STUDIES ON THE OCCURRENCE OF ECTOCOMMENSAL CILIATES ON *Penaeus (Fenneropenaeus) indicus* H. MILNE EDWARDS IN RELATION TO WATER QUALITY PARAMETERS IN SHRIMP CULTURE PONDS AT VYPEEN ISLAND

DISSERTATION SUBMITTED BY

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IN PARTIAL FULFILMENT FOR THE DEGREE OF
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भाकृ अनुप
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POST GRADUATE PROGRAMME IN MARICULTURE
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COCHIN - 682 014

Dedicated to my Loving Parents

Certificate

Certified that the dissertation entitled "**STUDIES ON THE OCCURRENCE OF ECTOCOMMENSAL CILIATES ON Penaeus** ***(Fenneropenaeus) indicus*** **H. MILNE EDWARDS. IN RELATION TO WATER QUALITY PARAMETERS IN SHRIMP CULTURE PONDS AT VYPEEN ISLAND** " is a bonafide record of work done by Miss **Nisha P.C.** 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 "**STUDIES ON THE OCCURRENCE OF ECTOCOMMENSAL CILIATES ON Penaeus (Fennero penaeus) indicus H. MILNE EDWARDS IN RELATION TO WATER QUALITY PARAMETERS IN SHRIMP CULTURE PONDS AT VYPEEN ISLAND** " 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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सारांश

दशपद कवचप्राणियों विशेषता: संवर्धित झींगों में से दस एक्टोकमान्सल पक्षमाभियों की रिपोर्ट की गई । पक्षमाभियों को पानी के गुणता-प्राचलों के साथ सापेक्ष कराने का प्रयास किया गया । इस अध्ययन के लिए पेनिअस इंडिकस को चुना गया । वाइपीन द्वीप के चुने गए दो झींगा संवर्धन तालों में यह अध्ययन का कार्य चलाया गया । इन दोनों स्थानों के झींगों पर किए गए अध्ययनों में परिपक्षमाभी जूताम्नियम सबसे प्रमुख एक्टोंकमन्सल देखा गया । रोग ग्रसित झींगों की संख्या और जूताम्नियम उपनिवेश बहुत अधिक दिखाया पडा जिनका औसत क्रमश: 83 % और 53 % था । यह भी देखा गया कि पानी के विभिन्न गुणता-प्राचलों में स्थावर पक्षमाभियों की प्रचुरता और प्रचलन पर कुल निलंबित ठोस पदार्थों (टी.एस.एस) और विलीन ऑक्सिजन की सान्द्रता का अत्यधिक प्रभाव पडता है । जूताम्नियम की उपस्थिति और प्रचुरता का टी.एस.एस मूल्य से सीधा संबन्ध देखा गया । इसके विपरीत विलीन ऑक्सिजन की सान्द्रता कम होने पर जूताम्नियम की प्रचुरता और प्रचलन अधिक देखा गया । अधिक लबणता परास में दिखाए पडने पर भी पक्षमाभी कम लवणता पसंद करते है । पी. इंडिकस में पक्षमाभी ग्रसन पर लवणता और पानी की गुणता का प्रभाव जानने के लिए भी एक अध्ययन चलाया गया । पानी का विनिमय नहीं होने वाले टैंकों में जुताम्नियम की प्रचुरता और प्रचलन अधिक था जिसका औसत 74 % और 22 % था और नियंत्रित स्थितियों में ये क्रमश: 37 % और 7.4 % था । लवणता 30 % वाले टैंकों की तुलना में 13 % वाले टैंकों में पक्षमाभियों की संख्या अधिक थी । इस से यह मालूम पडता है कि ये कम लवणता पसंद करते हैं । मध्य और पार्श्व क्लोमों की तुलना में पश्च क्लास में पक्षमाभियों का उपनिवेश अधिक देखा गया ।

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PREFACE

Of the marine fish landings of 2.6 million metrictons in 1994-1995 in India, the penaeid prawns, which mainly contribute to precious foreign exchange are steady at 1,73,000 metrictons. Thus, a quantity of 51,000 mt in 1978-1979 and 57,800 mt in 1989-1990, has almost doubled, expanding our cash reserves through export of this very important commodity. An important factor, contributing to this major improvement was the role played by aquaculture or more precisely, its metaphor, the brackish water shrimp culture which is synonymous to the Blue Revolution, and itsrise into an industry.

Aquaculture is an important intervention of man, in trying to increase proteinaceous food for the geometerically increasing population of today. It also helps in reducing fishing pressure on the depleting resources from nature; the existance of which is threatened by an ever increasing fishing fleet. With the expanding global market, including exports, the additional quantity of shrimp through aquaculture, has helped in increasing the much needed revenue from developed countries such as The United States and Japan, where shrimp is a habitual menu preferred for its low cholesterol white meat.

The picture of this ever expanding shrimp culture industry, is not rosy. It has its share of problems that accompany intensification. One major problem is the outbreak of diseases, especially due to improper management of the culture envioronment. It affected many world leaders in shrimp culture, like China, and Thailand. India had a similar experience during which entreprenuers faced massive losses.

The aquatic environment offers the greatest intimacy between itself and the organisms which it bathes and whose life it supports. Thus it is the environment that determines the health and growth of the culture animals. Abuse of this often results in stress which would invariably lead to diseases of animals. Many protozoan diseases are directly connected to poor water quality, which helps them to thrive well and make the cultured animals an easy target. Hence, more and more investigations are needed to relate the various diseases with the existing water quality.

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INTRODUCTION

Crustaceans have attained recognition in today's world as primary animals for aquaculture. Those that have attained this distinction are the penaeid shrimps, which are in the fore-front; lobsters; and the fresh-water prawns of of the genus *Macrobrachium* , crayfish and some of the crabs like the Blue crab in the United States, and Mud crab in the South East Asian region. Commercial scale culture of penaeid shrimps have been achieved, and that of others are in the experimental, developmental or pilot plant stages. The intensification has held responsibility to problems that the industry is facing, which are various. Almost without exception are the disease problems that have surfaced. Commercial crustacean culture is plagued by various disease causing agents, including Protozoa.

Crustaceans serve as hosts to symbiotic, commensal, parasitic and pathogenic representatives of all major taxa of Protozoa. Studies of microsporidian epizootics in shrimp (Visoca, 1943; Lightner, 1977), crayfish (Pixell - Goodrich, 1956), amoebic epizootics (Johnson, 1977) dinoflagellate infection (Newman and Johnson, 1975; Couch, 1983) and ciliate protozoan outbreaks in shrimps, crabs and cray fish (Couch, 1978; Overstreet, 1973; Lightner, 1975; Scott and Thune, 1986) demonstrate the periodic and chronic impact of Protozoa. Ciliated protozoans are frequently associated with commercially important species of decapod crustaceans as commensals, parasites or pathogens. Many of them are also known to harbour several species of ectocommensalic ciliates, especially peritrichs (Kane, 1965; Lightner, 1977; Sindermann, 1977; Fisher, 1977)

Owing to the commercial importance of decapod crustaceans, various studies on the association between this group and ectocommensal citiates have been conducted, which help to spot-light the importance of such associations. The stalked peritrich, *Zoothamnium sp* and *Epistylis sp* have been seen in the Pink Shrimp (*Penaeus deorarum*), White shrimp (*Penaeus setiferus*), Brown shrimp *(Penaeus aztecus)*, Blue shrimp (*Penaeus stylirostris*), Mexican white shrimp (*Penaeus vannamei*) and the Central American White Shrimp (*Penaeus occidentalis*) (Lightner, 1977). High infestations of the peritrichous citiates of the genera *Lagenophrys* and *Epistylis* were seen on moulting blue crabs (*Callinectes sapidus*) from Chesapeake Bay (Couch, 1967). The peritrichous ciliate *Epistylis*, *Lagenrophrys* and the suctorians *Acineta* and *Cothurnia*, were seen in the gills of nearly 94% of crayfish (Scott and Thune, 1986).

