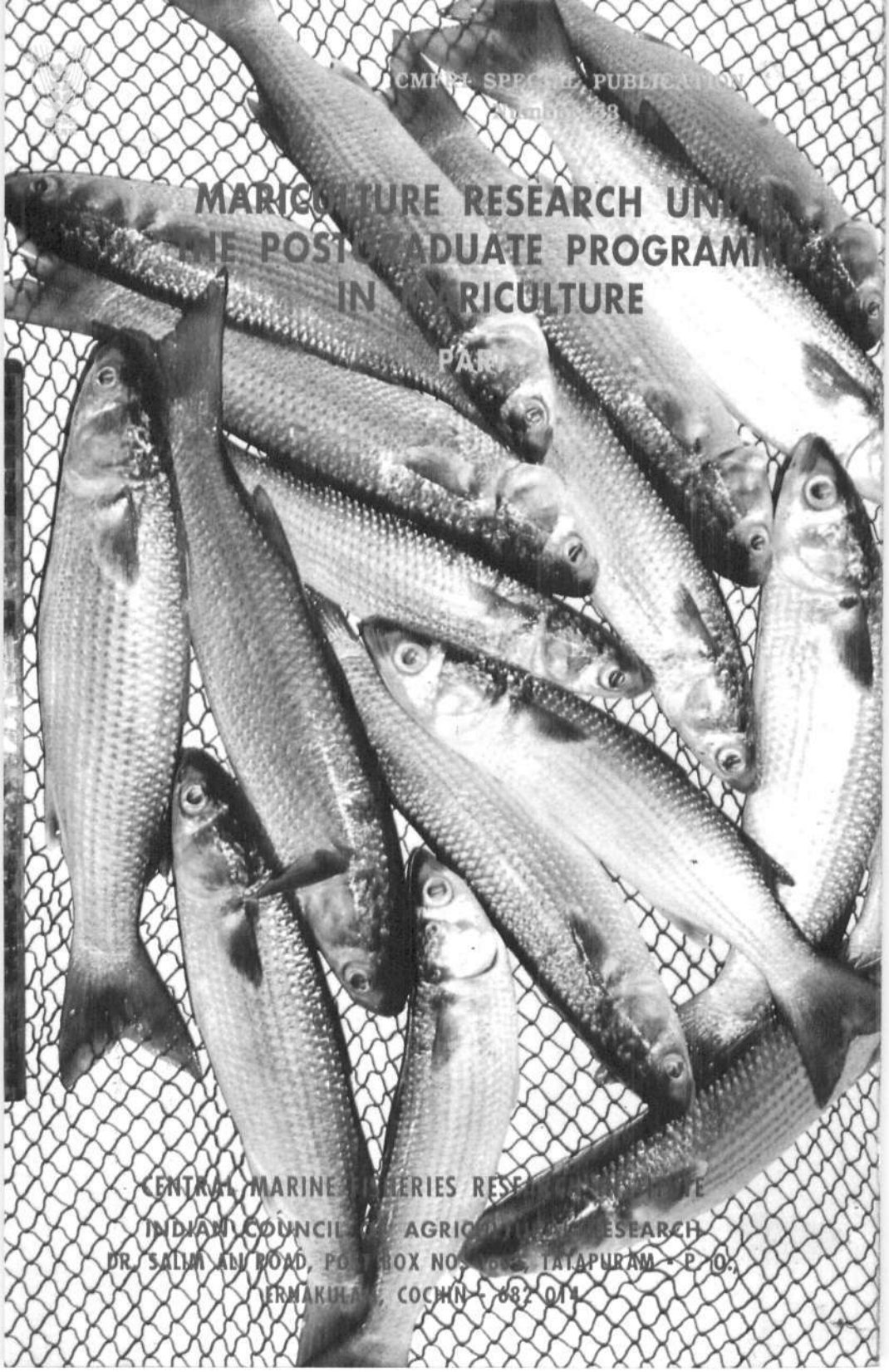

CMFRI SPECIAL PUBLICATION

Number 28

MARICULTURE RESEARCH UNI[T]
IE POST[-G]RADUATE PROGRAMM[E]
IN [M]RICULTURE

PA[RT]

CENTRAL MARINE FISHERIES RESE[ARCH] INSTITUTE
INDIAN COUNCIL [OF] AGRICU[LTURAL] RESEARCH
DR. SALIM ALI ROAD, PO[ST] BOX NO. [1603], TATAPURAM - P.O.,
ERNAKULA[M], COCHIN - 682 014

The Centre of Advanced Studies in Mariculture commenced in 1979 at the Central Marine Fisheries Research Institute, Cochin under one of the Sub-projects of the ICAR/UNDP project on 'Postgraduate Agricultural Education and Research, is now continued as a regular **Postgraduate Programme in Mariculture**. The main objective of the PGP in Mariculture is to catalyse research and education in mariculture which forms a definite means and prospective sector to augment fish production of the country. The main functions of the Programme are to :

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— improve the quality of postgraduate education in mariculture;

— make available the modern facilities, equipments and the literature;

— enhance the competence of professional staff;

— develop linkages between the Centre and other institutions in the country and overseas;

— undertake collaboration programmes; and

— organise seminars and workshops.

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Cover Photos by Shri M. Kathirvel.

MARICULTURE RESEARCH UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE

PART 2

CMFRI SPECIAL PUBLICATION
Number 53

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
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OXYGEN REQUIREMENT OF PRAWN LARVAE IN THE HATCHERY SYSTEM*

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Introduction

The rearing of prawn larvae under controlled conditions has had limited success, due largely to insufficient knowledge of their environmental and nutritional requirements. Factors such as dissovled oxygen, the temperature regime, the salinity and other physico-chemical characteristics of water play significant roles in the survival and the growth of the larvae. Adequate knowledge of all these parameters and their effects on larvae is essential to successfully culture them.

Oxygen consumption is often used as an indicator of sublethal environmental stress on the metabolism of the organism. It is of basic importance in defining the energy budget of the animal. Marine organisms show varying rates of oxygen consumption according to their physiological activity and ecological demand. A knowledge of the specific requirement of each larval stage is therefore necessary to successfully rear them in hatcheries. Hence a study on the oxygen requirements of different stages of hatchery reared prawn larvae was taken up.

Material and methods

The larvae for each experiment were obtained from a single brood, hatched out and reared at the laboratory. Three stages of larvae were used in the experiments *viz.* Nauplius (0.36 - 0.48 mm), Protozoea (1.40 - 1.55 mm) and Mysis (3.39 - 3.58 mm).

*Prepared by the Editorial Committee.

Each experimental run lasted for 12 hrs and sampling was done at hourly intervals. The experiments were conducted at ambient temperature of 30 ± 1° C. The temperature of the water was 29 ± 0.5° C and the salinity was 34.5 ± 1‰. The experiments were carried out in water of known oxygen concentration. The difference in oxygen content at the beginning and at the end of the experimental time was taken as the amount of oxygen consumed by the larvae. The number of animals per bottle was determined according to the size of the experimental animals and consisted of Nauplius - 100, Protozoea - 50 and Mysis - 50.

In each experiment, the animals were counted and transferred to 25 ml beakers containing filtered sea water by repeatedly sucking up 5 - 20 individuals in a narrow glass pipette. Filtered and aerated sea water was siphoned into 36 bottles of 250 ml capacity, taking care to prevent the entry of air bubbles and the animals from the beakers were transferred to them. To serve as controls, 12 bottles of 250 ml capacity were filled with water, but no animals were added. All the experimental bottles were covered with a black cloth in order to eliminate errors due to possible photosynthesis.

Dissolved oxygen content and salinity were determined by the Winkler Method and standard Argentometric Method respectively as given by Strickland and Parsons (1968, *Fish. Res. Bd. Canada Bull.*, **167** : 1-111). The results obtained in the present study ware subjected to analysis of variance test (Baily 1981, *Statistical methods in biology*. The Chaucer Press, Bungay, Suffolk, Pp. 99-114) in order to determine the significance of variability between larval stages and between the experimental hours.

Results and discussion

The oxygen consumption per animal was found to be 0.0533 µl/hr in nauplius, 0.1361 µl/hr in protozoea and 0.2969 µl/hr in mysis. The respiratory rate was 4.4375 µl/mg/hr in nauplius, 3.3191 µl/mg/hr in protozoea and 2.8011 µl/mg/hr in mysis. The oxygen consumption increased with increase in size and progress in developmental stage of the larvae. The respiratory rate declined with increase in body weight of the

individual. The increase in oxygen uptake in each stage is of the order of 2.55 times from nauplius to protozoea and 2.18 times from protozoea to mysis. The hourly variation of oxygen uptake was significant in all the three stages during the twelve hours of experimentation. There was significant difference in the rate of oxygen uptake between the stages.

