect water samples from a depth of about 10cm above the bottom of the pond.

PLATE - II

Experimental prawn *M. dobsoni*

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Sampling of Prawn: Sampling of prawns (*M.dobsoni*) was done every week using cast net. They were transferred to a well -aerated 12 ltr plastic bucket containing the pond water, for live examination. About 20 prawns were randomly selected for analysis.

Sampling of water and Sediment: Ambient water temperature was recorded at the site itself using a mercury thermometer with 0-50 calibrations. Water samples were collected in two 1ltr. polypropylene bottles for the analysis of the nutrients and total suspended solids (T.s.s.). Bottles for nutrient analysis was fixed immediately with 1ml chloroform. All the bottles were washed twice with ambient water before sampling. To estimate the dissolved oxygen (d.o.) 125ml reagent grade corning bottles with B.O.D. stopper was used. To estimate the d.o. content of the water, bottle was immersed into the water. Care was taken not to entangle any air bubble while collecting the water. The bottles were re-stoppered inside the water column. The oxygen bottle was immediately fixed by 1ml each of winkler A and winkler B. Precipitate formed was allowed to disperse uniformly through out the bottles. Water sample for salinity estimation was collected in 100ml glass bottle and stoppered tightly. All the water samples were chilled during transportation.

How D.O was determined

Sediment sample for pH determination was collected using a p.v.c. pipe of 1inch diameter. pH was determined using Universal Indicator solution (Glaxo India Ltd.)

Examination of Prawns: Live samples of *M.dobsoni* were brought to the laboratory immediately after collection. Size and sex of the prawns were recorded. Since ciliates are seen concentrated mostly on the gills, only gills were examined for occurrence of ciliate infestations. Branchiostegite or gill cover on the left side of the prawn was cut open to expose the crescent shaped gills. It was observed the gills increased in size from anterior gill to the posterior gill. The gills were seen attached to the thorasic wall at the middle of its length only and the point of attachment is called gill -root. Gills were dissected out using a forceps and placed on a glass slide containing a few drops of pond water. The gills were grouped into three-anterior, middle and posterior. They were examined under a compound microscope after putting a cover slip. The number of ciliates per gill were counted and the individual ciliates were identified using standard keys (Kudo, 1966).

Prevalence of ciliate infestation in the population of *M.dobsoni* was calculated as the total number of infested prawns divided by the total number of prawns examined. This value when multiplyed by 100 will give the percentage prevalence of ciliate infestation in *M.dobsoni* for that day. Similarly abundance was calculated as the total number of ciliate observed in the gills divided by total number of infested prawns. For the calculation of total number of ciliates, their numbers on the anterior, middle and posterior gills were pooled together, for all the animals and divided by number of infested prawns.

To study the size prefarence of ciliate about 101prawns samples were analysed for infestation. The prawns were grouped into various size groups of 10mm size and prevalence and mean intensity of infestation was calculated as mentioned earlier.

Laboratory analysis of the water sample: Water samples brought from the field were analysed for the various hydrographic parameters. Determination of salinity, d.o.,t.s.s., phosphate and nitrate were made using standard procedures.

DO, T.S.S

P.T.O

Analysis

The water samples for oxygen, salinity, nutrients and total suspended solids, brought back to the laboratory were analysed as follows

Salinity

Reagents required :

1. Silver Nitrate (24.5 gm/litre)
2. Potassium Chromate - (10%) 10 gms in 100 cc.
3. *Standard* Sea Water

Procedure :

Pipette out 10 cc of Standard Sea water into a 250 cc conical flask. Add 4 drops of potassium chromate solution and using a mechanical Stirrer titrate aganist silver nitrate solution. Repeat to concordance. Pipette out 10 cc of the sea water sample into the conical flask and proceed as above.

Salinity is calculated as follows :

Let Volume of *Silver nitrate for 10 cc standard* Sea water = V1

Volume of silver nitrate per 10 cc Sample = V2

Salinity of Standard Sea water = S

$$\text{Salinity of sample} = \frac{V_2 \times S}{V1}$$

Dissolved Oxygen

Reagents

1. Sodium Thiosulphite solution (1.25 gms in 1 litre).
2. Starch solution - 1gms starch made into a paste with distilled water and diluted to 100 cc, boiled and kept.
3. Winkler Solution - A (20 gms of Manganese chloride in 100 ml water).

4. Winkler solution - B (41 gm of sodium hydroxide +25 gm of potassium iodide in 100 cc water).

5. Concentrated hydrochioric Acid.

6. Standard potassium iodate. (Accurately weigh out 0.1784 gm of potassium iodate into a 1 litre volumetric flask and dissolve and make up to the volume. This is 0.005N).

7. *Potassium Iodide.*

Procedure

Collect the water sample in a 125 ml glass stoppered bottle without entangling any air bubbles. Take out the stopper and add 1cc each of Winkler - A and winkler - B solution. Close the bottle. Shake the bottle gently till the precipitation formed is evenly distributed. Allow to settle. Then add 2ml conc. hydorochioric acid, close the bottle and gently shake till the precipitate is completely dissolved.

Pipette out 10ml of potassium iodate solution into a conical flask. Add 1 gm of potassium iodate and 2 ml of conc. hydrochioric acid. Dilute to 100 ml and titrate against sodium thisulphate solution till the blue colour disappears. addition of indicator ?

Pipette out 100 ml of the preserved sample and titrate against std. sodium thiosulphate as above.

Calculation

Calculate the normality of potassium iodate as $N_1 = \frac{\text{Weight / litre}}{35.67}$

Titrate value of thiosulphate for 10ml of potassium iodate = V_1

Calculate normality of thiosulphate as $N_2 = \frac{N_1 \times 10}{V_1}$

Hence amount of dissolved oxygen in ml/litre = $\frac{V_1 \times N_2 \times 8 \times 1000 \times R}{100 \times 1.429}$

(Where 1.429 being weight of 1ml of oxygen in miligrams. R is known as the corrections factor and which is roughly equal to 1.01 in majority of the cases)

Reactive Phosphorus

Reagents

1. Ammonium molybdate solution

   15 gms of A.R. quality Ammonium molydate in 500 ml distilled water. Store in plastic bottle, keep away from sunlight.

2. Sulphuric Acid Solution

   140 ml of A.R. quality sulphuric Acid added to 900 ml of distilled water.

3. Ascorbic Acid solution.

   Dissolve 27gm of ascorbic acid (A.R. quality) in 500ml distilled water. Freeze the solution and for use then and bring to laboratory temperature. After use again freeze the solution. Store the solution in frozen state and for use bring it to room temperature.

4. Potassium Antimony tartrate solution.

   Dissolve 0.34 gm of good quality of potassium Antimony tartrate in 250ml distilled water.

5. Mixed Reagent.

   Mix together 50 ml of Ammonium molybdate. 125 ml of Sulphuric Acid, 50 ml of Ascorbic Acid and 25 ml of Antimony tartarate solution. Mix well and the solution can be kept for 6 hours. And the above quantity is sufficient for about 50samples.

Procedure.

To 10ml of sample at laboratory temperature add 10 ml of Mixed Reagent . After 5 minutes and preferably within the first 2-3 hours measure the extinction of the solution. in a 10 centimeter cuvette at a wave length 8850^0 A units in a spectrophotometer.

Phosphate Standard.