Most of the ectocommensal ciliates belong to the subphylum Ciliophora of the phylum Protozoa. Organisms of class Ciliata, under ciliophora, possess, as the name implies, cilia, in atleast one stage of their life. Sessile stalked ectocommensals are seen under the subclass Pritrichia, eg: colonial forms like *Zoothamnium*, *Epistylis* and single forms like *Vorticella*, which are bell shaped, and have an adoral row of clila along the peristome; and Suctoria eg: *Acineta* and *Stentor* whose adults attach by a stalk and possess cilia only in their young stages. Suctorians sieve the water for food, with tentacles. The host merely acts as a substratum. Thus these are not pathogens in the ture sense. But there is ample evidence that these ciliates are one of the main causes for mortalities of commercially important decapods in culture fields, especially, when these ectocommensals heavily infest the site of respiration, i.e. the gills thus reducing the respiratory surface. This happens more frequently in stressed conditions. Although stalked sessiles are found attached to gills, appendages and body surface, it is more advantageous for those like *Zoothamnium* to attach to gills than to the other regions, since the branchial chamber is protected by an overlying carapace or branchiostegite. Also, the gill chamber provides a constant flow of water across the gill surface, enabling these protozoans to feed on a steady current of bacteria, the principal food or diet of colonial peritrichs (Sleigh, 1973). The ectocommenals utilize the cuticular surface of decapods, merely as a substratum for attachment. Histological lesion of the gills, appendages, or general body surface have not been demonstrated at the site of attachment of a colony of *Zoothamnium sp* (Overstreet, 1973). The stalks of the colonies of this protozoan attach to the surface of the cuticle and do no mechanical damage to the cuticle. There is no foreign body response by the shrimp's haemocyte at the site of attachment (Overstreet, 1973). The Scanning and Transmission Electron Microscopic study of the ultra-structure of *Zoothamnium* and its infestation on the gills of *Penaeus aztecus* and *Penaeus setiferus*, by Foster etal. (1978) supports previous histological evidence (Lightner, 1975) that *Zoothamnium sp* neither penetrates the cuticle or elicits a haemocyte response.

In healthy environment, penaeid shrimps can toterate a large number of these ectocommensals, with no apparent effect. However, heavy infestation of *Zoothamnium* on gills of crowded pond-reared shrimps, in conjugation with low dissolved oxygen levels have been implicated in mass mortalities of cultured stocks in Texas in Louisiana (Johnson etal., 1973; Overstreet, 1973). This is due to suffocation of the shrimp, from the dense colonies of citiates on the gills, that reduce the effective respiratory surface (Lightner, 1975)

Experimental infestation of *Zoothamnium rigidum* on reared individuals of *Metapenaeus monoceros* and the Tanaidacean, *Apsedeus chilkensis* (Chilton) showed colonies larger than those from the nature. This shows the probable conduciveness of stagnant water and overcrowding for infesation (Santhakumari and Gopalan, 1980; Overstreet, 1973). Information from such studies show that epicommensal ciliate infestation is related to the water quality. For aquaculturists, water quality refers to the qulality of waters, that enables successful propagation of the desired organisms (Boyd, 1988). Various parameters that define the quality of the culture environment inluence the growth of the culture animals as well as that of the ectocommensals. Scott and Thune (1986) found significant association between the incidence of ectocommensal citiates on the gills of crayfish *Procambarus clarkii* (Girard) and water quality variables indicative of primary productivity. Turbidity was significantly related with the incidence of peritrichous ciliates *Cothurnia* and *Epistylis*.

Most peritrichs feed on bacteria and must have proper substratum and water temperature for reproduction and growth. Unfortunately, the practice of intensive culture of a variety of shrimp species providesthose exact conditions, ie, an abundance of substrata (host cuticle surface) and concentrated bacterial population due to organic wastes and other factors associated with large numbers of shrimp, closely crowded for maximum yield. Many a times, good water quality has been recommended as an important preventive measure (Johnson, 1978; Johnson etal., 1973). Rigid water control, filtration and, sterilization of incoming waters and removal of organic detritus should be applied as preventive measure (Lightner, 1975).

Little work has been done to correlate water quality parameters with ciliate infestation in penaeids. Hudson and Lester (1991) found correlation between water quality parameters and the presence of ectocommensals in *Penaeus japonicus* from a commercial Prawn farm at Cleveland, Brisbane, Australia. *Zoothamnium* showed positive correlation with temperature and stocking density. Sawyer et al. (1976) suggested that the suctorian *Ephelota* found on lobsters and rock crabs may be useful as an indicator species classification of freeliving ciliates according to the saprobic systems of water quality of their environment, was proposed by Sladeck (Foissner, 1988).

Penaeus (Fennero penaeus) indicus H. Milne Edwards is one of the best suited species for aquaculture and ranks second only to *Penaeus monodon* in its commercial culture. Wide ditribution, fast growth and production of seeds in hatcheries have made its culture easier. This "hard-cash cropping" shrimp species is cultured in all the maritime states with the exception of Maharashtra. The existing culture practices are traditional and semi intensive.

Hence *Penaeus indicus* was chosen for the present work due to its commercial importance. The study elucidates the relationship between the occurence of ciliates on this commercially important species with the existing water quality parameters such as temperature, salinity, hydrogen ion concentration, dissolved orthophosphate and nitrate and total suspended solids (T.S.S.). An experiment was also conducted to see whether stress from salinity and poor water quality had any effect on the occurrence and abundance of the ciliates on the Indian White Shrimp.

PLATE I

A: Station I. Pond at Narrackal

B: Station II. Pond at Puthuveyppu

MATERIALS AND METHODS

The site for the present study was confined to 2 different locations on the 24km. long Vypeen Island, Kerala, which is flanked by the Cochin back waters on its eastern side and by the Arabian sea on the west.

Station I :

A pond of area 0.04ha., at the Central Institute of Brackish water Acquaculture, Narakkal was chosen as station I. The 0.5 to 1.0m deep pond had a single sluice gate, and drew water from the near-by brackish water canal. The stocking density in the pond was very low. Natural stocking of seeds was done depending on the tidal influx. The pond bottem was predominently clayey (Pl. I A).