The above results are only basic oxygen requiremtent under ambient conditions of temperature and salinity for nauplius, protozoea and mysis of *Penaeus indicus*. Further knowledge on the variation in oxygen consumption during environmental changes particularly in salinity, temperature and oxygen tension is necessary in order to definitely determine the oxygen requirement of these individuals. It is felt that further work in this field will add to the above information which is so vital for successful hatchery operations for prawn seeds.

CMFRI Spl. Publ., 1993; 53 : 4 - 6

CARBONDIOXIDE EQUILIBRIA AND NUTRIENT AVAILABILITY IN CULTURE ECOSYSTEM*

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Introduction

In India, aquaculturists depend mainly on natural source of water for raising the animals. Hence a sound knowledge about the conservative and non-conservative properties, is very essential for a proper management of our tide-fed farms. A number of gases are found to be dissolved in natural waters namely hydrogen, oxygen, carbondioxide, ammonia, etc., among which oxygen and carbondioxide occupies the prime position. The fluctuation in the parameter such as carbondioxide and oxygen has got a very good impact on water quality management. The other factors such as temperature and pH has synergistic effect on these above mentioned factors. The various forms in which the different elements and compounds are present in water also have very significant role to play in the management. When compared to the natural waters, the culture ponds contain more number of organisms for a standard volume of water. The importance of carbondioxide as a contributor to the fitness of natural waters depends essentially on three factors. In the first place it serves more less, purely in the chemical sense, to buffer the environment against rapid shifts in alkalinity and acidity. A second contribution of carbondioxide pertains to regulating biological process in aquatic communities. The third and most important contribution is that it contains carbon, one of the versatile elements which facilitates the formation of various important biological compounds by tetravalent bonding.

The weak carbonic acid formed by the reaction between water and carbondioxide reacts with lime stone to form soluble

*Prepared by the Editorial Committee.

calcium bicarbonate. The solution of bicarbonate remain stable only in the presence of certain amount of free or "equilibrium carbondioxide". The free carbondioxide represents the carbon-dioxide in carbonic acid plus that in the simple solution. Thus in establishing the water quality criteria of cultured water, acidity and alkalinity are of importance.

There is generally a trend to maintain constant pH in sea and the adjacent water which can be extended by the buffering capacity of sea water with respect to dissolved carbondioxide. It has been inferred that the increase in carbondioxide must be counter-balanced by the other processes that tend to increase the pH and the total alkalinity so that the net balance result in a fairly constant pH. Thus it can be inferred as said above that the studies of productivity in terms of nutrient content and availability can be remarkably facilitated by a side by side study of pH and carbondioxide content. By measuring some of these easily measurable parameters periodically, the quality of the water bodies can be kept up and thereby increasing fish production.

Material and methods

Two ponds at Narakkal at a distance of approximately 250 m from the sea were selected as regular collection sites for the studies.

The diurnal variations for the major nutrients and carbondioxide was conducted twice during the three months of study. For these studies two ponds in Narakkal Prawn Hatchery Laboratory site itself were selected.

The parameters *viz.* atmospheric temperature, water temperature, pH, salinity, dissolved oxygen, free carbondioxide, nitrate nitrogen, nitrite nitrogen, reactive phosphorus, total alkalinity (by direct calculation from salinity) were measured and analysed by the methods described by Strickland and Parson (1968, *Bull. Fish. Res. Bd. Canada,* **167** : 127).

Statistical methods : All statistical analyses were carried out according to Snedecor and Cochren (1967, *Amer. Iowa,* pp. 539).

For the present study 't' test are used to find out the level of significance for the changing pattern of free carbondioxide with other parameters such as dissolved oxygen, pH, water temperature, salinity, alkalinity, for the entire period of three months. Linear regressions and simple correlation were worked out for the above parameters.

Results and discussion

Intermittent low and high temperature values were observed which mainly depend on the monsoon precipitation. Super saturation with respect to dissolved oxygen was observed in both the ponds and these high oxygen values corresponded to low carbondioxide contents. The ponds were uniformally well oxygenated during the period of investigations. The time of observation during the day was found to be significant especially in the case of carbondioxide and dissolved oxygen contents. The pH value showed a general decrease with time although intermittent high values were also observed. The bicarbonate alkalinity rather availability was found to be high during the peak monsoon season. The overall alkalinity of the pond waters was also low during the peak monsoon. This high bicarbonate alkalinity during the monsoon is congenial to healthy phytoplankton growth, since plants depend mainly on bicarbonate ion as the source for carbon. The nutrient availability in the ponds did not show a definite pattern although there was a rough direct relationship between phosphate and nitrate. Nitrite content showed an inverse relationship with that of nitrate. The carbondioxide equilibria were observed to be mostly dependent on water temperature, pH and time of observation and very little correlation was observed with the biological cycle of nutrients. The diurnal variation studies in the concerned ponds also, more or less confirmed the relationship between carbondioxide content, dissolved oxygen and pH, especially the observations during the night period.

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EFFECT OF HYDROGEN SULPHIDE ON JUVENILES OF *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Prawn culture in ponds is distinguished from all other aquaculture, because of the particular behaviour of prawns which spend greater part of the day embeded in bottom soil. It is clear that the nature of the pond bottom will greatly influence their growth and survival. It is common to see the bottom mud blackened by hydrogen sulphide produced by bacterial activity. The growth and survival of prawns are adversely affected by such a polluted pond bottom, leading to poor production of prawns.

Since very little factual data are available on the effect of hydrogen sulphide on the cultivable species of penaeid prawns, the present study was undertaken to get some information on the toxicity of hydrogen sulphide to different size of juveniles of the Indian White Prawn *Penaeus indicus*, a prime candidate species for culture in coastal brackishwater ponds. The effect of hydrogen sulphide found in the bottom soil on the feeding and burrowing habits of this prawn were also studied and the results are discussed in the context of hydrogen sulphide concentrations.

Materials and methods

Test organisms : Juveniles of *Penaeus indicus* (ranges 20-25 mm 40-45 mm and 80-85 mm) were used for the experiments. Maximum care was exercised to avoid injury to the animals. All the prawns used for the experiments were in the intermoult stage. Test animals were acclimatised for 24 hr under laboratory conditions.

Lethal toxicity bioassays : a. 24 h LC_{50} experiments : To find the 24 hr median lethal concentration to three size groups were exposed to hydrogen sulphide concentration of 1 mg/l to 14 mg/l in flow apparatus. For experiments on 20-25 mm and 40-45 mm size groups, 10 prawns were kept in the test chamber for each hydrogen sulphide concentration tested. For the 80-85 mm size group five prawns were used for each run. The hydrogen sulphide stock solution was prepared by dissolving a known quantity of analar grade $Na_2S.9H_2O$ in one litre of oxygen free distilled water. The test water had a salinity of 32-33 ppt, pH 8.1-8.3 and temperature 28.0-28.5°C. The behaviour of the prawns in the animal chamber was closely observed through out the experimental period. Animals that lay on the side and showed no movement of the appendages were considered dead. Dead animals were removed from the chamber and examined for any discolouration or damage to gills, etc. b. Effect of pH on LC_{50} : As pH of the medium is known to affect toxicity of hydrogen sulphide to animals, using the same equipment and procedures described above, the 24 hr LC_{50} for 40-45 mm juveniles of *P. indicus* were determined at four different pH levels *viz.* 5.9-6.3, 6.9-7.3, 8.1-8.3 and 8.8-9.3.

Weight loss due to hydrogen sulphide toxicity : During the course of the above experiments it was found that the prawns exposed to hydrogen sulphide concentrations for 24 hr were flaby to touch, unlike the normal prawns which appeared turgid to touch. To see whether this flabiness could be due to loss of water from the body of the prawns, the following experiments were conducted.

Ten healthy juveniles of *P. indicus* 80-85 mm in total length, acclimatised in sea water of salinity 30 ppt were selected. The individual wet weight was taken. Then five of them were introduced into the animal chamber of the flow apparatus. The sea water flowing through had a salinity of 30 ppt, pH 8.1-8.3 and hydrogen sulphide concentration of 1.5 (± 0.5) mg/l. The other five were kept in a beaker through which hydrogen sulphide free sea water from the same reservoir was made to flow. At the end of 24 hr the wet weight of the prawns were

again taken. Using the same size of the prawns acclimatised to 15 ppt sea water another experiment was conducted with hydrogen sulphide concentration and pH, same as in the previous experiment. The weight loss in the lower salinity was compared with the weight loss in 30 ppt salinity.