Dissolve accurately 0.816 gm of anhydrous potassium dihydrogen phosphate in 1000 ml of distilled water. Store in a dark bottle with 1 ml of chloroform. 1 ml of the solution = 6µg. at. / ltr phosphate phosphorus. 1 ml of this solution is made upto 100ml. From this 5ml is taken and diluted to 100ml. 100 ml sample is taken in a conical flask, and 10 ml of mixed reagent is added to the standard and sample. After 10 minutes the colour comparison of these 2 solutins is made in a Spectrophotometer.

The strength of the colour developed being proportional to amount of phosphate, calculate the phosphate concentration in sample using the standard strength of the standard potassium phosphate solution.

Nitrate

Reagents

1. Phenol solution.

   Dissolve 46 gm of dry A.R. quality phenol in 1000 ml. of distilled water. It is stored in a glass bottle tightly.

2. Sodium hydroxide.

   Dissolve 20 + 0.5 gms of A.R. quality sodium hydroxide in distilled water. Cool and dilute to 2000 ml.

3. Buffer Reagent.

   Pippete out 25ml of phenol solution into a dry beaker and add 25 ml of sodium hydroxide solution. The solution is stable for one hour.

4. Copper sulphate solution.

   Dissolve 0.1 gm of A.R. copper sulphate in 1000 ml of distilled water.

5. Hydrazine sulphate solution.

   Dissolve 14.5 gm of A.R. quality hydrazine sulphate in 2000ml of distilled water. Store in a dark glass bottle. The solution is stable for one month.

6. Reducing Agent.

   Mix 25ml of copper sulphate solution and 25ml of hydrazine sulphate solution in 50 ml measuring cylinder. The solution is stable for one hour.

7. Acetone.

8. Sulphanilamide solution.

   Dissolve 5 gm of sulphanilamide in a mixture of 50 ml con. hydrochloric acid and about 300 ml distilled water. Diluted to 500 ml with water. It is stable for many months.

9. NI-Naphthyl Ethylene Diamine Di-hydrochloride solution (N.N.E.D)
   Dissolve 0.5 gm of N.N.E.D. is 500 ml distilled water. Store the solution in a dark bottle.

10. Standard Nitrate solution

    Dissolve 1.53 gm of analytical reagent quality potassium nitrate in 1000 ml. 1ml=15.0 ug. at / nitrogen. Dilute 5ml of this solution to 250 ml with water. It is stored in dark bottle.

Procedure

Measure out 50 ml of the sea water sample with a 50ml measuring cyliner into a 250 cc conical flask, when (sample should acquired room temperature). And 2 ml buffer reagent and mix. After the buffer has been added to all teh samples, add with rapid mixing 1.0 ml of reducing agent and keep the flasks away from sunlight in a dark place for about 20 hours. Add 2 ml of acetone, and after 2 minutes add 1 ml of sulphanilamide solution. After 2 minutes, but not later than 8 minutes add 1.0 ml of N.N.E.D solution and mix. — Method of Estimation?

Statistical Analysis:

Statistical analysis of the data was done using Carl Pearson's correlation coefficient Correlation analysis was performed for different water quality

PLATE - III

Experimental set up

parameters and ciliate prevalence and abundance; and between the water quality parameters. Wherever significant correlations were observed, regression analysis was done. The values obtained were analysed for the test of significance.

Laboratory Experiment:

To find out the role of stress at different salinity conditons on the occurrence of ciliates on *M.dobsoni* an experiment was conducted for a period of 10 days commensing from 9th to 19th June 1997. About 120 *M.dobsoni* of the size group 30-60mm were selected for the experiment. Prior to experiment, 30 animals each were acclimatized to the different salinity conditions of 13ppt, 20ppt and 30ppt.

Animals were stocked in four 60ltr fibre glass tanks. 30 animals each were kept at 13, 20 and 30ppt salinity conditions for a period of 10 days. They were given 50% water exchange per day except the tank with deteriorating water quality. Clam meat was given *ad libitum* as food for these prawns, during the period of the experiment. Excreta as well as uneaten food were removed by syphoning. The tank with deteriorating water quality, kept at 20ppt salinity was also stocked with 30prawns. All the tanks were given very good aeration through out this experimental period.

Five prawns each were sampled with 2days interval from all the four tanks towards the end of the experimental period. The length and sex of the prawn, prevalence and abundance of infestation in different salinity conditions were calculated as mentioned earlier.

PLATE - IV

Zoothamnium sp. and **Vorticella* sp. attached on to the gill-filaments X20.

**Acineta* sp. attached to the gill-filament X20

RESULT

Estuarine Environment:

During June 1997, water temperature in the culture pond ranged from 30-34°C, salinity 13.4-25.4 ppt, dissolved oxygen 2.8-7.6 ml per ltr, total suspended solids(T.s.s) 49-226 mg per ltr, pH 7.5-8.0, phosphate 12.0-30.7µg. at/ltr and nitrate 1.35 -2.03µ g.at/ltr (Table -1). These values did not vary significantly from surface to bottom as the depth was less than 1m in the pond. The mean values of salinity, dissolved oxygen and T.s.s. were 20.2 ppt, 5.2ml per ltr and 136 mg per ltr respectively, while those of phosphate and nitrate concentrations were 20.5µg.at/ltr and 1.7µg.at/ltr respectively in the culture ecosystem.

Ciliate Occurrence in *Metapenaeus dobsoni*

The ciliate population noticed in the gills of *M.dobsoni* consisted the groups of *Zoothamnium(81%), Acineta(16.9%), Vorticella(2%),* and *Epistylis(0.1%)* in their order of abundance (Plate IV&V) A total of 101 specimens of *M.dobsoni* were examined. The size of the prawn *M. dobsoni* ranged from 31-90 mm. The occurrence of ciliates in the different size groups of *M.dobsoni* in the culture pond is given is table-2. The examination of ciliates in the size groups of *M. dobsoni* did not show any size group preference by the ciliates. In the over all prawn population of culture pond, the mean values showed that the ciliates where more abundant in the size group of 31-40 mm, 51-60mm and 81-90mm and very less in the size group of 61-70mm (Table -3). Highest mean values of abundance in the population were observed in the size group of 81-90mm in the present investigation.

Among the infested prawns the size group of 51-60mm were found to be infested more with ciliates and less abundance of ciliates was recorded for the size group of 71-80mm (Table -4)

TABLE - I

HYDRO GRAPHIC FEATURES OF THE PRAWN CULTURE POND AT PUTHUVEYPPU - IN JUNE 1997

Date	Temperature (°C)	Salinity (ppt)	Dissloved oxygen (ml / l)	Total Suspended solids (mg / l)	pH of Sediment	PO_4 (µg.at./l)	No_3 (µg. at./l)
2/6	33	22.4	5.3	48.8	7.5	20.3	2.03
4/6	34	25.4	6.6	112.0	7.5	30.7	11.7
9/6	34	18.7	2.8	203.2	7.5	27.5	1.75
17/6	32	13.4	7.6	91.2	8.0	12.2	1.73
23/6	30	21	3.5	226.0	7.5	12.0	1.35
Mean	33	20.2	5.2	136.0	7.6	20.53	1.7

TABLE - 2

PREVALENCE OF CILIATE INFESTATION AMONG DIFFERENT SIZE GROUPS OF PRAWN - *M. dobsoni.*

Size group (m. m)	No. of Specimens analysed	No. of infested specimens	Prevalence (%)
31 - 40	7	4	57.1
41- 50	36	11	30.5
51 - 60	26	4	15.4
61 - 70	14	2	14.3
71 - 80	10	4	40.0
81 - 90	8	3	37.5

TABLE - 3

ABUNDANCE OF CILIATE INFESTATION AMONG DIFFERENT SIZE GROUPS OF PRAWN - *M. dobsoni* in the population.