Station II :

The perennial pond at the Fisheries Station, Kerala Agricultural University, Puthveyppu, was chosen as station II. The 0.1 ha, 0.75 - 1m deep pond had a sluice opening at one end, drawing water from a feeder canal. The pond bottom was silty - clayey. Here also the stocking density was very low (Pl. I B)

Period of Sampling :

Station I:

Sampling was done during May - June, 1997. Samples were taken on the 8th and 27th of May and the 2nd,16th , 26th, of June. Except on the 27th of May which was taken at 3.00 P.M., evening, all the other samples were made before noon, preferably between 9.30 AM and 11.00 A.M.

Station II :

Samples from station II were collected, starting from the 4th to the 23rd of June, 1997. Sampling was made on the 4th, the 9th, 17th and 23rd. All the samples were taken between 9 AM and 11 AM.

Method Of Sampling

The shrimp sampling were made just after the water samples were taken so also as to get a clear picture of the interaction between the existing conditions and the ectocommensal infestation, as well as not to disturb the pond bottom.

The water samples were collected for the analysis of the following parameters :

1. Temperature

2. Salinity

3. Dissolved oxygen

4. Hydrogen ion concentration

5. Nutrients

6. Total suspended solids

Of these, the samples for the analysis of nutrients, salinity, total suspended solids (T.S.S.) and dissolved oxygen (after fixing the oxygen with Winkler A and Winkler B respectively) were brought back to the laboratory for analysis. The water temperature and, the Hydrogen ion concenration of the pond sediment were measured at the site of sampling. Shrimp samples were examined microscopically on location. Utmost care was taken not to disturb the pond bottom during sampling.

Sampling of Water :

A 0-50^{0}C calibrated mercury thermometer was used for reading the water temperature. Samples for the analysis of salinity were taken in 25ml plastic bottles.

For dissolved oxygen, samples from the surface as well as the bottom were taken. For this, 125ml Corning bottles with tight fitting stoppers were used. For surface sampling the bottles were tilted to the side, just at the surface, so that water flowed in, through the sides. Care was taken not to entrap air bubbles. Once full, 1ml of Winkler A and 1ml of Winkler B was added to fix the existing oxygen in the sample. The bottles were closed and shaken well. The bottom samples were taken by immersing the stoppered bottles and opening just above the pond bottom. To the full bottle 1ml. each of Winkler A and Winkler B were added and stoppered. The samples were brought to the laboratoryfor analysis.

The "Universal Indicator solution" (Glaxo India Ltd) was used to measure the hydrogen ion concentration of the pond sediment. The sediment was taken using a 1 inch pipe to get 10 cm deep sample. 1gm of the sediment was diluted with 10+2 ml of distilled water, shaken vigorously and kept to settle. To 10ml of supernatent solution, 4 drops of indicator solution was added, shaken vigorously and the colour developed was compared with that given in the chart to obtain the pH value.

500 ml clean dry plastic bottles were used to take bottom samples of water for nutrient analysis. The closed bottles were immersed and opened just above the pond bottom. To the samples, 2-5drops of chloroform were added to preserve them during transportation. On reaching the laboratory the samples were immediately kept in a deep freezer for further analysis.

One litre clean plastic bottles were used to take water samples for the analysis of total suspended solids.

Analysis

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

SALINITY

Reagents :

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

Procedure :

Pipette out 10ml of Standard Sea water into a 250 ml conical flask. Add 4 drops of potassium chromate solution and titrate aganist silver nitrate solution. Repeat to concordance. Pipette out 10 ml 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 ml

Standard Sea water = V1

Volume of silver nitrate

for 10 cc Sample = V2

Salinity of Standard Sea water = S

$$\text{Salinity of sample} = \frac{V2 \times S}{V1}$$

DISSOLVED OXYGEN

Reagents

1. Sodium thiosulphite solution (1.25 gms in 1 litre)

2. Starch solution - 1gm 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 Hydrochloric 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 1 ml eachof 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. Hyd rochloric 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. Hydrochloric acid. Dilute to 100 ml and titrate against sodium thiosulphate solution till the blue colour disappears using starch as an indicator.

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

Calculation

Calculate the normallity of potassium iodate as

$$= \frac{\text{Weight/litre}}{35.67} = 0.005 = N1$$

Calculate normality of thiosulphate as = N_1 x 10 divided by

Titrate value of thiosulphate for 10ml of potassium iodate = N2

Hence amount of dissolved oxygen in ml/litre

$$= \frac{\text{ml.thio x n2 x 8 x 1000 x R}}{100 \text{ x } 1.429}$$

(Where 1.429 being weight of 1ml of oxygen in miligrams. R is known as the correction 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

4. Potassium antimony tartrate solution

Dissolve 0.34 gm of good quality of potassium Antimony tartrate in 250 ml 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 50 samples.

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 ina 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 cylinnder. 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. N1-Napthyl Ethylene Diamine Di-hydrochloride solution (N.N.E.D) Dissolve 0.5 gm of N.N.E.D. in 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.

PLATE II

Penaeus indicus

1ml=15.0 ug .at/l 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 the 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.

Sampling of Shrimp

To get an exact picture of how the existing environmental conditions influenced the ciliate infestation in *Penaeus indicus*, the shrimps were sampled immediately after the water sampling. From both the stations, the shrimpswere sampled using a cast net and their gills microscopically examined for ciliates at the respective stations. During the examination, the shrimps were kept alive in clean pond water with aeration (Pl.II).

Total length of the shrimp was taken from the tip of the rostrum to the tip of the telson. The gills on the left side were dissected out using fine scissors. The gills were arranged on a clean glass slide in groups of three. The gills were then examined under a compound microscope. 10 animals each were examined on all sampling days from both the stations. Colonies of ciliates of the gills were counted and recorded group wise.

The various ectocommensal ciliates encountered were identified from the standard keys (Kudo, 1966)

The Prevalence (%) was calculated from the total number of shrimps examined and the number of shrimps infested with ciliates

$$\text{Prevalence (\%)} = \frac{\text{Number of infested shrimps} \times 100}{\text{Total number of shrimps examined}}$$

PLATE III

Experimental set up

In order to get a clear picture of their degree of infestation, the average number of ciliates per gill (on the left side) were also taken for each sample.

$$\text{Abundance} = \frac{\text{Total number of ciliate colonies}}{\text{Number of infested shrimps}}$$

Correlation Studies

The various water quality parameters analysed were then studied for correlation, between themselves as well as with prevalence and abundance of ciliates. For this Karl Pearson's method was used, the Pearson's coefficient of correlation being

$$r = \frac{\text{Covariance of X.Y}}{\sqrt{\text{Covariance of X x Covariance Y}}}$$