To see whether the weight loss was due to catabolic loss of organic matter, the following experiment was conducted. Fifteen *P. indicus* 80-85 mm in size acclimatised to 30 ppt salinity were weighed to find the initial wet weight. Individual dry weights were also taken. The percentage of dry weight over the initial wet weight was calculated for these prawns and taken as reference values. Then five animals were kept as control and five were kept in the experimental chamber and the experiment was performed as mentioned earlier. Two sets of experiments were conducted with sea water of 30 ppt and 15 ppt. At the end of the experiment the individual percentage dry weight over the initial wet weight was estimated for the experimental and control groups and compared with the reference group.

Behavioural response to hydrogen sulphide charged substratum : To study the behavioural response of the juvenile *P. indicus* to hydrogen sulphide containing substratum the following experiments were carried out in an aquarium tank with two incompletely partitioned compartments. One compartment was provided with washed sea sand, another with a mixture of sea sand and dry powdered clay in the ratio 3:1 and third compartment provided with wet black hydrogen sulphide containing soil taken from the pond and filled with 30 ppt sea water. Ten *P. indicus* 80-85 mm in total length were introduced into the aquarium tank. The animals were fed twice a day with clam meat. The animal distribution in the three compartments were noted three times a day at 0200, 1000 and 1800 hrs. The experiment was continued for seven days. If there is no preference for or avoidance of any substratum the animals will be distributed uniformly in all the compartments.

Feeding behaviour on different types of substratum : Three aquarium tanks were taken, one of them was provided with washed sea

sand, another with sand and dry powdered clay. The third tank was provided with wet bottom soil of the pond containing hydrogen sulphide. In each tank 33 ppt sea water was filled and ten juveniles of *P. indicus* 80-85 mm size were introduced. The animals were fed with pellatised diets twice a day. Left over pellets were collected and counted. From this, the number of pellets eaten by the animal was noted.

Ecology of culture pond : The fluctuations in the ecological parameters were monitered at fortnightly intervals. Samples of surface and bottom water were taken from these stations to estimate temperature, dissolved oxygen, pH, salinity and hydrogen sulphide. Soil samples were also taken from the fixed stations to determine the total sulphide, pH and Eh.

Results and discussion

The hydrogen sulphide toxicity bioassays conducted with different sizes of juveniles of *P. indicus* have shown that the 24 hr LC_{50} declined with increase in size of prawns. The calculated LC_{50} values have 7.22, 6.44 and 3.35 mg H_2S/l for 20-25 mm, 40-45 mm and 80-85 mm size groups respectively at a pH range of 8.1 - 8.3.

It was also evident that the LC_{50} values declined sharply with decrease in pH *i.e.* H_2S becomes more toxic to prawns at lower pH levels. The LC_{50} was 6.83 mg H_2S/l at pH 8.9-9.3, 6.4 mg H_2S/l at pH 8.1-8.3, 3.10 mg H_2S/l at pH 6.9-7.3 and 0.47 mg H_2S/l at pH 5.9-6.3.

During the course of the 24 hr LC_{50} experiments, some general observations were made on the appearence and behaviour of the prawns subjected to different concentrations of H_2S. After 2 to 3 hrs of exposure they were sluggish and showed restricted movements. Once they fell to the bottom they showed circular movement on the bottom in the lying down position. Further all prawns exposed to H_2S showed blackening of gills. At a concentration of 1-2 mg H_2S/l the blackening became apparent almost 20 hr after exposure, whereas at 5 mg/l the gills became black 8 hr after exposure. Another observation was that

the prawns subjected to H_2S concentrations showed intensification of body colour. An interesting observation was that the prawns were soft to touch and the pleopods were white when exposed to H_2S concentrations in the flow apparatus. These prawns were examined under the microscope and found that the pleopod setae and the gill filaments were covered with a profuse growth of a *Leucothrix* like filamentous bacteria.

The marked reduction in LC_{50} values at lower pH levels recorded during the present experiments is in agreement with results obtained by earlier workers who attributed the greater toxicity of sulphides at lower pH to the fact that dissolved sulphides at low pH levels exist mainly as un-ionised H_2S which is more toxic to animals than the ionised forms; HS^- and S^{--}. At pH 9.0 only 1% exists as un-ionised and at pH 5.0, 99% of sulphide are present as un-ionised H_2S.

During the present study period the concentrations of dissolved sulphides in the bottom water of the ponds were far lower than the 24 hr LC_{50} H_2S levels calculated for *P. indicus* and so would not have been lethal to the prawns. The highest concentration recorded in the ponds was 0.11 mg H_2S/l. In the natural condition high levels of H_2S was negatively correlated with pH of water and soil and also with dissolved oxygen. Temperature showed positive correlation with H_2S in water and soil. Low values of Eh was noted in stations where the sulphide concentration was high.

The prawns, being demersal forms which spend their time on bottom either burried in the mud or browsing for food among the sediments, will certainly be affected if the H_2S level is increased by diffusion of H_2S from the sulphide rich soil especially during the summer months when the water temperature is high leading to accelerated growth of anaerobic bacteria in the mud.

Even if the H_2S level in the pond are not high enough to cause acute lethal effects, it should be remembered that concentrations as low as 0.0007 to 0.003 ppm un-ionised H_2S if persisting for a long period could produce chronic toxic effects

such as retardation of growth, lowering of fecundity and inhibition of spawning in fish and invertebrates. In the amphipod *Gammarus pseudolimaneus* food intake declined when H_2S level reached 0.05 ppm. In the aquarium experiments conducted during the present study feeding of *P. indicus* juveniles was markedly inhibited on pond soil rich in total sulphides and the prawns also did not moult eventhough no H_2S could be detected in the aquarium water.

The build up of H_2S in the pond soil and bottom water apart from affecting natural prawn food production in the ponds and inhibiting the feeding response, would have reduced the area available to the prawns for burrying and resting during the day time, as the prawns clearly avoided the H_2S charged substratum during the aquarium experiments. Thus the sulphur cycle in the ponds during the hot months seems to create an environment of stress in which lack of natural food, sublethal effects of H_2S toxicity, low dissolved oxygen tension and reduction in living space are important factors.

In fact it was during this period that the prawns stocked in the ponds were affected by soft prawn disease a very common phenomenon in Vypeen Island. The flabbiness of the experimental prawns noted during the 24 hr LC_{50} experiments is very similar to the conditions of the prawns during early stages of the soft prawns syndrome. The weight loss observed during the toxicity experiments appears to be mainly due to osmotic loss of water through the gills. When the experimental animals exposed to H_2S for 24 hr, suffered an average weight loss of 7.3% at 30 ppt salinity and 2.5% at 15 ppt salinity and the gills became black due to exposure to H_2S. This blackening may be indicative of damage to the gill epithelium which plays an active role in osmoregulation. When the gill epithelium is damanged by exposure to H_2S, water is lost from the body by osmosis to the external medium which at 30 ppt salinity is hyperosmotic compared to the body fluids of penaeid prawns.

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STUDIES ON SULPHUR BACTERIA IN THE PRAWN CULTURE ECOSYSTEM

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Introduction

Sulphur in its various organic and inorganic forms is essential for all living organisms. Availability of sulphur can limit the productivity of the aquatic environment and has been linked to decreased productivity of fish. The major reserve of the element in sediment is unlocked only through biological decomposition. Microbiol transformations of sulphur in the bond is governed to a large extent by environmental factors that effect composition and activity of the microflora.

Sulphur bacteria are the groups which oxidise or reduce sulphur or its inorganic compounds and the genus *Desulfovibrio* and *Desulfotomaculum* play a key role in sulphur cycle. Sulphate reduction alone accounts 53% of total mineralisation of organic matter in brackishwater sediment.

Objectives

The present investigation was taken to study the bacterial parameters and physico-chemical parameters in two different prawn culture ponds (one perennial and one Pokkali field) for a period of 4 months from June to September 1984 to find out the factors responsible for the quantitative variations in the hydrogen sulphide production in the culture ecosystem.

Material and methods

Samples were collected fortnightly from two field points from each pond (Pond A & B) located at Narakkal (10°01' N-75° 16'E) between 0800 hrs and 0900 hrs and were brought to the laboratory within two hours in an icebox. Samples were subjected to bacteriological analysis within 3 hrs of collection.

Other chemical parameters of mud and water were found on the same day of collection. Samples were kept in a refrigerator at 4°C till the time of bacteriological analysis.

Results and discussion

Total heterotrophs in both the ponds showed a similar trend of variation. In Pond 'A' total heterotrophic counts in the sediments varied from 11 x 10^5/g to 23 x 10^6/g of mud and in water from 15.5 x 10^4/ml to 21.0 x 10^5/ml during August and June. In Pond B in the sediments the variation was from 15 x 10^5/g to 29.5 x 10^6/g of mud during the same period. The total heterotrophs varied from 18 x 10^4 to 23 x 10^5/ml in water during September and June. The maximum value of 29.5 x 10^6/g of sediment was observed during the first fortnight of July. A sudden decrease in bacterial population was noted in August in both the ponds.