Size group (m m)	No. of Specimens analysed	No. of ciliates (Total)	Average intensity of infestation / specimens the population.
31 - 40	7	19	2.71
41- 50	36	71	1.97
51 - 60	26	8	0.57
61 - 70	14	12	1.20
71 - 80	10	26	1.20
81 - 90	8	26	
	101	206	

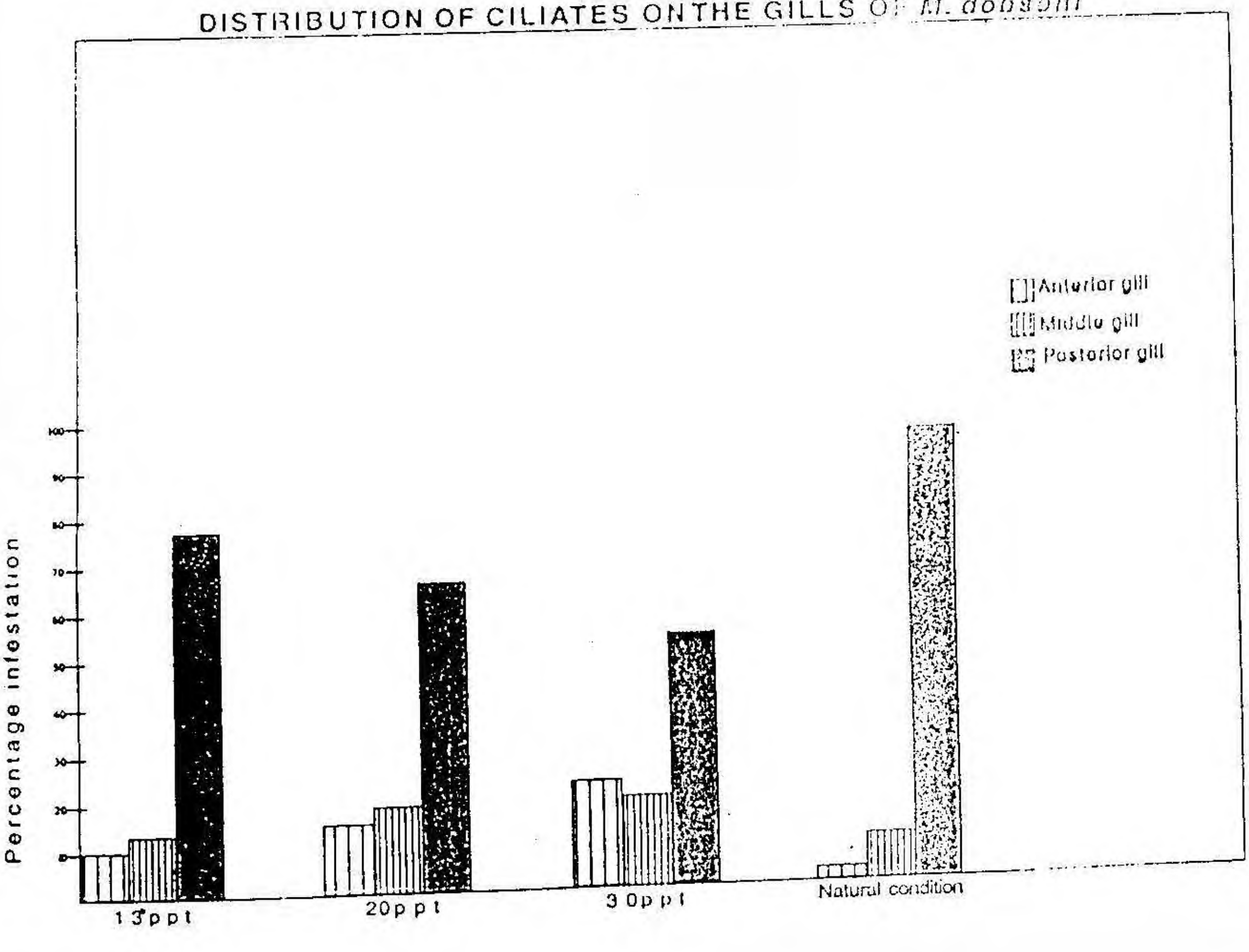
TABLE - 4

ABUNDANCE OF CILIATE AND ITS DISTRIBUTION AMONG DIFFERENT SIZE GROUPS OF INFESTED PRAWNS - *M. dobsoni.*

Size group (m m)	No. of infested specimen	No. of ciliates (Total)	No. of ciliates per specimens or Ab undance
31 - 40	4	19	4.75
41- 50	11	71	6.45
51 - 60	4	70	17.50
61 - 70	2	8	4.00
71 - 80	4	12	3.00
81 - 90	3	26	8.67

FIGURE - 1

DISTRIBUTION OF CILIATES ON THE GILLS OF *M. dobsoni*



The result of experiments conducted in the laboratory for 10 days to study the salinity preference of the ciliates at 13, 20 and 30 ppt are given in table-5. The results showed that the intensity of ciliate infestation in *M.dobsoni* at 30 ppt was high in the population in general and among infested animals (Table-5).

The experiments conducted to study the location preference of ciliates in the anterior, middle and posterior gills of *M.dobsoni* at 13, 20 and30 ppt salinity under laboratory conditions showed that the posterior gills were concentrated with ciliates than the middle and anterior gills (Table-6). The observation of prawns in the natural condition of pond showed their preference to posterior gills. The data also showed that the number of infested prawns were relatively more in the laboratory reared conditions as compared to the pond ecosystem.

The ciliate prevalence and abundance on *M.dobsoni* in the pond showed positive correlation with turbidity ($r = +0.4706$ and $r = 0.3358$ respectively). With the dissolved oxygen of pond water, abundance ($r = -0.756$) and prevalence ($r = -0.68$) showed negative correlation, but not to any significant levels. When correlations were tried in between water quality parameters, it was observed that temperature and d.o., T.s.s. and d.o., T.s.s. and nitrate are negatively related whereas temperature and phosphate, temperature and nitrate showed a positive trend.

In the experiment conducted to find out the role of deteriorating water quality on the occurrence of ciliate infestation, it was found that as the water quality becomes bad, suctorians - *Acineta sp* appear in the culture tank (tables 7).