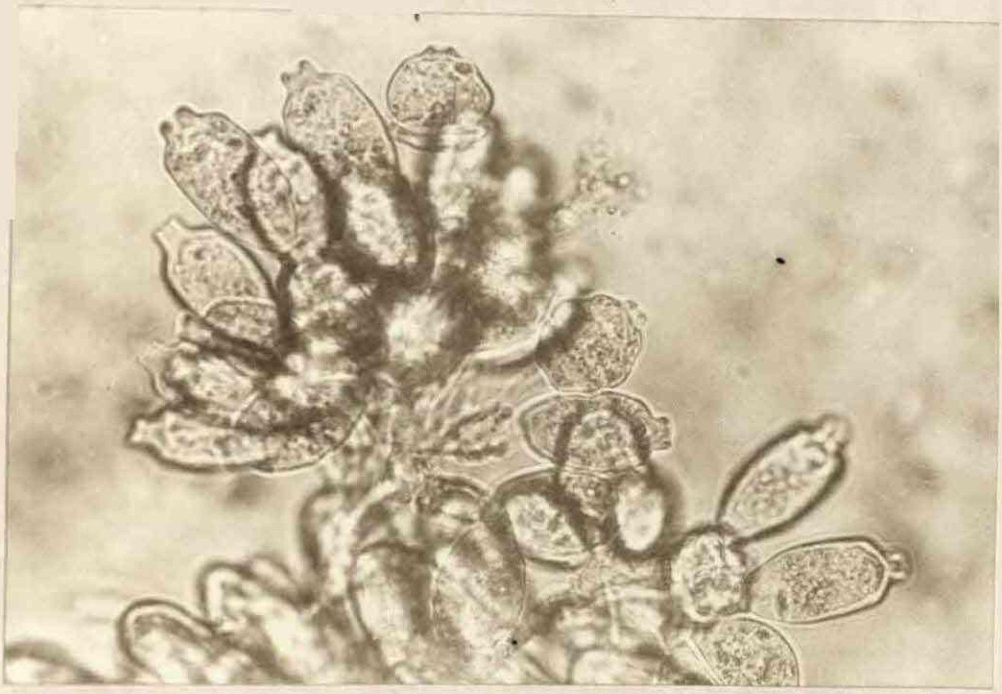
To see whether there was any preference for site of attachment on the gills, the average number of colonies on the anterior, middle, and posterior were recorded separately.

<u>Experiment</u> :

(Pl.III)

To obtain a better view on ciliate infestation under stressed conditions such as poor water quality and different water salinities, an experiment of two weeks duration was conducted at the field laboratory of the Central Institute of Brackish Water Aquculture, Narakkal. Shrimps ranging from 45mm to 75mm were selected. After an acclimation of 3days, the shrimps were stocked in four 380 litre rectangular fibre glass tanks, filled up to 360 litres and provided with good aeration. The shrimps were stocked at 35 per tank. The salinity of the control and the tank with no water exchange was at 20 ppt. In the other two tanks, the animals were acclimatised to 13 ppt and 30 ppt salinity. Except the tank in which stress from water quality was studied, in all the other tanks 50% of the water was changed daily; adding fresh filtered water. The animals were fed <u>ad libitum</u> on clam meat. The left over feed and faecal matter were siphoned out daily. Samples of 5 shrimps each were taken from all the tanks at regular intervals and the gills examined for the prevalence and abundance of ciliate colonies.

PLATE IV



Zoothamnium sp. attached on to the gill filament

RESULT

Both the ponds were abundant in the number of ectocommensal ciliates, showing a value of more than 80% incidence. The ectcommensal ciliates encountered during the period of study, were *Acineta, Vorticella* and *Zoothamnium*, of which *Zoothamnium* was the predominant species. *Zoothamnium* was noticed through out the study period, while the single forms made their appearence only towards the fag end of the study, and that too in negligible numbers.The Zoothamnium with minimum 2 trophonts were found atached to the gills. They occurred in large numbers, when compared to the other stalked ciliates. (Pl. IV).

Station I:

Samples of shrimp from this pond revealed that the pond was highly infested with *Zoothamnium* colonies. Both the prevalence and the abundance recorded on the various sampling days were high, an average of 84% and 60 numbers respectively. The abundance ranged from about 20 colonies on average per gills (on the left side) to a high value of 140. High values of prevalence were recorded, the values reading as high as 100% in the initial samples. All in all, the pond showed a high abundance and prevalence (Table 1).

Temperature values didnot show high fluctuations, and showed a gradual decline through the sampling period. The highest recorded was 38^{0}C and the lowest, 31^{0}C (Table 1). Salinity ranged from 11ppt to 18 ppt. The pH of the pond sediment showed a constant value of 7.5 except for 7.0 (on the 2nd of June).

Dissolved oxygen of the surface waters showed a range from a maximum of 7.7ml per ltr to a minimum of 5.47 ml per ltr, during the period of study. The bottom water samples showed an average value of 4.28 ml per ltr.

Of the nutrients analysed, the values of phosphate were within a range of 4.1

Table I

Prevalence and Abundance of ciliates on *P indicus* and the water quality parameters at station I

Date	Prevalence (%)	Abundance (nos.)	Temperature (^0C)	Salinity (ppt)	Dissolved. O_2 (ml/ltr)		pH	Nutrients (µg at/ltr)		TS S (mg/ltr)
					Surface	Bottom		PO_4	NO_3	
8/5	100	140.2	33	18.0	7.7	*	7.5	4.1	*	123.2
27/5	100	71.0	38	13.7	*	*	7.5	7.9	1.0	307.2
2/6	50	31.3	35	14.7	7.2	5.7	7.0	8.1	1.2	201.6
16/6	90	33.0	33	11.0	6.1	5.5	7.5	4.5	1.4	126.4
26/6	80	19.6	31	13.0	5.5	1.7	7.5	12.2	1.2	168.0

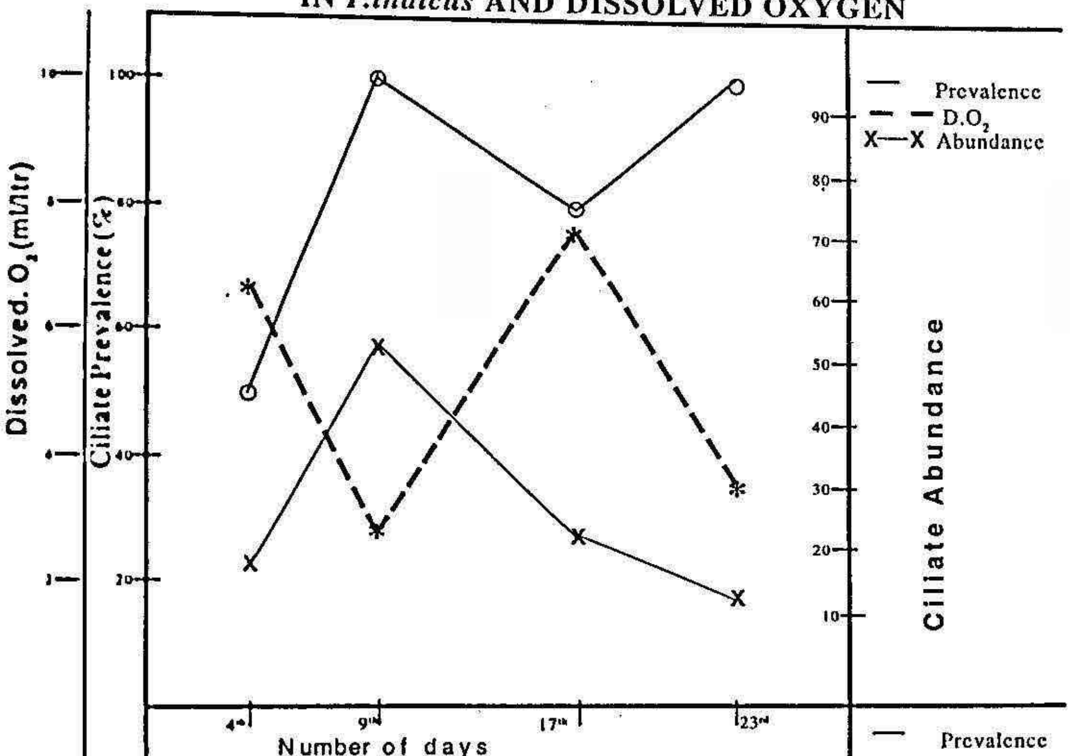
Table II

Prevalence and Abundance of ciliates on *P indicus* and the water quality parameters at station II