In Pond A the sulphate reducers varied from 2.45 x 10^2/g to 6.6 x 10^2/g of mud. The maximum number was observed during June and a minimum value of 2.45 x 10^2/g of sediments was recorded during July, August and September. In Pond B the variation was from 2.45 x 10^2/g to 20.8 x 10^2/g of mud during August and June respectively. A sudden decrease of sulphate reducers was noted in July.

Multiple regression analysis of the results showed that in both the ecosystems salinity, sediment Eh and total heterotroph have correlation with sulphate reducers. Standard partial regressions analysis showed correlation with environmental parameters in the order, sediment Eh, Salinity, total heterotrophic populations in sediments and water. In pond B however the order was found to be total heterotrophic populations in water and sediments, sediment Eh and salinity.

Results of the biochemical tests on 18 isolates of sulphate reducers showed that all the isolates were gram negative asporogenous/rods and motile. Their morphology varied from small curved rods to straight rods. All the isolates produced H_2S. Sodium chloride tolerance test showed that they failed to

grow in 7% and 10% solutions. All the isolates grow in NaCl concentrations of 0%, 1% and 3%.

Conclusion

The results of the biochemical tests were compared with the taxonomic scheme given in the Bergey's manual of determinative bacteriology (1974). It was found that the species involved in the process of sulphate reduction in Ponds A and B were *Desulfovibrio desulfuricans* and *Desulphovibrio aestuari* and the standard partial regression analysis showed positive correlation of these species with Eh of the environment.

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INFLUENCE OF HYPOXIA ON METABOLISM AND ACTIVITY OF *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Exposure to low levels of oxygen (hypoxia) is a common event in the life of many animals and it has been proved that the level of dissolved oxygen in the surrounding medium has a definite effect on the oxygen consumption in Crustacea. Since metabolism is directly reflected on respiration, the uptake of oxygen by the animal is taken as a measure of its metabolism. In ammoniotelic animals like prawns, a measure of ammonia excretion is important as a measure of protein degradation. In addition to this, behavioural changes especially that indicated by random activity are important in studying energy utilization and survival.

In the present study, oxygen consumption, ammonia excretion, ammonia quotient* and random activity of three size groups (40-50 mm, 70-80 mm and 130-140 mm) acclimated to three temperatures (28, 32 and 36°C) have been investigated at different levels of ambient oxygen. There recovery metabolism of the prawn has been studied after subjecting the animal to hypoxia.

Materials and methods

Male and non-ovigerous female intermoult prawn belonging to the above mentioned size groups were collected from the

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* Ammonia Quotient (AQ) = $\frac{\text{Volume of ammonia excreted}}{\text{Volume of oxygen consumed}}$

grow out ponds of the Institute and maintained in plastic lined pools of 200 litre capacity. Only healthy prawns acclimated for seven days in the experimental temperature were used. During this period they were fed *ad lib* with clam meat and later starved for 24 hours before experiment.

Apparatus : The experimental apparatus consisted of two units, an electronic counter and a transparent plastic respirometer. In the electronic counter twenty small photocells in two rows were fixed along side vertically at 180° C to each other in the inner hollow of the annular respirater, so as to face two focus - lights fixed just outside the periphery of the respirometer. The interruption of the light beam by the animal was sensed by a transister amplifier which in turn actuated the electronic relay. The relay triggered the main contactor and thereby the counter. The random activity counts recorded by the electronic counter were counter-checked by observing the movements of the animals visually.

A modified annular Fry's Respirometer of 3000 ml capacity was used for the experiments. In addition to this a recirculation system was deviced. It consisted of a 200 litre capacity rectangular overhead tank and reservoir tank kept at ground level. Water from the reservoir was pumped to the overhead tank which was flushed through the respirometer. Compressed air was bubbled in both the reservoirs to maintain oxygen concentration of the water near air saturation.

Experimental Design : The following series of experiments were done.

1. Routine metabolism in normoxic condition (in ambient oxygen concentration near air saturation).
2. Influence of hypoxia on metabolism and random activity.
3. Recovery metabolism after hypoxia.
4. Influence of temperature on (1), (2) and (3).
5. Influence of size on (1) (2) (3) and (4).

Experimental procedure

Routine metabolism in normoxic condition : The experiment was conducted for 60 minutes in the respirometer. The initial and final water samples were taken for estimating the dissolved oxygen and ammonia. The random activity counts recorded by the electronic counter were noted after each run. For conducting experiments at higher temperature (32 and 36°C), the electronic counter was seperated from the respirometer and the respirometer was kept immersed in a tank where the temperature was adjusted to the desired test temperature.

Influence of hypoxia on metabolism and random activity : At the start of the experiment, samples were taken and thereafter with an interval of 60 minutes samples were taken and an equal amount deoxygenated water was added to the respirometer. During the last run, the final samples were collected only after the animal reached the asphyxial oxygen level as indicated by the equilibrium loss of the animal. Then the respirometer was flushed with air saturated water to revive the animal.

Recovery metabolism after hypoxia : The former phase of the experiments was followed for a few hours after the animal was asphyxiated. Air-saturated water at the specific temperature was flushed through the respirometer for 30 minutes to revive the animal from asphyxia. At the end of the 30th minute, samples were collected and the circulation of water through the respirometer was cut off. After the closure period of 60 minutes, final samples were collected and the respirometer was opened to the circulating water for 30 minutes after which another period of closure followed.

Dissolved oxygen was analysed by the unmodified Winkler method, ammonia by Phenol-hypochlorite spectrophotometric method and salinity by Standard Argenotitric method.

Results

The routine oxygen consumption and random activity of

TABLE 1. *Mean oxygen consumption, ammonia excretion, Ammonia Quotient and random activity in ambient oxygen concentrations near air saturation in three size groups of* Penaeus indicus *acclimated to and tested at 28, 32 and 36°C*

Size Group (mm)	Temperature (°C)	Mean Ambient Oxygen (mg/l)	Mean Oxygen Consumption (ml/m/hr)	Mean Ammonia Excretion (ml/m/hr)	Mean AQ	Mean Random Activity (counts/hr)
40-50	28	5.01	535.0	8.90	0.0165	26.6
	32	4.958	685.7	11.06	0.0160	32.7
	36	4.967	903.8	13.72	0.01512	34.6
70-80	28	5.02	350.4	17.08	0.4780	8.8
	32	5.07	465.0	18.87	0.0407	15.0
	36	4.96	659.8	19.97	0.0288	17.0
130-140	28	5.02	286.3	20.87	0.0727	7.2
	32	5.03	400.5	22.74	0.0566	12.6
	36	5.01	507.9	23.61	0.0467	14.2

TABLE 2. *The asphyxial level of oxygen concentrations of three size groups of* P. indicus *at three different temperature*

Size of prawn (mm)	Temperature (°C)	Asphyxial oxygen concentration (mg/l)
40-50	28	0.54
	32	0.77
	36	0.92
70-80	28	0.62
	32	0.79
	36	0.91
130-140	28	0.98
	32	1.00
	36	1.11

P. indicus decreased with increasing body size (weight) at all the three temperatures studied (Table 1). The rate of ammonia excretion increased with increasing body weight. The AQ values also increased with increase in body weight indicating that relative protein utilization is more in larger prawns. The rate of oxygen consumption and ammonia excretion and random activity increased with increase in temperature in three size groups of prawns studied. But the AQ values showed a reverse trend suggesting that relative protein utilization is less at higher temperatures. The oxygen consumption of three size group of prawns decreased with decrease in ambient oxygen at all the three temperatures tested. Therefore, *P. indicus* can be considered as an "Oxyconformer". The asphyxial levels of the prawns were influenced by body size and temperature (Table 2). The asphyxial levels of smaller prawns are found to be lower than larger ones. The asphyxial level of *P. indicus* increased with increase in temperature. The rate of ammonia excretion increased with decrease in ambient oxygen in all the size groups of *P. indicus* at all the three temperatures studied. Size of prawn and temperature also influenced the rate of ammonia excretion. Irrespective of the size groups and temperatures tested AQ values have shown a definite increase with decrease in ambient oxygen. The increased protein utilization as indicated by high AQ values may be of value for the prawn in acid-base balance and also in conservation of Sodium. The random activity of *P. indicus* decreased with decrease in ambient oxygen. Decrease in activity under hypoxic exposure, though it may not lead the animal to escape, would allow it to conserve energy. With some tolerance to low oxygen, the limited source of oxygen and energy conserved might be of value in survival. All the size groups of *P. indicus* accumulate oxygen debt at all the three temperatures studied. The oxygen debt is either fully or partially repaid during the recovery phase.