TABLE - 5

SALINITY PREFERENCE AND INFESTATION RATE OF CILIATES PER GILL OF M. DOBSONI [EXPERIMENT DATE 9 - 19 JUNE 1997]

Salinity of the medium	No. of Prawns examined	No. of infested prawns		No. of ciliates noticed	Mean intensity of infestation per animal (in total)	Mean intesnity per infested animal
		number	% prevalence			
13 ppt	15	11	73.1	127	8.47	11.54
20 ppt	14	8	57.3	105	7.50	13.12
30 ppt	15	11	73.1	238	15.87	21.64

TABLE - 6

OCCURRENCE AND AREA PREFERENCE OF CILIATE INFESTATION ON THE GILLS OF *M. dobsoni* [DURING JUNE - 1997]

	Salinity of the medium	No. of Prawns examined	No. of infested prawns		No. of ciliates observed		
			number	% prevalence	Antenion gills	Middle gills	Posterior gills
IN CAPTIVITY	13 ppt	15	11	73.1	12	17	98
	20 ppt	14	8	57.3	16	20	69
	30 ppt	15	11	73.1	57	52	129
IN NATURE	13 - 25 ppt	101	29	28.7	2	1	73

Table 7 showing the effect of water quality on the appearance of suctorian *Acineta sp.* in *M.dobsoni* towards the end of the experiment (Expt. date - 9 to 19 June)

TREATMENT	PREFERENCE (%)		
	Zoothamnium sp.	*Vorticella sp.*	*Acineta sp.*
	0	0	0
Control	60	40	0
	20	20	0
	60	0	0
No water change	20	0	0
	40	20	40

Table II
Prevalence and Abundance of ciliates on *M dobsoni* and the water quality parameters

Date	Prevalence (%)	Abundance (nos.)	Temperature (^0C)	Salinity (ppt)	Dissolved. O_2 (ml/ltr)	T.S.S. (mg/ltr)	pH	Nutrients (µg at/ltr)	
					Bottom			PO_4	NO_3
2/6	0	0	33	22.4	5.3	48.8	7.5	20.3	2.03
4/6	20	3	34	25.4	6.62	112.0	7.5	30.7	1.7
9/6	58	25	34	18.66	2.8	203.2	7.5	27.5	1.75
17/6	20	1	32	13.4	7.6	91.2	8	12.2	1.73
23/6	36	8	30	21.0	3.5	226.0	7.5	12.0	1.35
Average	**28**	**7.33**	**33**	**20.2**	**5.2**	**136.0**	**7.6**	**20.5**	**1.71**

PLATE - V

Ciliates concentrated on to the anterior free tip of the gill ×3.5

DISCUSSION

The development of experimental and commercial culture of penaeid prawns has been accompanied by the occurrence of diseases of infectious and non-infectious etiologies. Among the more serious diseases are those caused by non-infectious epicommensal organisms.

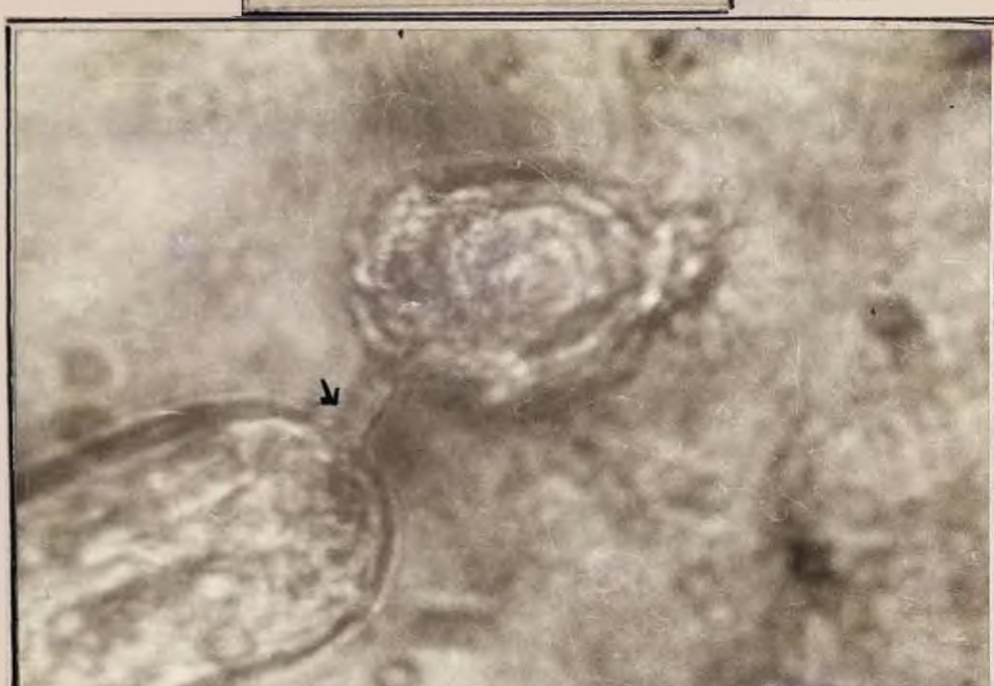
In the present investigation prevalence of epicommensalic ciliate infestation varied from 0 - 58% in the pond. Gills of infested prawns when examined under the microscope revealed the presence of a large number of dichotomously branching colonies of *Zoothamnium sp* with occasional prevalence of *Vorticella sp, Acineta sp* and *Epistylis sp. Zoothamnium sp* (more than 80%) was the most prevalent through out the period of study. Overstreet, 1973; Johnson,1973; Lightner, 1975; Couch, 1978; Foster *et al.*, 1978 observed that among the different species of ciliates found associated with shrimps the most conspicuous is *Zoothamnium.* The study conducted by Santhakumari and Gopalan (1980) on the estuarine shrimp *Metapenaeus monoceros* and the tenaidacean *Apseudes chilkensis* from cochin backwaters revealed that of the different species of ectocommensalic cilites, the heaviest infestation was that of *Zoothamnium.* The protozoan epibiont *Zoothamnium* has been reported to preferentialy located on the gill lamellae of shrimps while other protozoan epibiont do not show site specificity (Hall, unpublished data). Stalked peritrich *Zoothamnium sp, Vorticella sp, Epistylis sp* are generally found attached to the gills, appendages and body surfaces (Overstreet, 1973, 1978). Soni (1986) reported mortality due to heavy infestation of Zoothamnium sp in the culture pond at Valappu (Cochin, Kerala).

Relatively higher concentration of ciliates were observed on the anterior body parts of the prawn such as rostrum, carapace and gills. Dense growth of larger colonies of ciliates were observed on the gills (more than 90%) through out the period of study. Concentration of more ciliates at the anterior part of

PLATE - VI

Ciliates (*Zoothamnium sp* & *Vorticella sp.*) attached to the pleopod of prawn (*M.dobsoni*) x20

Photograph showing the superficial attachment of *Acineta sp.* on to the gill-lamellae x40



This photograph is not necessary

the body of the host might be due to the need for taking advantage of the respiratory current of the host for food and respiration (Santhakumari and Gopalan, 1980).

Attachment of the ciliates on to the body parts of the prawn does not appear to cause any mechanical damage to the host tissue as the attachment was found to be only superficial. About 22% of the sample (n=238) of *Penaeus aztecus* and *P.setiferus* from Gulf of Mexico carried suctorian (*Ephelota sp*) but they did not appear to cause any histological damage (Fontaine, 1985).

When the prevalence of the ciliate infestation among the different size groups were analysed, it was found that, in general ciliates do not show any size specificity for the prawns. However, the present observation showed that the chances of being infested is relatively more for smaller sized prawns of the size group 31-40 mm. Overstreet (1973) also observed high infestation rate in small sized brown shrimp.

When the ciliate abundance in the whole population was worked out it was observed that abundance is very less for prawn samples of size group 61-70mm. Ciliates were found more abundant among prawn population of size group 31-40mm 51-60mm and 81-90mm in the present investigation. But when the mean intensity of infestation per infested prawns were calculated, it clearly indicated the size preference of the ciliates to prawns samples of 51-60mm size. That means, once infested by ciliates, the number of ciliates increase in considerable numbers in this size group. Out of the 4 prawns examined of this size group (51-60mm) about 70 ciliates were observed, indicating their maximum abundance in this size group.