Date	Prevalence (%)	Abundance (nos.)	Temperature (^{0}C)	Salinity (ppt)	Dissolved. O_2 (ml/ltr)		pH	Nutrients (µg at/ltr)		TS S (mg/ltr)
					Surface	Bottom		PO_4	NO_3	
4/6	50	18.6	34	24.5	6.8	6.6	7.5	30.7	1.71	112.0
9/6	100	52.2	34	18.6	2.9	2.8	7.5	27.5	1.75	203.2
7/6	80	21.3	32	13.4	8.2	7.6	8.0	12.2	1.73	91.2
23/6	100	11.5	30	21.0	3.9	3.5	7.5	12.0	1.35	225.6

Figure. 1: STATION II

DISTRIBUTION OF CILIATE PREVALENCE, ABUNDANCE IN *P.indicus* AND DISSOLVED OXYGEN



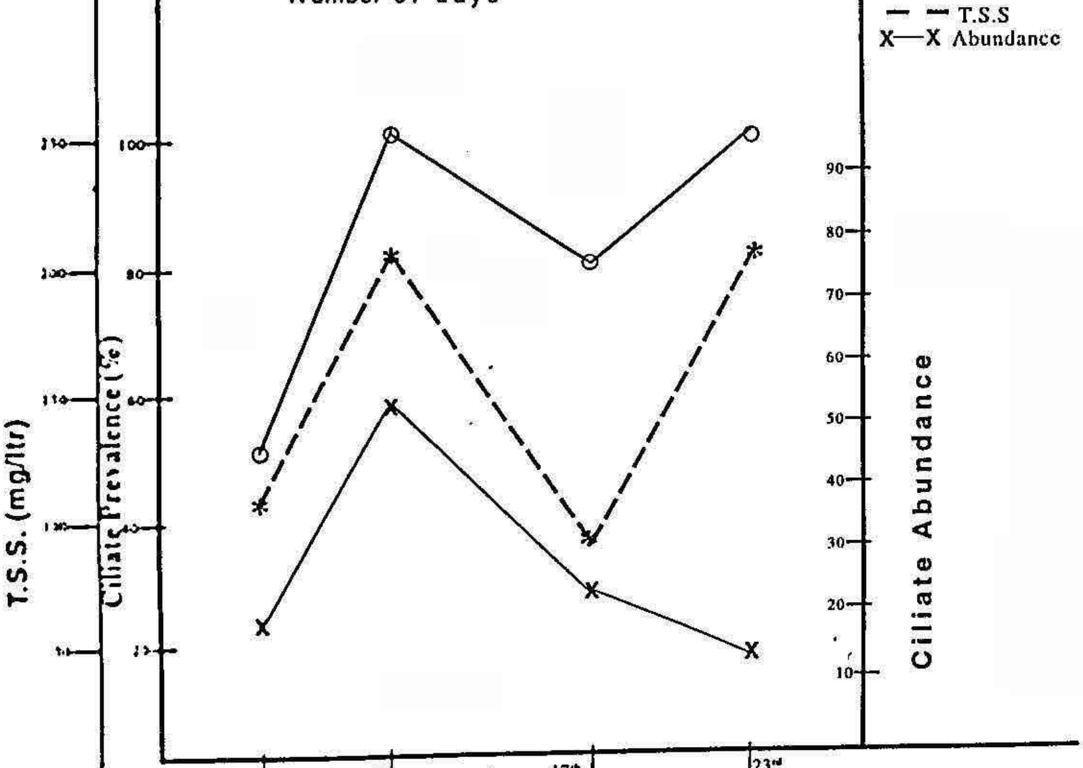


Figure. 2: DISTRIBUTION OF CILIATE PREVALENCE ABUNDANCE IN *P.indicus* AND T.S.S

μg at per ltr to 12.2 μg at per ltr. The range for the nitrate concentration was from 1.0 μg at per ltr to 1.4 μg at per ltr. The phosphate: nitrate was in the ratio 6:1.

The concentration of the total suspended solids in the pond ranged from 123.2mg per ltr to 307.2mg per ltr. It has an avaerage value of 185.2 mg per ltr. It gave a high correlation value of 0.8479 with temperature.

Station II :

The ciliate infestiation at this station recorded an average 82% prevalence and abundance 30. The prevalence ranged from 50% to 100%, while abundance from 11.5 to 52.2. Intially, both the abudance and prevalence were of low valves, which showed a gradual increase afterwards. At the end of the period of study, the prevalence increased to 100% though there was a gradual decrease in the abundance (Table II).

Of the water quality parameters, temperature ranged from 30°C to 34°C during June 1997, and didnot show much fluctuation. Salinity showed a decrease from 25.4ppt to 13.4 ppt. It rose to 21.0 ppt within the next 6 days. The prevalence as well as the abundance showed a negative trend with salinity. The hydrogen ion concentration of the pond sediment, ranged from 7.0 to 8.0 (on the 13^{th} day). On all the other days it remained at a value of 7.0, both values being normal for aquaculture.

The dissolved oxygen for the surface and bottom waters were more or less similar. The surface recorded a high value of 8.23 ml per ltr and a low value of 2.9ml per ltr. Dissolved oxygen of the bottom samples ranged from 2.8 ml per ltr to 7.58 ml per ltr. Both the prevalence and the abundance showed a negative trend with respect to oxygen values (fig.1). The oxygen values had a negative relationship with the concentration of total suspended solids, the values of 2.8 ml per ltr and 3.38 ml per ltr being recorded at higher T.S.S. values and vice versa. They showed high negative correlations, that for surface oxygen being significant (5%), with a correlation coefficient of 0.9507 and, for the bottom a value of 0.9312.

Phosphate values showed a minimum of 12 μg at per ltr and a maximum of 30.7

μg at per ltr. There was a sharp decline in the phosphate values, during the period of study. Nitrate values were more or less stable, ranging from 1.35 μg at per ltr to 1.75 μg at per ltr. Both nutrients showed good relations with temperature (-PO_4 r = 0.899; - NO_3 r = 0.867). The abundance of ciliates showed a positive relation with the nutrient concentration.

Total suspended solids in the pond ranged from 91.2 mg per ltr to 225mg per ltr., a general increase being recorded over the period of study. The T.S.S. value, at a low of 112mg per ltr showed an increase to 203.2mg per ltr on the 9th of June. It then dropped to the lowest value recorded, 91.2mg pet ltr on the 17th, and then increased to 225.6mg per ltr on the 23rd. Interestingly the prevalence also showed a similar trend as that of the increasing T.S.S. values (fig 2). At the lowest T.S.S. value of 91.2mg per ltr both the surface and bottom samples showed the highest oxygen concentration values of 8.23ml per ltr and 7.58 ml per ltr respectively. At the highest T.S.S. value of 225.6 mg per ltr both the oxygen samples showed their minimum values of 2.9 ml per ltr and 2.8 ml per ltr respectively. The values of T.S.S. showed a positive trend with temperature.