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EFFECT OF PARTICLE SIZE IN THE COMPOUNDED DIETS ON THE PELLET STABILITY AND FOOD CONVERSION EFFICIENCY IN *PENAEUS INDICUS*

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Introduction

The water stability of an aquatic feed plays an important role in determining the overall performance of the feed. Feed pellets which disintegrate faster facilitate rapid leaching of nutrients, especially the micronutrients leading to non-availability to the animal. This leads to pollution of the water and economic loss. The water stability of the feed depends primarily on the binding material used. However, the method of preparation of the feed also contributes considerably to its stability. Invariably, in formulating experimental diets and practical feeds, a variety of ingredients with different properties are used. Grinding the raw materials to an uniform particle size is essential for preparing a homogenous feed mixture. The impact of particle size of the ingredients on the stability and the digestibility of the feed is not known. In this context, the effect of particle size of ingredients on the pellet stability in the water and the digestibility of an experimental (purified) diet and a practical prawn feed was evaluated for the prawn *Penaeus indicus* in the present study.

Materials and methods

To study the impact of particle size of ingredients on the pellet stability, food conversion ratio, digestibility and growth of prawns, a research diet and practical prawn feed were selected.

Research diet : The research diet consisted of the purified ingredients caesin, sucrose, starch, cod-liver oil, cellulose, vitamins and minerals and other additives. Chromium oxide was included for digestibility determination and sodium alginate was used as the binder. Among the solid ingredients obtained, only caesin was in granular form and all the others were fine powder. Hence only caesin was prepared into different particle size as follows. It was first dried in the oven at 60 ± 2°C for 12 hours and powdered in a micropulverizer, using 1.0 mm mesh sieve. The powdered material was sieved through seven different standard sieves of 500, 420, 300, 250, 212, 100 and 50 micron mesh sizes with the help of a mechanical sieve shaker and prepared into samples of the respective particle sizes.

Practical feed : The practical feed consisted of prawn waste, mantis shrimp (*Oratosquilla nepa*), fish meal, groundnut oil cake and tapioca. Among the ingredients, prawn waste, mantis shrimp and groundnut oil cake were seperately prepared into six samples of particle sizes 500, 420, 300, 250, 212 and 100 microns as described under the research diet. Since fishmeal and tapioca were available in fine powder form, they could not be prepared into different particle sizes.

Preparation of diet and feed : In the case of research diet, seven diets, 1, 2, 3, 4, 5, 6 and 7 were prepared into dry pellets of 3 mm diameter having 500, 420, 300, 250, 212, 100 and 50 micron particles of caesin respectively, whereas in the case of practical feed, seven feeds, 8, 9, 10, 11, 12, 13 and 14 (mixed sizes of particle obtained without sieving) were prepared as dry pellets (3 mm diameter) having the similar descending order of particles. Starch in the tapioca served as binder in practical feed. The pellets were made with the aid of a hand pelletizer. They were cut into pieces of approximately 5 mm length.

Water stability of pellets : Thirty pieces of the pellets of each diet and feed were taken into previously dried and weighed pouches of bolting cloth (No. 30) and the weight of the pellets was

recorded. The pouches were slowly immersed in troughs containing 25 L of water having 8 cm depth and placed in a petridish at the bottom of the trough. Simultaneously for each diet, nine pouches were lowered in the trough. At the end of one hour the petridish along with the pouch was gently taken out, the physical shape of the pellets was noted, the adhering salt was carefully washed away and dried in the oven at 60°C for 24 hours.

The loss in weight of the pellets was recorded. Similarly the pellets which remained in water for 2, 3, 4, 5, 6, 8, 12 and 24 hour time intervals were treated and the loss in weight was calculated. The experiment was repeated thrice and the average loss in weight in each case was determined. The salinity, temperature and pH of the water used in the troughs are same as reported under the feeding experiments.

Feeding experiments : For determining the digestibility, growth and food conversion ratio of each group of pellets, hatchery reared juveniles of the prawn *Penaeus indicus* with an average length and weight of 33 mm and 0.331 gm respectively were stocked at the rate of 6 animals in circular troughs of 30 l capacity. There were three replicates for each treatment. The prawns were fed at the rate of 20% body weight initially and feeding was regulated according to the left over food, each day. The animals were fed twice daily, dividing the feed in equal portions. Water management in the troughs was carried out by removing the sediments, providing aeration and changing complete water once in five days. The salinity of the water was maintained at 15 ± 1 ppt, temperature at 28° ± 1°C, oxygen at 4.1 ml L^{-1} and pH 8.2 ± 0.2. The duration of the feeding experiment was 30 days. Separate feeding experiments were conducted with research diets and practical feeds.

For determining the digestibility, faeces were carefully collected and pipetted on bolting cloth and gently washed with distilled water. The samples were dried in the oven at 60°C for

12 hours. Faeces accumulated over the period of feeding experiments, were analysed for Chromium oxide content.

Results

Physical characteristics of the constituent particles of feed in the pellets affected cohesion between them and thus play a determining role in the stability of the pellets in the water. Results of the present study clearly indicated that in the purified diet, weight loss of pellets become lesser with the increase in particle size from 50μ to 212μ, but more from 212μ to 500μ. Minimum weight loss was exhibited by diet No. 5 with 212μ particle size ($P<0.05$). The changes occurring in the physical shape of pellet with time reveal that the smaller the ingredient particle size, the more the pellet is stable. Only diets 5, 6, 7 in increasing order of stability could retain shape upto 6 hours.

Larger spaces among the particles of larger sizes, which reduces cohesion between them are most likely responsible for lower stability in diets 1 to 4. Diet 5 and 7 having finer particle will have faster leaching of nutrients thus resulting in more loss of weight though remaining intact in shape.

In the practical feeds the difference in weight loss among diets was found to be insignificant ($P>0.05$) except for diet 14 (mixed particles size) which lost the maximum amount of weight. Diet 10, 11, 12, 13, & 14 were able to retain physical shape upto eight hours while diets 8 and 9 started disintegrating at the end of three hour.

Food conversion ratio vs particle size : FCR was significantly better with research diet having 212μ particles size, most likely due to better retentivity of shape and less weight loss.

Digestibility vs particle size : Digestibility of 96.7% (at 500μ particle size) and 94.23% (at 212μ particle size) were observed with purified and practical diets respectively. Digestibility decreased with particle size with practical diets. The digesti-

bility at 212μ particle size was significantly more as compared to that of other (lower as well as higher) particle sizes.

Growth vs particle size : Growth was significantly better ($P<0.05$) with the feed having 212μ particle size in both sets of experiments.

In the light of the results of the present study, particles of 212μ seems to be optimum for the preparation of compounded diets for *P. indicus* in terms of both water stability and growth of prawn *P. indicus*.

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EFFECT OF CERTAIN ENVIRONMENTAL FACTORS ON DEVELOPING EGGS AND EARLY LARVAE OF THE MULLET *LIZA PARSIA* (HAMILTON-BUCHANAN)*

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Introduction

Liza parsia (Hamilton-Buchanan) is one of the common mullets in the Cochin Estuary and constitutes a thriving fishery in the estuarine and brackishwater systems of India. The techniques of artifical propagation and larval rearing have been perfected to a great extent. However very little information is available at present regarding the relationship between the earlier life stages and the environmental factors of mullets compared to other fishes. Such information on the developing embryos and early larvae in relation to environmental factors, acquired through carefully planned laboratory experiments is an inevitable need for continued research in improving our techniques of larval rearing of mullets. Hence it was felt worthwhile to carry out a series of experiments to study the influence of salinity, pH and light on the developing embryos and early larvae of *Liza parsia* (Hamilton-Buchanan) induced to spawn in the laboratory.

Material and methods

Spawners collected were hand stripped at proper time and fertilized with required quantity of fresh milt by wet method of fertilization with very little quantity of control sea water. Once fertilized, the eggs were transferred to a clean plastic tub having enough clean normal sea water having the same salinity in which the fish spawned. For all experiments eggs within one hour of fertilization in the blastomere stage were used.

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*Prepared by the Editorial Committee.

Two series of experiments were designed and conducted for each parameter. In the first series the fertilized eggs at the blastomere stage were transferred to various levels of the respective parameter, the percentage of hatching noted and the newly hatched larvae were transferred to fresh solutions of the same qualities as the incubating solutions and is referred to as *Test - to - Test Series.*

The range of salinity tested were 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51 and control of 31 ppt which is the salinity in which the fish has spawned. The various ranges were prepared as mentioned by Shapiro (1961, *Science,* 133 : 2063 - 2064). The ranges of pH tested during the present investigation were 5, 6, 7, 8, 9, 10 and control (8.15). Lower ranges of pH were prepared by adding the required volume of 1 N HCl drop by drop in required quantity of seawater with thorough mixing and pH adjusted by 1 N NaOH.