The number of ciliates should correlate with quality of the water and indicate the conditions that are suitable for bacteria, particularly those associated with elevated organic matter and other nutrients in the pond (Couch, 1983). Culture practices often provides those exact conditions that is, the abundance of substrate (host - cuticular surface) and concentrated bacterial populations due to organic wastes.

When the various water quality parameters were analysed, it was observed that dissolved oxygen (d.o) value fluctuated between 2.8-7.6ml per ltr (Table 1). The supersaturation d.o. value indicates planktonic bloom in the pond. There were 2 alternate peaks and drops in the d.o. values indicating planktonic bloom and die off. The first peak is soon followed by a fall in the d.o. value as the plankton die. It takes some time for the d.o. value to rise again as the algal scum formed from algal die off will deteriorate the water quality making the conditions unfavourable for the growth of plankton. The second peak values of d.o. which is the maximum value recorded, is observed as the algae blooms again. The death of algal bloom again brings down the d.o. value. The supersaturation of d.o. values in the culture pond observed on 2 occasions in June (4th and 17th) could be more likely due to the excess blooming of photosynthetic phytoplankters. This has probably led to the subsequent reduction of oxygen values to a low level in few days causing an unhealthy environment, more likely by the death of the short lived photo synthetic organisms which has resulted in the increase of total suspended solids (t.s.s.) values. The rate of decomposition of plant materials are increased if protozoans are present to graze the bacteria (Mann, 1982). The low values of d.o. indicates a reduction in the photo synthetic activity of the planktons, consumption of oxygen by organic matter contributed by planktonic decomposition and respiration by benthic organisms including prawns. So as the plankton become more abundant, rate of oxygen production and consumption increases (Boyd, 1985).

Abundance and prevalence of ciliate infestation is found to be much related to the d.o. content of the water. Johnson *et al.*, (1973) reported mortality of pond held shrimps due to the presence of large number of *Zoothamnium* on the gills and a reduction in the oxygen levels. Death of prawn due to heavy infestation of *Zoothamnium* usually coincides with periods of low concentrations of d.o. in the water, a common condition following several warm overcast days or following decomposition of large algal blooms (Overstreet, 1975). Soni (1986) reported mortality of penaeid shrimps due to heavy infestation of *Zoothamnium* when the d.o. level was as low as 2.36 ppm.

Relatively high correlation was obtained between d.o. and T.s.s., more likely due to plankton die off (FIGURE). Maximum T.s.s values were recorded when the d.o. values showed a decline (table 1). T.S.S. peaks were soon followed by d.o. peaks, then drop in the T.s.s. which indicates that T.s.s. is mainly contributed by organic matter produced by algal die off. Brownish colourations of the pond water was observed associated with algal die off giving a high T.s.s. value for the day.

Maximum abundance and prevalence of ciliates were observed when d.o. values were the lowest. Plankton die off and subsequent putrifaction of the water makes the situation congenial for the bacterial multiplication in the system. High T.s.s. values also reveal organic degradation by bacteria. The increase in T.s.s. values favours in the adherence of bacteria more and more in the water medium which may serve as food for those ciliated protozoans to promote the ciliate population in the environment as evidenced by the occurrence of relatively more number of ciliates on these two days (9th and 23rd) with relatively higher concentration (>200mg per ltr) of T.s.s. in water. So, as the bacterial load increases, ciliates which feed on bacteria flourish in the system. Peak values for abundance and prevalence of ciliate infestation obtained under conditions of low d.o. and high T.s.s. Experimental infestation of *Zoothamnium* in shrimp and the effect of stress produced by high turbidity and low d.o. conditions were carried out by Overstreet (1973) and in tenaidaceans by Santhakumari and Gopalan (1980) revealed that high turbidity of the medium result in fall in the d.o. values which intorn cause mortality of the population.