Experiment :

In the experiment conducted, to see the effects of stress from poor water quality and salinity variations on the ciliate infestation, the control showed a steady prevalence. The first day of sampling showed 40% prevalence which rose to 50% on the 5th day of the experiment. After that, it remained at a value of 40 % till the end of the experiment. The abundance showed high fluctuation. The average number of colonies counted per gill rose from 2 on the first day, to 13.5 on the 5th day and fell to a low value of 4 on the 7th day. It further rose to 8.5 on the final day of sampling. (Table. 3).

In the tank with no water exchange the percentage of prevalence was 100 in the beginning. But the value dropped to 60% on the 5th day of experiment. It then picked upto 100% after 2 days, finally decreasing to 25% after 3 days, thus showing high fluctuation.

TableIII

EXPERIMENT

Treatment	Date	No.of Shrimps Examined	No. of shrimps infested	No.of ciliate colonies on the group of gills				Prevalence (%)	Abundance (nos.)
				Anterior	Middle	Posterior	Total		
Control	13	5	2	1	0	3	4	36.8	7
	17	4	2	0	0	27	27		
	19	5	1	1	0	3	4		
	21	5	2	2	2	13	17		
Water Quality	13	5	5	72	50	69	191	73.7	22
	17	5	3	0	0	10	10		
	19	5	5	28	37	41	106		
	21	4	1	0	0	1	1		
13ppt	13	5	5	42	37	140	290	75.0	30
	17	5	4	18	2	7	27		
	19	5	5	4	28	43	75		
	21	5	1	3	10	25	38		
30ppt	13	5	5	16	15	61	92	55.6	16
	17	4	2	17	3	16	36		
	19	5	3	1	4	31	36		
	21	5	0	0	0	0	0		
Grow out pond	17	10	8	3	0	163	166	90.0	13.9
	23	10	10	21	0	63	84		
		20	18	24	0	226	250		

The abundance also showed a similar trend, recording a high abundance of 38.2 on the Ist day and then decreasing to 3.33 after a gap of 4 days. It again increased to 21.2 and decreased to a low value of 1.

In the tanks of 13 ppt and 30 ppt, the condition seen was different from that of the control. When the prevalence increased and steadied in the control, high fluctuations were seen in both the tanks. But on an average, the prevalence in the tank of 13 ppt water was more when compared to that of 30 ppt. In the latter, the prevalence reached a value of zero, quite unlike the other instances.There was also a marked difference in the abundance in both the tanks. In the tank with 13 ppt water, the abundance decreased but, unlike those in other tanks, it steadily increased (Fig3,C). In the tank with 30 ppt water, the abundance decreased steadily from 18.4 to the lowest value of zero . Thus from the 3 stressed conditions, the hightest abundance was seen in the tank with 13 ppt water, and lowest in 30 ppt.

During the examination of the shrimp gills, it was seen that the porterior group of gills were maximum infested when compared to the other 2 groups (Fig4).

Figure - 3

EXPERIMENT : CILIATE ABUNDANCE AND PREVALENCE

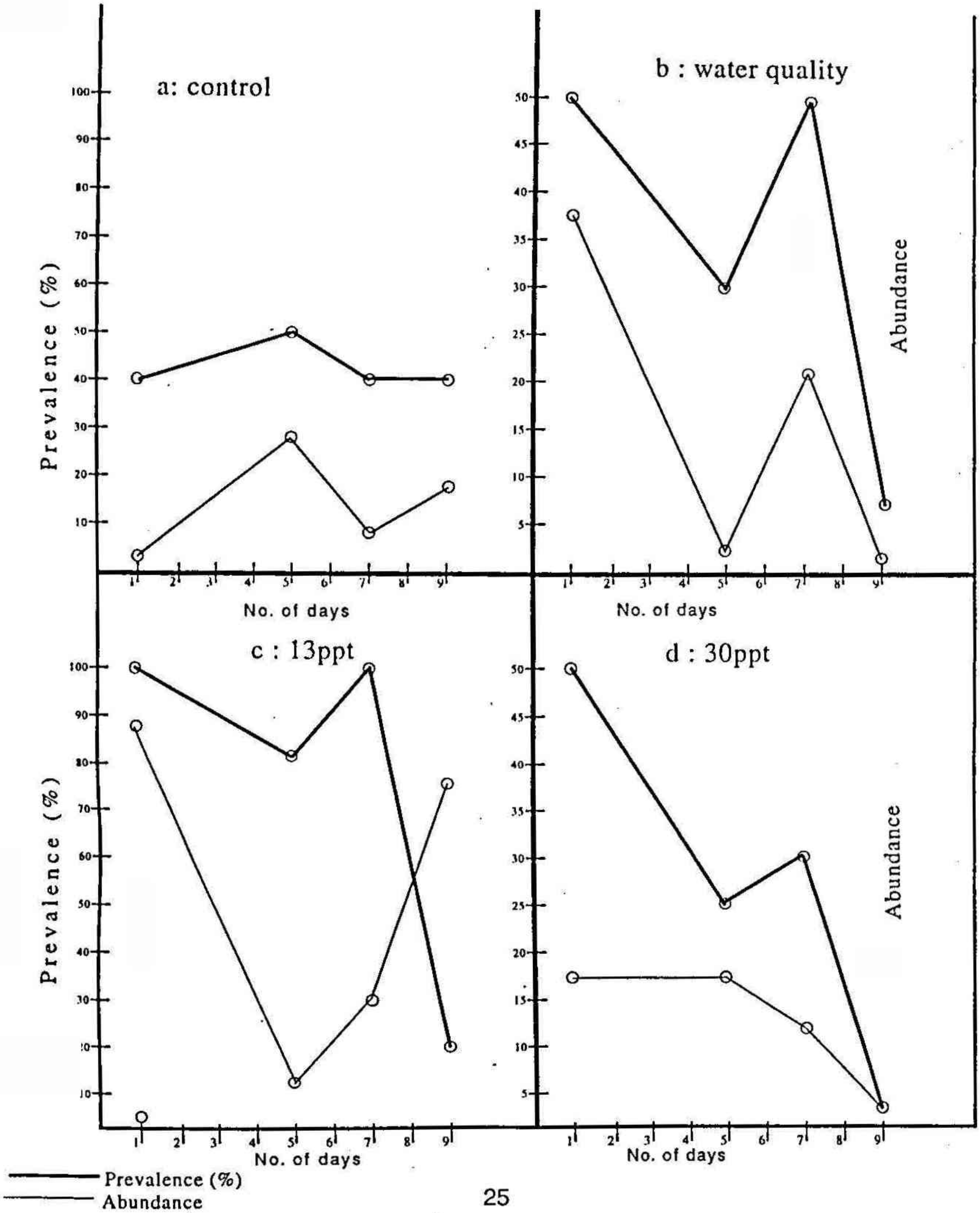
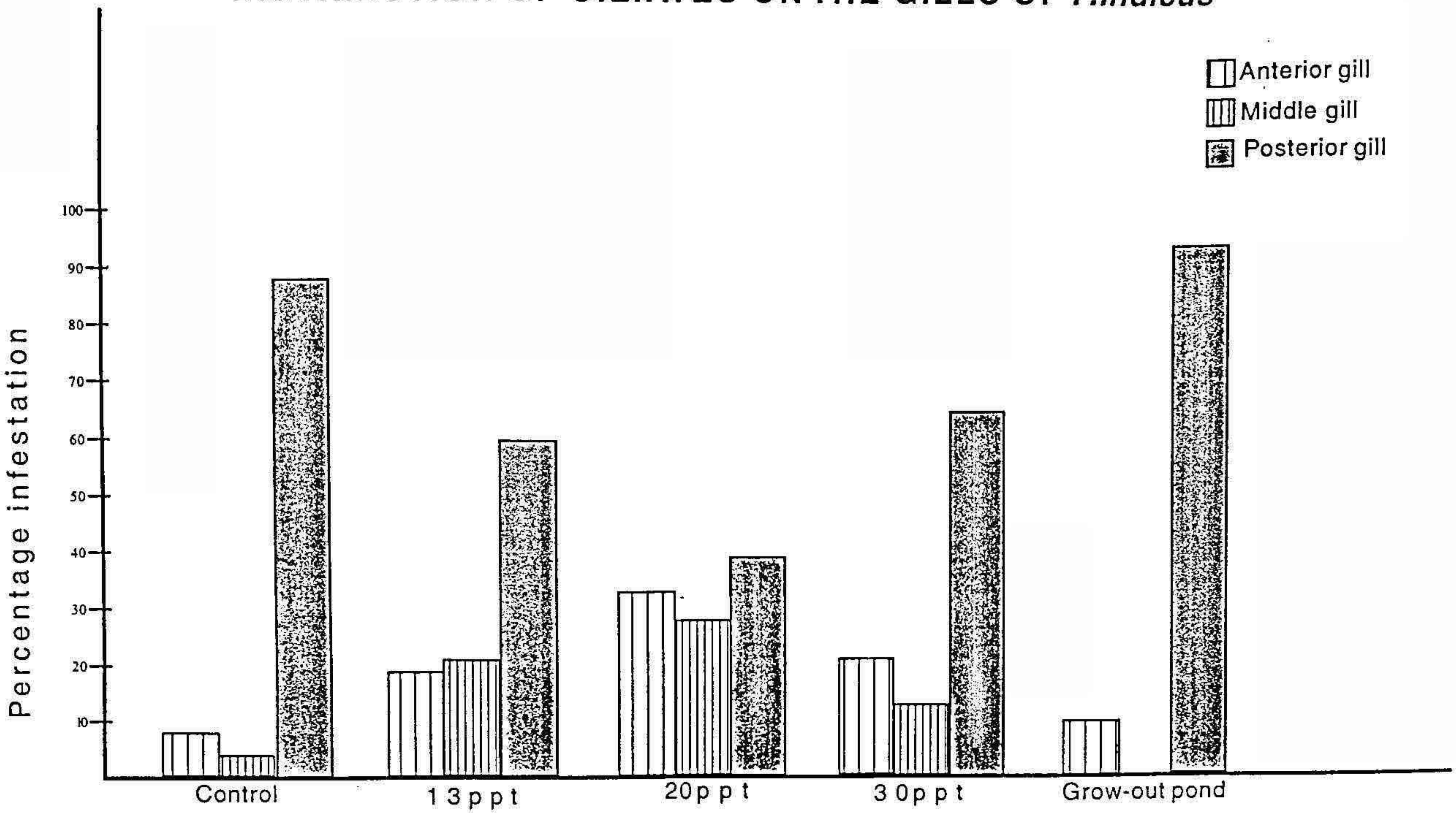