The light intensities tested during the present investigation were Zero lux (complete darkness), 14, 24, 95, 240, 500, 1700 lux, control (14.5 lux) and direct sunlight. The constant light intensity were obtained by using different power bulbs at a constant distance (80 cm) from the surface throughout the experimental period. Polynomial regression analysis of the data was carried out, following Snedecor and Cochran (1967, *Amer. Iowa,* pp. 539).

Results

The viable eggs of *Liza parsia* fertilized in sea water of 31‰ and transferred within one hour in the blastomere stage to different salinity ranges from 0 to 51‰ (at intervals of 3‰), showed hatching success in 6 to 51‰. The hatching sucess (in the range 18 to 51) was found to increase with an increase in salinity to an optimum salinity of 26.63‰ and decrease with further increase in salinity. The lower and higher extremes of salinity caused higher mortality of developing eggs, embryos and hatched larvae and abnormalities in the hatched surviving larva. When these larvae (hatched in the different ranges *viz.* 6-51‰) were further transferred to a second series of containers

containing the same ranges of salinity, survival of larvae for more than 3 days was observed in ranges 6-39 with the optimal success at 24.92‰ (in the range 9-42‰). Another series of experiments, larvae hatched in sea water of 31‰ when transferred to different salinity ranges 0 to 51‰ within 6 hours of hatching, larval survival for more than 72 hours was observed in the ranges 3-39‰. The survival showed no correlation with salinity. In both the experiments on larval survival (*i.e.* test-to-test series and control-to-test series) the 2 day old larva showed less tolerance to the higher ranges of salinities and in general the optimal survival value of salinity for larvae shifted to lower values compared to the same value for the hatching success. The early larvae were more tolerant to lower salinity ranges than developing eggs.

When eggs fertilized in normal sea water of pH 8.15 were transferred at the blastomere stage within 1 hour to different pH ranges 5 to 10 (with intervals of '1') hatching occurred in ranges 5-9. Only one larva hatched in pH value 5 and this also died immediately. The low range of pH *viz.* 5 and the high range of pH *viz.* 10 caused the maximum mortality of developing eggs and embryos, thus reducing the hatching success. The hatching success (in the range 5-10) increased with an increase in pH values till the optimal point of 7.5 and decreased with further increase in pH. The larvae hatched in the respective pH ranges were further reared in fresh pH solutions having the same pH values. Survival of more than 70 hours was then observed only in pH ranges 7-9 with the optimal peak survival at pH 7.8. When larvae hatched in normal sea water with pH of 8.15 were transferred around 10 hours of hatching to the different pH ranges 5 to 10, survival for more than 3 days was observed only in pH ranges 6.0 to 8.15 with the peak survival at the optimal point of 7.03. In the rearing experiments of early larvae (test-to-test and control-to-test) the larvae showed better survival in lower pH values and the optimal survival value of pH in the early larvae (in control-to-test series) was lower to that of the hatching period. The alkaline pH of 9 showed its detrimental influence on the 2 day old larvae.

In experiments on light fertilized eggs in blastomere stages when transferred to containers exposed to different light intensities varying from 0 to 1700 lux and direct sunlight (average lux above 42050), hatching was observed in all the light intensities. The results showed that the optimal low light intensity of 109 lux is more conducive to hatching success and that light is not a factor needed for hatching. Direct sunlight was observed to be harmful for hatching success by causing high mortality on developing eggs and killing the newly hatched larvae within 8 hours of hatching. In the range 0 to 500 lux, light showed a close influence on hatching, the hatching increasing from 0 lux to an optimal lux of 109 and then decreasing with further increase in light intensity. Freshly hatched larvae, when transferred to various containers kept exposed to various light intensities from 0 to 1700 lux and direct sunlight, larval survival after 72 hours was observed only in light intensities 0 to 500 lux. Larvae surviving for 72 hours in complete darkness showed abnormal body transparency.

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STUDIES ON THE EFFECTS OF TEMPERATURE AND pH ON THE POSTLARVAE OF *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

The effect of temperature and pH are particularly pronounced in shallow coastal zones and estuaries. Under normal conditions the pH and temperature will not be harmful to any organism. In view of the increasing industrial pollution threatening the aquatic ecosystems, studies about the tolerance limits of temperature and pH will provide essential information for the proper management of resources in the inshore waters and the prawn and fish culture which is gaining considerable importance in recent years. So, the purpose of the present study was to examine the effect and tolerance limits of temperature and pH on *P. indicus* postlarvae under simulated conditions in the laboratory. Specifically this study was aimed at determining the LC-50 and LD-50 values of pH and temperature respectively.

Material and methods

Acute toxicity tests : Postlarvae (PL-1 to PL-25) of *P. indicus* were obtained from the Prawn Culture Laboratory of CMFRI at Narakkal. For the growth rate studies, the same were collected from surf waters. These larvae were acclimatised in 30‰ salinity at zoom temperature for at least 6 hours before the experiment was initiated.

pH : The test waters with different pH (3, 4, 5, 6, 7, 8, 9, 10 & control) were prepared by adding 1N Hydrochloric acid and 1N Sodium hydroxide solution for lower and higher ranges respectively. These waters were kept in separate closed bins for more than two weeks and every day, the varying pH adjusted till it got stabilised. For experimental purpose, 1 litre capacity

glass beakers filled with 800 ml of test water was used. The pH was measured by pH meter.

Temperature : Temperature of the sea water was controlled by using temperature control unit which consists of Jummothermometer, heating coil, relay system (temperature cut-outer) and heater. The Jummothermometer and heating coil were immersed vertically in a 75 litre capacity aquarium tank filled with sea water. The beakers of 1 litre capacity with 800 ml sea water was immersed in the aquarium tank. The temperature inside the beaker and the tank were tested for uniformity with ordinary standard mercury thermometer. The temperature ranges of 30, 32.5, 35, 37.8, 40.8, 44.1°C and control (26-28°C) were used.

Ten postlarvae were subjected to each beaker and the whole system was aerated. For each dose, triplicate sets were maintained. The experiment media were changed every 24 hours.

Duration : For acute toxicity test, the animals were exposed to 96 hours and for growth rate studies, it was 15 days. The weight and length of 10 random samples were taken just before starting the experiment and also the same studies were conducted on the experimental animals. The larvae were fed with minced prawn meat of about 10% of the body weight twice in a day.

Experiment on growth rate : Effect of temperature on growth was found out with different temperature ranges of 26-28°C (control), 30°C, 32.5°C and 35°C. This was conducted in 5 1 capacity glass beakers filled with 3.5 1 of sea water. Half of the water was changed every day and the water was completely renewed every alternate days. The animals were weighed and measured individually before introducing into the test waters. The length of the animals was measured every 5 days interval from the respective days at which the experiments started.

In all the experiments, the test waters were analysed before starting and after every 24 hours of the experiment for oxygen, salinity and ammonia.

Statistical analysis : LC-50 (concentration in which 50% of the

animal is killed) and LD-50 (lethal dose for 50% organisms) values for pH and temperature respectively were estimated by weighted-probit analysis. For growth rate studies, the effect of different levels of temperature has been tested by analysis of variance.

Results

Acute toxicity test for pH

PL-1 : In pH 3, postlarvae were very active for five minutes soon after the introduction into the media. Then they slowly became inactive and started crawling on the bottom after 15 mts. Within one hour of exposure, all the larvae died. In pH 4, the larvae were not as active as in the former case. Evenafter 24 hours of exposure 3% of the larvae survived and complete mortality occurred within 37 hours. The mortality rate decreased to 17% in pH 5 and 10% in pH 6 & 7 after 24 hrs exposure. In pH 5, complete mortality was observed only after 56 hrs whereas in pH 6, 7, 8 and 9, evenafter 96 hrs of exposure only 57, 40, 33 and 40% of mortality respectively were occurred. The minimum mortality rate was in pH 8 and it again reached to the maximum at pH 10.

PL - 5, 10, 15 & 25 : In pH 3 and 10 complete mortality was observed after 24 hrs of exposure. In pH 4, 40% survived in PL-5 & 10, 80% in PL-15, 60% in PL-25 after 24 hrs of exposure. After 48 hrs, there was 100% mortality in PL-5, 88.34% in PL-10, 60% in PL-15 and 90% in PL-25. Whereas after 96 hrs, 100% mortality was observed in PL-10 and 94% in PL-15 and 25. In pH 5, 23.4% mortality was noticed in PL-5 and no mortality in the rest of stages after 24 hrs. After 96 hrs of exposure, 66.7% died in PL-5, 20% in PL-10 and 15 and 24% in PL-25. In the waters of pH 6, 7, 8 and 9 there was no mortality in any of the stages.