FIGURE - 2
DISTRIBUTION OF DISSOLVED OXYGEN, TOTAL SUSPENDED SOLID AND OCCURRENCE OF CILIATES

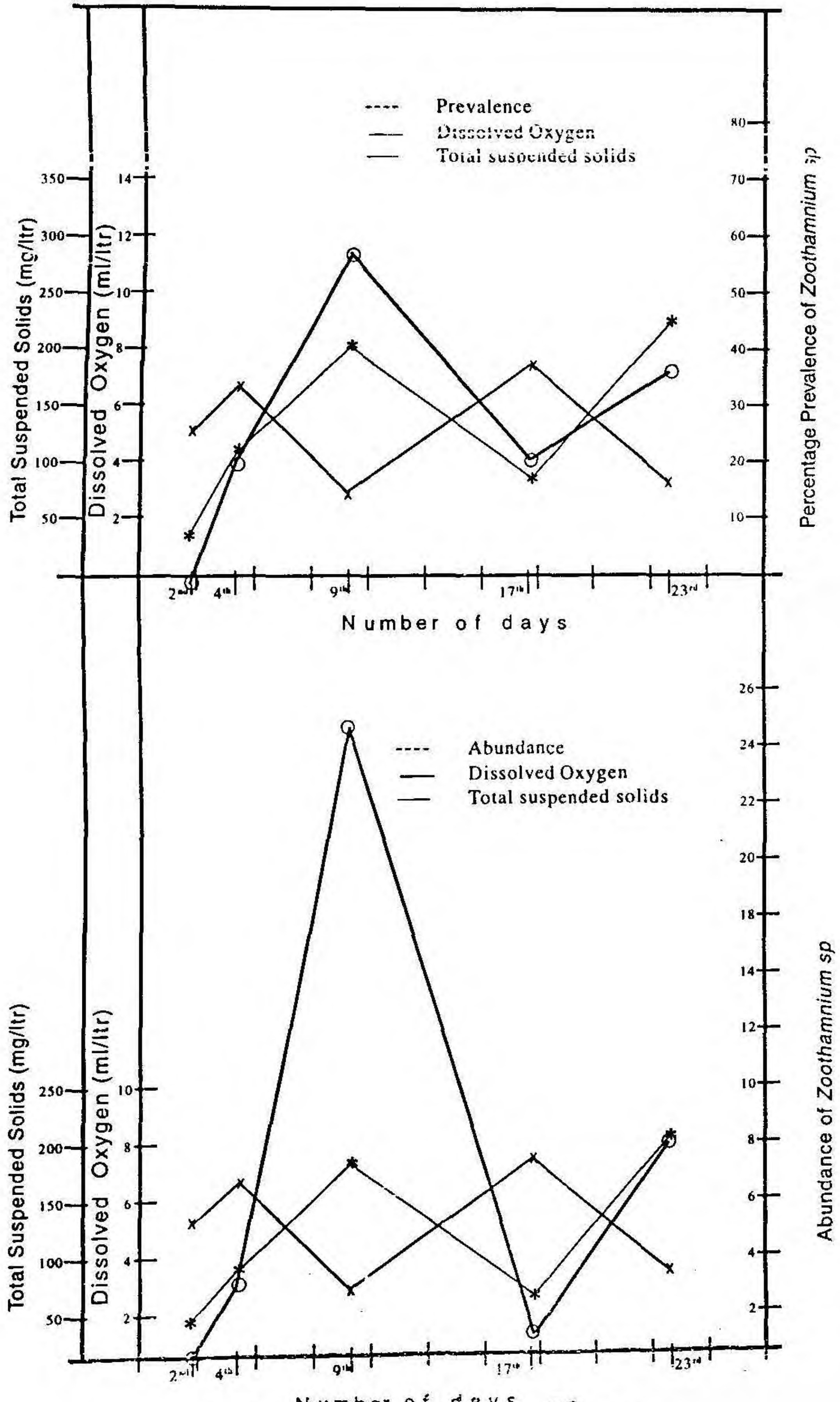
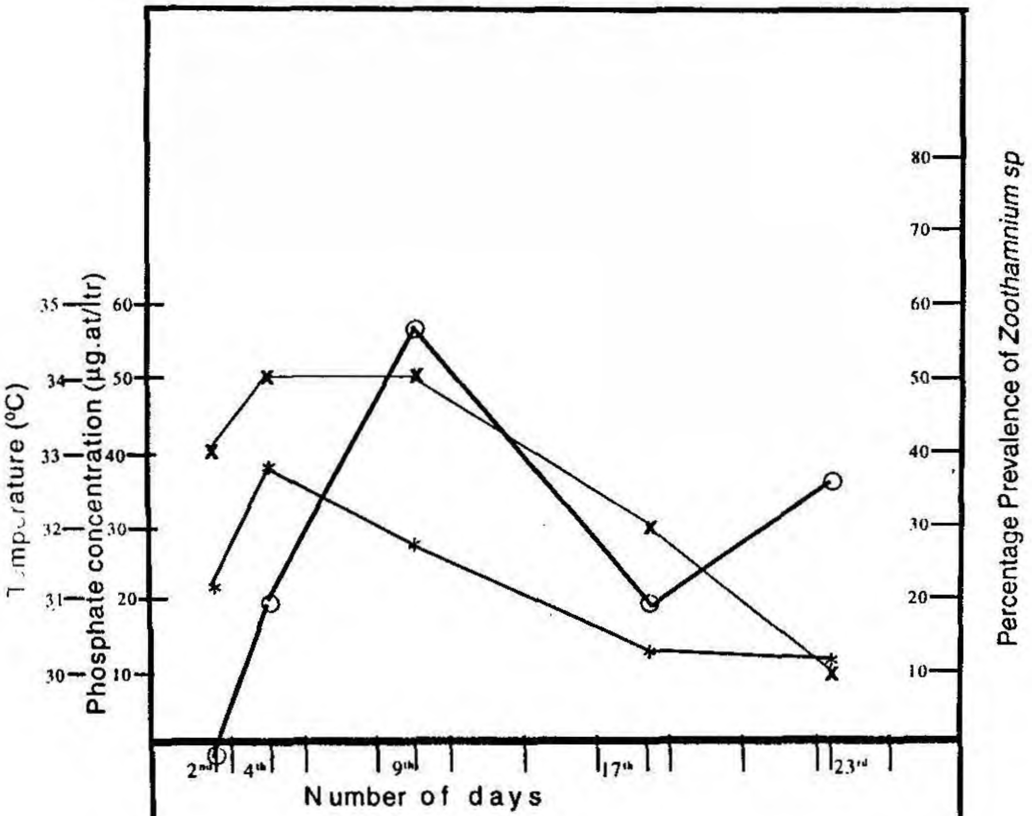
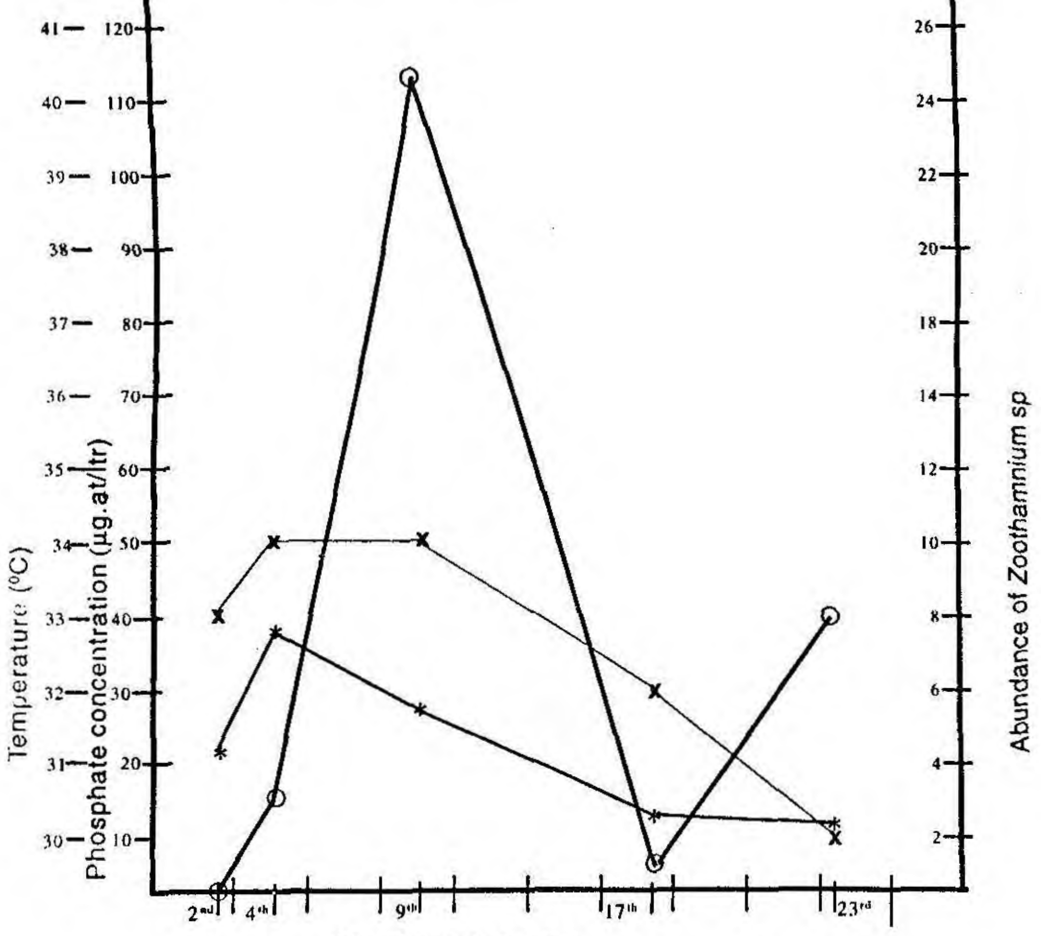


FIGURE - 3
DISTRIBUTION OF TEMPERATURE, PHOSPHATE AND OCCURRENCE OF CILIATES





Temperature showed wide diurnal variation during the period of study, the parameter is treated to compare the abundance of the ciliates. Salinity is found to play little role in ciliate infestation, as infestation was observed both at high and low salinity conditions.

During period of study phosphate valued varied between 12-30.7. µg.at./ltr. Phosphate and nitrate existed in the pond water in the ratio of 12:1 during the period of study. Bacterial activity is enhanced by increased availability of phosphate in the system (Mann, 1982). The occurrence of phosphate and nitrate in the water through out the period of study indicated that these nutrients however do not function as the limiting factor for the reduction of photo synthetic phytoplanktons.