FIGURE - 4
DISTRIBUTION OF CILIATES ON THE GILLS OF *P.Indicus*



DISCUSSION

The study on the occurrence of ectocommensal ciliates on *Penaeus indicus* in the culture ponds, revealed that these commensals flourished in the site of study. Of the ectocommensals encountered, the predominant form was of the genus *Zoothamnium* from both the sites. This reflected upon, the high abundance and prevalence values from the ponds, an average of 83% and 43, respectively. These values were contributed mainly by *Zoothamnium*, as the other ectocommensals encountered namely *Acineta* and *Vorticella* were in negligible numbers. There have been reports of the occurrence of ectocommensal ciliates in culture ponds of crustaceans. An accont by Overstreet (1973) on the common and rare organisms and diseases in the Northern Gulf of Mexico and from the ponds at Grand Terre, Lousiana shows the predominance of the ectocommensals ciliates of the genus Zoothamnium associated with the white shrimps (*Penaeus vannamei)* and brown shrimps (*P aztecus).* Ciliate protozoans of the genera *Epistylis, Acineta, Lagenophrys,* and *Cothurnia,* were found to be associated with cray fish (Procambarus clarkii [Girard]) culture in commercial ponds (Scott and Thunes, 1986). 94% of these crayfish were infested and 65% of them had more than 100 ectocommensals on the gill surface. Santhakumari and Gopalan (1980) observed the peritrichous ectocommensals *Zoothamnium rigidum* (Precht) and the heterotrich, *Stentor coeruleus,* to be associated with the estuarine shrimps *Metapenaeus monoceros* (Fabricius) and tanaidacean *Apseudes chilkensis* (Chilton) from the Cochin back waters. Nurdjana *et al.,* (1977) reported that *Zoothamnium* was the most common ciliate to appear in larval rearing tanks as well as nursery tanks. Couch (1967) highlighted the infestations of the genera *Lagenophrys* and *Epistylis* on moulting blue crabs *(Callinectes sapidus).*

These previous accounts strengthen the possibility of the conduciveness of the culuture ponds, as in Narrackal and Puthveyppu, for the high population of ectocommensals ciliates. As Couch (1986) states, it could be due to the prevailing environmental parameters in the culture field-such as the abundance of substrata in the form of the culture animals and the rich baterial populations, which is the principal diet of colonial peritrichs like *Zoothamnium* (Scott and Thune, 1986; Sleigh, 1973) assciated with high organic load in such environments. Such factors make culture environements appreciable to ciliates and thus shows their occurrence in relation to water quality.

In the present study, the ciliate infestation showed a positive relation with temperature. The temperature recorded at the stations did not show much fluctuation and showed an average value of 33^{0}C. The temperature seemed not to influence the ciliate prevalence and abundance to a great extent. Couch (1973) found that optimum temperature for the culture of the prawns, is often adequate or optimum for the ectocommensal ciliates. But the study by Hudson and Lester (1991) on the ectocommensal infestations in *Penaeus japonicus* and its relation to water quality parameters showed that lower than optimum level of temperature are also adequate for their growth. In the study, the occurrence of *Zoothamnium* at higher temperature during summer months may show that these ciliates can tolerate both high and low temperatures.