LC-50 values of pH : In acidic media, the LC-50 values of PL-1 to 25 varied from 3.7 - 4.7 after 24 hrs of exposure, 4.2-5.6 after 48 hrs and 4.45-5.71 after 96 hrs. In alkaline media, the LC-50 value for PL-1 was 9.5, 9.4 and 9.15 after the periods of 24, 48 and 96 hrs respectively. In all other stages, this value was 9.5.

Sublethal effects : The arrangement of chromatophores on cuticle was disturbed by the different grades of pH solutions. In low acidic waters the pigments were concentrated and in high alkalinity it was in expanded condition after 96 hrs of exposure.

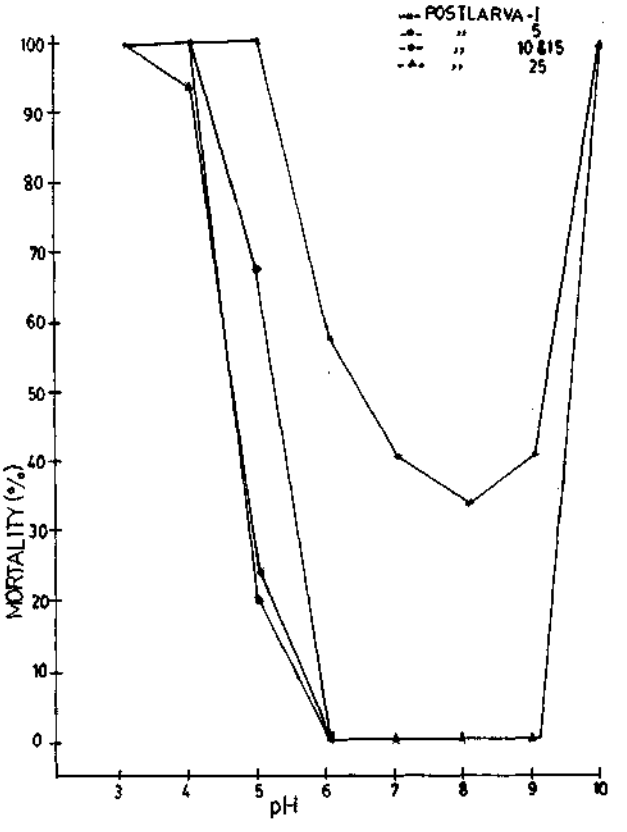

Fig. 1. Comparison of the susceptibility of different stages of postlarvae (1, 5, 10, 15 & 25) after 96 hrs of exposure in different pH.

A reduction in growth was observed in the postlarvae reared in waters of pH 4, 5 and 6. They were not as healthy as from the control. The high alkaline water even affected the swimming activity of the larvae.

Acute toxicity test for temperature

PL-1 : At this stage the larvae were highly susceptible to temperature variations. There was 17% mortality in control (26-28°C) whereas in 30°C it was only 7% after 96 hrs of exposure. Then the mortality rate again increased from 32.5°C onwards. The mortality rate in 32.5°C and 35°C were 40% and 93% respectively after 96 hrs. The larvae survived only for 40 hrs in 37.8°C. When the temperature of the water was increased to 39°C, the larvae became very active till it reached to 40°C. At 40.8°C they survived only for 20 mts. At 44.1°C, the larvae died within 5 mts and its body curved in the shape of 'C' in both the temperature of 40.8 and 44.1°C.

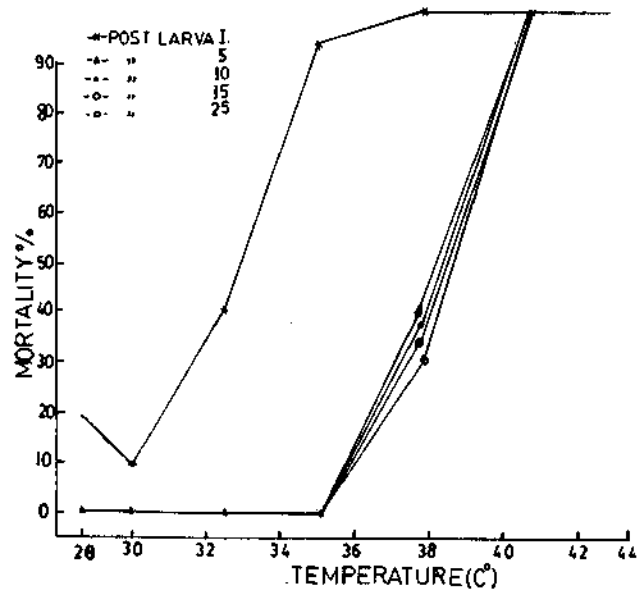

Fig. 2. Comparison of the susceptibility of different stages of postlarvae (1, 5, 10, 15 & 25) after 96 hrs of exposure in different temperature.

PL-5, 10,15 & 25 : From PL-5 onwards, they were more resistant than the earlier stages. There was no mortality in control waters and upto 35°C. At 37.8°C, there was a gradual decrease in the

mortality and it varied from 40% in PL-5 to 30% in PL-25. At 40.8°C, there were 100% mortality in all the stages.

LD-50 values : In PL-1, the LD-50 value was very low ranging from 32.27 - 34.9°C after 24-96 hrs of exposure. In other stages, it varied from 38.15 - 38.5°C.

Experiment on growth rate : It is evident from this experiment that the temperature level which was not lethal in 96 hrs of exposure can be lethal in long term exposures. The 35°C was not lethal in acute toxicity test whereas it was lethal when the animal was exposed for longer period. When the temperature was increased, the growth in length also increased upto 32.5°C. The further increase in temperature retarded the growth of the animal. In the case of weight, it increased only upto 30°C and started reducing in high temperatures.

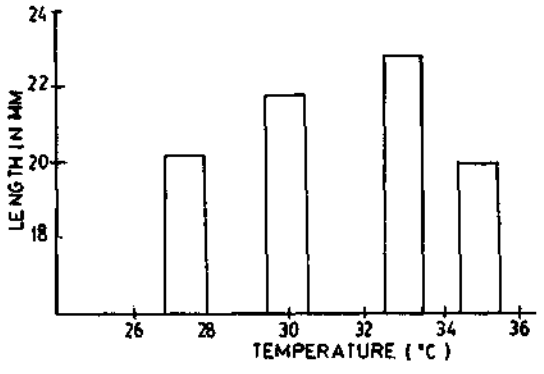

Fig. 3. Effect of temperature on length of postlarva.

The analysis of variance showed that the growth rate was not significant in different temperature ranges. This may be due to the reduction in growth in high doses. That means, the increase in growth at 30.0 and 32.5°C is compensated by the retardation of growth at 35°C. Biologically the data indicated that the maximum growth in length is taking place at 32.5°C and growth in weight at 30°C. The minimum growth and maximum food intake were observed in 35°C.

Discussion

pH and temperature have a pronounced effect on *P. indicus* postlarval survival and growth. The present study showed that the pH values between 6 and 9 was not lethal in short term exposures from PL-5 onwards whereas PL-1 is highly susceptible to the slightest environmental changes. In the present study the lethal pH were 3, 4 and 5. The pH less than 6.7 was lethal to the crustacean *Acartia tonsa*. The extreme alkalinity is also lethal to organisms. At pH 10, complete mortality was observed in the case of *P. indicus* postlarvae within 30 mts.

For PL-1, the median lethal concentration after 24, 48 and 96 hrs of exposure were 4.73, 5.6 and 5.71 respectively. For PL-5, the lower median lethal concentration of pH was 4, 3, 5.1 and 5.4 after an exposure of 24, 48 and 96 hrs respectively. In PL-10, this value further decreased to 4.15, 4.3 and 4.65 in the same order of exposure as above. In PL-15, the respective values were 3.7, 4.2 and 4.6. But in PL-25, these values were slightly higher being 3.8, 4.55 and 4.45. In tests using bluegill sunfish (*Lepomis macrochirus*) of different size groups, Cairns and Scheier (1958, *Ind. Waste*, 3 : 126) found that the median lethal pH value for 4 days exposure were 3.6, 3.6 and 3.5 for fish with mean length of 3.9, 6.7 and 14.2 cm respectively. But Lyod and Jordan (1964, *Int. J. Air. Wat. Pollut.*, 8 : 393-403) found no correlation between sensitivity and size of rainbow trout of any one age group, but a positive correlation existed between age and sensitivity. In the present observation also, it was found that the LC-50 value decreased with age and increased with duration of the experiment. In alkaline waters, the median lethal pH for PL-1 decreased with durations of 24, 48 and 96 hrs to 9.5, 9.4 and 9.15. In all other stages, the LC-50 value was 9.5. Cairns and Scheier (1958, *Ind. Wastes*, 3 : 126) found that the 4 days median tolerance limits of pH value for bluegill sunfish were 10.5, 10.5 and 9.9 for fish with mean lengths of 36, 61 and 142 mm respectively. In brown trout, Carter (1964, *Eff. Wat. Treatment. J.*, 4 : 484-486) recorded 9.6 as median ph value for a period of 20 hrs.