The experiment conducted to find out the salinity preference of ciliate on *M.dobsoni* at different salinity conditions of 13ppt, 20ppt and 30ppt showed that infestation is low at 20 ppt salinity than at 13 and 30 ppt salinities. Prevalence of ciliate infestation is only 57% at 20ppt where as chances of being infested is almost same (73%) at both the salinity extremes (13 and 30ppt). When the number of ciliates observed at different salinity conditions of 13ppt, 20ppt and 30ppt were compared, it was observed that, maximum number of ciliate were observed at 30ppt salinity values. This indicates the preference of ciliate to get multiplied and established at 30ppt salinity, where as at 20ppt salinity which is considered as the most preferred salinity condition for *M.dobsoni,* the number of ciliates observed was low. When the mean intensity of infestation in the population at different salinity conditions were compared, it was again proved preference of salinity conditions. Maximum values for the abundance of ciliate infestation among infested prawns again confirmed that salinity around 30ppt is the most preferred salinity condition for the ciliate according to the present study. Soni (1986) reported ciliate infestation at 31.2ppt salinity in *Penaeus monodon, P.indicus and Metapenaeus monoceros* from cochin back waters. However Santhakumari and Gopalan

(1980) reported increased rate of infestation during monsoon months as an indication to their prefarence to low salinity conditions. This might be due to difference in the species of ciliates which are tolerant to the respective salinity medium.

When the prevalence of ciliate infestation on *M.dobsoni* under natural conditions and under captive conditons were compared, it was evident that prevalence of ciliate infestation is very low (28.7%) in the month of June in the natural condition than under conditions of captivity. Out of the 101 samples analysis, only 29 animals were found infested by ciliates, where as in the captive conditions of different salinity values (13ppt, 20ppt and 30ppt) prevalence varied between 57-73%. In the natural condition salinity fluctuated between 13-25ppt. Soni (1986) reported that infestation of ciliates on prawns is moderate during pre-monsoon (April-June) and rare during other months. The hydrographic features of the culture pond in June 1997 provided an environment of transition of the pre-monsoon habitat entering into the onset of south west monsoon habitat, with the salinity decreasing from 13-25ppt in June. This would result in the survival of certain euryhaline ciliates alone in the habitat and this could be the probable reason for the occurrence of less number of ciliate infestation during the period of study. Couch (1985) also reported the seasonal prevalence of ciliate *Lagenophrys callinectes* in blue crab where he found a direct relationship between high prevalence of *L.callinectes* in summer months and high intensity of individual crab infestation of *L.callinectes*. However Santhakumari and Gopalan (1980) reported increased rate of infestation during monsoon months.

When the location prefarence of ciliates on the gills both in natural condition and captive condition were observed, it was found that ciliates are more concentrated on to the posterior gills than in the middle and anterior gills. Their number on the anterior and middle gills are rare in the natural condition of the pond. But in the experimental tanks, ciliates are seen slowly spreading from the posterior gills towards anterior and middle gills. Fairly good number of ciliates in the anterior and middle gills indicated that ciliates prevalence in *M.dobsoni* preferred higher salinity (figure -1). Experimental infestation of *Zoothamnium* in *M.monoceros* by Santhakumari and Gopalan (1980) showed that colonies of ciliates get established on uninfested shrimp in 3-4days when infested animals were put along with uninfested individuals.Relatively high rate of infestation with more dens colonies occurred on the reared individuals than those collected from the nature, which shows the probable conduciveness of stagnant waters and over crowding for infestation (Overstreet, 1973) as it is observed in the present study.

Prevalence of ciliate infestation *M.dobsoni* was about 28% in the pond in the present study. The attachment of the ciliates on to the prawn cuticular surface does not inflict any mechanical damage to the host, but high encrustation of the ciliates on the gill surface may, reduce the respiratory surface area of the prawn, leading to hypoxia and even to severe mortality as observed by Johnson (1973) and Overstreet (1983). The prevalence of ciliates infestation under conditions of high total suspended solids and depleted dissolved oxygen indicates that the occurrence of ciliate infestation in prawn is an indication of deteriorated water quality. It is more likely that some of the species of *Zoothamnium* may serve as indicator of deteriorated water quality. More studies on this aspect to these species level is desirable.

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SUMMARY

Epicommensalic protozoans associated with *Metapenaeus dobsoni* consisted of species of *Zoothamnium, Vorticella, Epistylis* and a suctorian *Acineta.*

The major observations made in the present study are,

1. The most prevalent ciliate observed was species of *Zoothamnium* (more than 80%) followed by *Acineta*(16.9%), *Vorticella* (2%), *Epistylis*(0.1%)
2. The prevalence of ciliate infestation was comparatively low in *M. dobsoni* during June in the estuarine environment (28.7%)
3. Infestation was found high in the gills (more than 90%) than in other external parts of the body.
4. Intensity of infestation was found high in the prawns of the size group 51-60mm.
5. Ciliates were found to show a preference to get infested in the posterior gills than on the anterior and middle gills.
6. Correlations were observed between water quality parameters such as dissolved oxygen and total suspended solids in the pond and ciliate infestation. It was observed that under conditions of high total suspended solids and low dissolved oxygen ciliate infestation is high.

REFERENCES

AQUA COP, 1977. 'Observations on diseases of crustacean cultures in Polynesia'. In: Proc. World. Maricult. Soc., 8. pp.685

BARRADAILE *et al.*, 1935. 'Invertebrate'

BHAXTER, J.H.S. & SOUTH WARD, A.J., 1990. 'Ciliates, flagelllates and suctorian'. In. Advances in marine biology. pp.384-385.

BROCK, J.A., 1983. 'Diseases (infectious and non- infectious), metazoan parasites, predators and public health considerations in *Macrobrachium* culture and fisheries'. In. C.R.C. Handbook of mariculture. Vol - I pp. 337 - 342.

BOYD, C.E. & PILLAI, V.K., 1985. Water quality in warm water fishponds, C.M.F.R.I., special publication no-22.

* CORLISS, J.O. & BROUGH, I.M. 1965. 'A new species of *Lagenophrys* (Ciliata: Peritrichide from the Jamaican crab *Metapanlias depressure.*' Transactions of Americal Microscopical Society, 84 pp.73-80.

* CORLISS, J.O., 1979. The ciliated protozoa, 2nd edition. Pergamon Press, Oxford, pp. 455.

COUCH, J.A., 1983. 'Diseases caused by Protozoa'. In Provenzano, A.J. (editor). The Biology of Crustacea. Vol:6, Academic Press, New York, NY pp. 79-111.

*DEBAISIEUX, P., 1959. '*Lagenophrys lunatus* Ima. (Ciliata: Peritricha).' Cellule 59 pp 359-383.

FISHER, W.S., 1977'. Epibiontic Microbial Infestation of Cultured Crustacean'. In: Proceedings of the 8th annual meeting. World Mariculture Society. pp. 673-677.

FOISSNER, W., 1988'. Taxonomic and Nomenclature rivision of Sladeck's list of ciliates.(Protozoa. Ciliophora) as indicators of water quality.' In: Hydrobiologia, 166. pp. 1-64.

FONTAINE, C.T., 1985. Journal of Invertebrate Pathology. 18(2) pp. 301-303.

FOSTER, C.A., SARPHIE, T.G. & HAWKINS, W.E., 1978. 'Fine structure of the peritrichous ectocommensal *Zoothamnium sp.* with emphasis on its mode of attachment to Penaeid shrimp'. In: Journal of Fish Diseases 1; pp. 321 - 335.