Santhakumari and Gopalan (1980) has observed that, the *Zoothamnium rigidum* infestations increased during the monsoon period and substantiated this to the low salinity of the period. Overstreet in 1973 found high infestations in the wild population of the brown shrimps *P aztecus* to occur more often at times of low salinities. In the present study ciliate colonies were prevalent durng all the salinities encountered. This may show the high tolerance they have to a wide range in salinity. But the negative relation obtained during this study supports the former observations by Santhakumari and Gopalan, and Overstreet, and showed that *Zoothamnium* seen on *P indicus* showed preference towards lower salinities.

As there was no substantial fluctuations in the pH at both the stations, the relation between this parameters and ciliates infestation in *P indicus* could not be found. However Nurdjana *et al.*, (1977) reported the high occurrence of ciliat es in hatcheries especially at pH values lower than 7.

Dissolved oxygen is the most important factor in the culture environment which has a profound influence on the well being of the culture animals. Shrimps being benthic animals,are known to tolerate low oxygen concentration to an extent. But mortalities of brown and white shrimps reared in ponds at Texas, during concentration of dissolved oxygen of 2.6ppm was attributed to the heavy infestations of *Zoothamnium* on their gills. The dissolved oxygen concentration encountered in the present study were relatively high for prawn culture sometimes showing supersaturation as on many sampling days. The average dissolved oxygen concentration was nearly 5.0ml per ltr. The ciliate prevalence and abundance showed a negative trend with the dissolved oxygen concentration. The highest prevalence was seen during the lower values of dissolved oxygen.

However, a high positive relation was observed between *Zoothamnium* colonies and the concentrations of total suspended solids. As the T.S.S. concentration increased, the *Zoothamnium* colonies were also found to increase. The high negative relation between dissolved oxygen and T.S.S. values showed the probablity of the occurrence of high organic matter in the ponds, as the ponds also show high phosphate value during the study. Thus the reduction in oxygen at high T.S.S. values could be due to the bacterial degradation of organic matter (may be as the result of the degradation of planktonic matter) which is substantiated by the high phosphate value due to decomposition, by bacterial activity. From the observation it can be inferred that one main cause for the infestation of *P indicus* was due to high organic content in the culture medium. Rise in infestation with turbidity due to organic matter has been mentioned by Hudson and Lester (1992), Scott and Thune (1986). Nardjana *et al.*, (1977) reported the increase in infestations by *Zoothamnium* in hatcheries as the result of high organic matter. Santhakumari and Gopalan in 1980 also observed the high incidence of *Zoothamnium rigidum* in *M.monocer os* and *A.chilkensis* during the instence of crowding and high organic content in the water. Couch (1967) highlighted the infestation of genera Lagenophrys and *Epistylis* on moulting blue crabs during the rise in the stocking density as well as organic content of the culture waters.

Of the nutrient concentrations, that of nitrate was at optimum levels. But the concentration of phosphate was very high and could be due to the high bacterial activity in the ponds. The high phosphate value, the high surface oxygen concentrations as well as the negative relation between oxygen and total suspended solids strengthens the possibililty of the bacterial activity and the phytoplankton in the pond.

Bacteria form the principle food for many peritrichous colonial ectocommensals (Scott and Thune, 1986; Couch, 1986; Sligh 1973). Thus the high incidence of peritrich *Zoothamnium* in the ponds show the probable abundance of bacteral population from the plankton die-off.

Experimental Studies

The fluctuations in the ciliate abundance and prevalence in the stress conditons of water quality and salinities, from that of the control, show the positive influence of stress. In stress conditions, the infestation increases, as has been seen in the works of Couch (1986), Lightner (1977), and Santhakumari and Gopalan (1980). Even though no mortalities were reported during the experiment, there could be every possibility that a low oxygen concentration could have detrimental effects. All the tanks showed higher prevalence and abundance, thus concerting the effect of stress. Another interesting feature that was seen in the experiment was the increase in the abundance in the 13ppt tank. When compared to 30ppt, it showed high abundance and prevalence with respect to the control. Not only that, the increasing abundance over the days showed that low salinity were favourable for these commensals.

The study on the prevalence of ciliates on the site of the gills also yielded interesting results. The occurrence of ciliate colonies on the posterior gills in almost all of the experimental animal as well as those from the samples from the ponds show the prefarence for the site of attachment among the gills. Couch (1986) has cited that among the sites of attachment, the gills are a better location, as they offer better protection as well as a better supply of food. The overlying branchiostegite offers protection to these sessile ciliates. The steady current of water filtered through the gills also help in providing a continuous flow of bacteria present in the medium, which is the principal diet of colonial ciliates (Sleigh, 1973). The ciliates use the gills merely as a place of attachment (Couch, 1986; Lightner, 1975). This information has been proved both histologically (Lightner, 1975) and electron microscopically by Foster *et al* (1978). Lightner found that there were no mechanical injuries at the site of attachment of the ciliates. There was no foreign - body response from the host haemocyte as well. The reason to this was explained by Foster *et al.*,(1978) through the first ever electron microscopic study of the attachment of *Zoothamnium sp.* on gills and pereopods of *Penaeus aztecus* and *Penaeus setiferus*. They found that the base of the stalk of *Zoothamnium* forms circular disc that fuses with the epicuticle but does not penetrate the underlying cuticle or epithelium. Thus the mode of attachment does not cause injuries or affect the growth of the animal. Thus they become detrimental to the shrimps when they block the area of respiration. Thus the suffocate the host by reducing the function of the gills, especially in stress conditions due to the deterioration of the culture environment.

The ectocommensal ciliates form an important group of organisms that cause problems to the shrimps and other crustacean cultures. Although they do not cause mechanical injuries or any other lesions on the sight of attachment, the massive mortalities of cultured invertebrates associated with these protozoans highlight their importance. Many studies have been made on the incidence of these ciliates when associated with such mortalities. But, more work has to be conducted to correlate the various environmental entities with incidence of ciliate occurrence and abundance in the various culturable animals, it infests. Many observations provide important information about the mortalities inflicted by the low oxygen concentration, during their infestations. But experiments and observations have to be made to see how these ciliates interact during such instances. This will help in the effective monitoring of, and the control of these vast occuring protozoans.

SUMMARY

The important observations made during the study are summarised below

1. More than 80% of the shrimps, *P. indicus* from the different stations, seemed to be infested with ectocommensal ciliates.

2. The ectocommensals encountered were of the genera *Zoothamnium, Vorticella* and *Acineta.*

3. Of the ectocommensals, *Zoothamnium* was the predominant, contributing to nearly 95% of the total number of ectocommensals ciliates.

4. The abundance, which is the number of ciliates per gill, was seen to be about 54 in each individual.

5. From the observation of water quality parameters, the dissolved oxygen,total suspended solids and salinity were found to influence the infestations in *P indicus.*

6. Oxygen concentration showed a negative relation with the infestation.

7. The concentrations of T.S.S. showed high positive relation with infestation and a negative with dissolved oxygen.

8. Infestation also showed a negative trend with salinity, although present in a wide range of salinity; this showsits euryhaline nature.

9. The experiment conducted helped to show that stress from the deteriorating quality of water as well as salinities 13ppt and 30ppt showed an increase in the infestations.

10. The abundance and prevalence at 13ppt strengthen the observation that a lower salinity is preferred.

11. The examination of gills showed the higher occurrence of the ciliates on the posterior ones.

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