FISHER, W.S., 1977. 'Epibiotic microbial infestation of cultured crustaceans'. In: Proc. Ann. Meeting, World Mariculture Society, 8: pp. 673 - 684.

FISHER, W.S., NELSON, E.H, STEEN BERGEN, J.F., & Lightner, D.V., 1978. 'Microbial Diseases of Cultured Lobsters : a review,' In: Aquaculture - 14. pp. 115.

*GUCATAN, R.Q., *et al.* 1979. 'A suctorian parasite of *Penaeus monodon* larvae'. In: Proceedings - 2nd Biennial Crustacean Health Workshop, Texas A & M University (TAMU - SG - 79 - 114) Sea Grant College Programme, College Station, Texas. pp. 202 - 213.

* HALL T.J., 'Ectocommensals of the freshwater shrimp *Macrobrachium rosenbergii* in culture facilities at Homestead, Florida,' In. Proceedings, 2nd

Biennial Crustacean Health Workshopm, Texas A & M University (TAMU - SG - 79 - 114) Sea Grant College Programme, College Station, Texas. pp.214.

HUDSON & LESTER, 1992. 'Relationships between water quality parameters and ectocommensal ciliates on prawn (*Penaeus japonicus* Bate) in aquaculture.' In. Aquaculture, 105 pp. 269 - 280.

ISAAC RAJENDRAN, A.D., VENKATESAN, V & VICTOR CHANDRA BOSE, S., 1982. 'Observations on the infestation of ectocommensalic protozoans, *Zoothamnium sp.* and *Epistylis sp.* on pond cultured prawn *Penaeus monodon* Fabricus'. In: Simposium on Diseases of Finfish and Shellfish, 1 - 3. March, 1982. Univer. Agric. soc., College of Fisheries, Mangalore, India.

JOHNSON, S.K., 1972. Fish diseases and there control. In: F.A.O. Aquaculture Bulletin, 4 (4) pp. 15.

JOHNSON. S.K., PARKER, J.C & HOLCOMB, H.W., 1973. 'Control of *Zoothamnium sp.* on Penaeid shrimps,' In: Proceedings World Mariculture Society, pp. 4. 321.

JOHNSON, S.K. 1974. 'Some marine microorganisms related to shrimp diseases'. In: Proceedings 5th Annual Workshop, World Mariculture Society. pp. 251 - 266.

JOHNSON, C.A., & BRADBURY, P.C., 1976. 'Observations on the occurrence of the parasitic ciliate *Synophrya* in decapods in coastal waters off the South Eastern United States.'

* JOHNSON. S.K., 1978. 'Some disease problems in crawfish and fresh water

shrimp culture'. Texas A & M University Extention, Fish desease diagnostic laboratory publication, FDDL - S11.

* KANE, J.R., 1965. 'The genus *Lagenophrys* Stein (Ciliatica: Peritricha) on Australian parastacidae'.In: Journal of protozoology, 12, pp. 109 - 122.

*KIDDER, G.W. & SUMMERS, F.M., 1935. Taxonomic and cytological studies on the ciliates associated with the amphipod family Orchestiidae from Woods Hole District, The stomatus holotrichous ectocommensals. In: Biological Bulletin, Woods Hole, 68. pp. 51 - 68.

KUDO, R.R., 1966. Protozoology, 5th edition, Charles C Thomas, Springfield pp. 1174.

* LIGHTNER, D.V., 1975. 'Some potantially serious disease problems in the culture of Penaeid shrimp is North America.' In: Proceedings 3rd U.S. - Japan meeting on Aquaculture, Tokyo, Japan, pp. 75 - 97.

LIGHTNER *et al.*, 1975. 'Some form of full diseases in penaeid shrimp.' In: Proceedings 6th Annual Meeting, World Mariculture Society. pp. 247 - 255.

LIGHTNER, D.V., 1977. 'Ciliate diseases of shrimps.' In: Sinderman, C.J. (Editor). Disease Diagnosis and control in North American Marine Aquaculture. Developments in Aquaculture and Fisheries Science, 6 pp. 56 - 60

LIGHTNER, D.V., 1983. 'Diseases of cultured Penaeid shrimp'. In: James P. Mc Vey and J.R. Moore (Editors), C.R.C. Handbook of Mariculture, vol.1.

Crustacean Aquaculture. C.R.C. Press. Boca Raton FL. pp. 289 - 320.

LIGHTNER, D.V., 1988. 'Protozoan fouling diseases of Penaeid shrimps.' In: Sindermann, C.J. & lightner, D.V. (Editors). Disease diagnosis and control in North American Marine Aquaculture, Elsevier, New York, NY.

MANN, K.H., 1982. ' Phytoplankton - based system.' In: Ecology of coastal waters, volume - 8, pp.83 - 124.

OVERSTREET, R.M., 1973. ' Parasites of some penaeid shrimp with emphasis on reared hosts.' In: Aquaculture, 2 pp. 105 - 140.

OVERSTREET, R.M., 1987. 'Solving parasite - related problems in cultured crustacean.' In : Journal of Parasitology, 17 pp. 309 - 318.

* OVERSTREET, R.M. & WEIDNER, E., 1978. 'Marine - Maladies? Worms, Germs and other symbionts from the Northern Gulf of Mexico.' Miss - Ala. Sea Grant Consortium Publication. pp. 78 - 012. Ocean Spring, Mississippi.

* PRECHT, H., 1935. Epizoen der Kieler Bucht. Nova Acta Leopoldina. 2: pp. 405 - 473.

* ROSEN, B.1970. 'Shell diseases of aquatic crustacean.' In: Sneiszko, S.F. (Editor), A symposium of diseases of fishes and shellfishes, Special publication No.5, American Fisheries Society, pp. 409 - 415.

HAMEED SAIT SAHUL, A., 1986. 'Studies on the pathobiology of penaeid larvae and post larvae. Doctoral thesis.

SANTHAKUMARI. V. & GOPALAN, U.K. 1980. ' The protozoan associates of some crustaceans.' Mahasagar, 13 (2) pp. 125 - 131.

SCOTT, J.R. & THUNE, R.L., 1986. ' Ectocommensal protozoan infestation of gills of red swamp crayfish *Procambarus clarkii* (Girard) from commercial ponds., In: Aquaculture, 55 pp. 161 - 164.

*SHOMAY, D.,1955. 'A contribution to the morphology, taxonomy and bionomics of the epizooic peritrichous ciliates *Lagenophrys labiata* Stokes.' 1887. unpubl. Doctoral Diss, Univ. of Illinois, Univ.Microfilms, Ann Arbor, Michigan.

GUPTA, S.P., 1987. Statistical Methods.

SPRAGUE, V. & COUCH, J. 1971. An annotated list of protozoan parasites, hyper - parasites, and commensals of decapod crustacea, In: Journal of Protozoology, pp. 18, 526 - 537.

SONI SUBHASH CHANDRA, 1986. 'Ciliate infestation.' Pathological investigations in penaeid prawns. Doctoral thesis. pp. 70 - 75.

STILLER, J.1953. 'Epizoische Peritrichen aus dem Balaton III. Hydrobiologia, 5 pp.189 - 229.

STIRLING, H.,1985. Chemical and biological methods of water analysis for aqua culturists, Institute of Aquaculture, University of Stirling. pp.1-118.

VISWANATH, 1979. A text book of zoology. pp. 7 - 59.

* Not referred in original