In the present study, larvae reared in low pH waters (4, 5, 6) were smaller in size when compared to the control. This stunted growth was more pronounced in older stages of post-larva. Trojnar (1977, *J. Fish. Res. Bd. Canada*, **34** : 574-579) also reported a reduction in normal length of prolarva of *Salvelinus fontinalis* reared in pH 5.2. According to Campbell (1961, *Salm. Trout. Mag.*, **161** : 47-52), there was no correlation between pH value (4.9-8.4) and growth rate of brown trout.

In temperature experiment, the postlarvae survived upto 35°C in acute toxicity tests. The temperature higher than 37.8°C was lethal to PL-1 and higher that 40.8°C was lethal to older stages (PL-5 to 25). Costlow and Bookhout (1970, *4th European Mar. Biol. Symp.*) studied the thermal death point on larvae of mudcrab *Rhithropanopeus harrisii* and got a highest survival rate at 30-35°C. Catedral (1977, *SEAFDEC*, **3** : 13-16) worked on the effect of temperature on the survival and growth of *P. monodon* larva. The results showed 73% survival at 29-32°C whereas it again increased to 78% at 32.34°C. In the present study, the best temperature for PL-1 was 30°Cand the upper median lethal dose recorded as 34.9°C after 24 hrs and 32.25°C after 48 and 96 hrs exposures respectively. In PL-5 to 25, the LD-50 value was higher varying from 38.51-38.15°C. Here also a marked variation in resistance can be seen in different age groups. The upper median lethal temperature for *P. indicus* from Tuticorin Bay was 41.7°C (Santhanam *et al.* 1980, *Indian J. Mar. Sci.*, 9 (4)). The growth rate studies of postlarva showed that the growth in length was maximum at 32.5°C and that of weight at 30°C. A reduction in growth rate was found at 35°C. Zein-Eldin and Aldrich (1965, *Biol. Bull. Mar. Biol. Lab., Woodshole*, **129** : 199-216) observed the same effect of temperature and salinity on brown shrimp *P. aztecus*. According to him, the growth rate increase with temperature between 15 and 32.5°Cand the growth rate decreased markedly at 35°C. Smith (1930, *Biol. Bull.*, 58 (2) : 193-202) studied the effect of temperature upon the chromatophore distribution of crustacea. The result of that experiment showed that at 36°C, the animals were mainly reddish and at 30-28°C shrimps were colourless. The lowest temperature capable of

expanding the chromatophore was found to be 35°C. The present study showed that there was no marked colour difference in *P. indicus* postlarva even at a high temperature like 37.8 and 40.8°C. But the chromatophores were highly expanded compared to lower temperatures of 35°C and below.

Conclusion

The present study showed that the optimum pH range for postlarvae of *P. indicus* was between 7.5 and 8.5. However, the LC-50 values varied from pH 5.71-3.70 for postlarvae 1.25. The optimum temperature was between 30 and 32.5°C. The LD-50 value for PL-1 to PL-25 was 32.27 - 38.51°C. The maximum growth in length attained at 32.5°C and maximum weight at 30°C.

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COLONIZATION OF THE MANGROVE *ACANTHUS ILICIFOLIUS* IN THE SEA ACCRETED REGIONS NEAR COCHIN

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Introduction

Mangroves are characteristic coastal vegetation and the community of tree and shrub along with faunal communities drawn from the adjoining estuaries and terrestrial system, form distinct specialised ecosystem. Apart from wood, timber and other produce of forests, the mangroves has been much valued as shore protectors, desalination agents, their high productivity and transport of organic food to other systems and capacity to recycle organic matter. From the fisheries point of view mangrove creeks and swamps form important nursery grounds for fishes and prawns. In the Cochin mangrove system *Acanthus ilicifolius* forms the predominant vegetation and have colonised traditional aquaculture fields and areas potentially suitable for such practices. Realising this importance and significance to coastal aquaculture, a study was undertaken especially to understand the different factors responsible for the colonisation, differential distribution, etc. of *Acanthus ilicifolius* in the sea accreted areas of Vypeen Island. A study on the germination requirement of the species too is attempted.

Materials and methods

The distribution pattern of *A. ilicifolius* in Vypeen Island, was made out through a reconnissance survey from the barmouth upto Narakkal. The mapping of the distribution of the species was done taking the Vypeen-Munambam Road as reference. Based on the survey three habitats were recognised as follows for taking up detailed study :

1. Large mangrove patches (>150 Sq. m.) dominated by *A. ilicifolius* where *Avicennia* sp. forms less than 5% by shoot number, situated immediately west of Murukkampadam.

2. *A. ilicifolius* in association with *Avicennia* (20-40% by shoot number), located on southwest portion of Vypeen Island.

3. Smaller patches (<50 sq. m) of *A. ilicifolius* occupying the canal or creek sides from Puthuvyppu to Narakkal.

The above three habitats were chosen as sampling site A, B & C. 3 sampling areas each in sites A & C and 2 in site B were fixed at random within the vegetational patches. In these sampling areas fortnightly sampling on both biological and environmental parameters were carried out from April to August 1984. For the study each time 3 quadrant of 50 cm x 50 cm were selected in each of the sampling area.

The shoot material in each quadrant was cut out 10 cms above ground level using a garden scissors and the following parameters were studied :

a. Shoot density : Number of shoots per unit area.

b. Standing crop : weight of the total shoots cut off.

c. Mean length and diameter of shoot-measured by vernier calipers and measuring tape.

d. Percentage composition of different phases : by number of young shoots by cloning, flowering shoots, fruiting shoots, drying and green shoots.

Soil samples from a depth upto 15 cm were collected from 3 spots at each area and pooled together in polythene bags. Separate soil samples were taken in air tight containers and the pH and Eh of the wet sample determined immediately on returning to the laboratory. The sample in polythene bags were air dried, ground and seived through 0.5 mm seive for different

chemical analysis. The electrical conductivity, exchangeable cations *viz.* Na^+, K^+ and Ca^{++} ions, chloride ions, total nitrogen and available phosphorus of the samples collected at each fornight were determined by standard methods. Water samples were also collected of which the pH and salinity were determined by standard methods. Air dried soil passing through 2 mm seive was analysed for grain size composition by the pipette methods and seive analysis.

The tidal fluctuation for specific peak at a particular spring tide was studied in the sampling areas by recording water level against marked fixed reapers at one hour intervals from rise to fall of the peak.

The seedling development and germination studies : The different aspects of natural seedling development and growth were studied in marked out quandrants every fifth day from 20th July 1984 onwards.

To study the effect of tidal inundation on germination and seedling growth in the field, three different areas with differential tidal effect were selected : (a) a creek side are inundated on an average for 12 days every moon phase, (b) an area where the inundation were around 8 days in a moon phase and (c) an area which is not inundated. Three quadrants each were marked out and 16 seeds collected from the plants of the same area sown in each quadrant by July 20th 1984 and the early development recorded every 5th day till the end of August 1984.

Similarly experiments were also conducted in the laboratory for studying the effects of different salinities and tidal inundation over germination and early development of the species. Soil from the mangrove habitat was collected and filled in shallow plastic troughs in laboratory close to glass windows. They were seasoned with brackishwater of 4 ppt.

The experiments for the inundation studies were conducted in troughs where simulated inundation twice daily for 3 hrs, 2 hrs, 1 hr and one with 24 hrs inundation and another

without any inundation (just kept damp) was maintained. Triplicates were maintained for each treatment, where ten seeds each were planted and studied for 22 days.

The experiments for effect of salinity were also conducted in similar troughs where a uniform inundation of 3 hrs twice daily of water of different salinities of 50‰, 40‰ 30‰, 20‰, 10‰, 4‰ and 0‰ were given. Germination and development for 19 days were recorded.

Results

The reconnaissance survey shows that the mangrove *Acanthus ilicifolius* shows a distribution with three different habitats.

a. Occurs in large colonies as in site A (b) in association with *Avicinnia* sp. or (c) as small patches along tidal canals.

The shoot density is high in site A and C (117 to 162/sq. m) compared to site B (90-64/sq. m). The shoot density reaches a peak by June end from a minimum in May. The standing crop of the species is highest in site A (8.4 to 13.21 kg/sq.m). The standing crop in site A shows two clear dips in mid May and mid July, while it is almost steady in A & C. The shoot length in sites A and C (0.93 to 1.2 m), from an initial higher value it drops down to mid May with a recovery till June. The low shoot length in site B shows an increasing trend over the period.

The appearance of young shoots in the plants expressed as percentage increase from a low (0 - 5.03%) from April-May to a peak (28.5 to 36.84%) in June-July. The flowering is in a peak in April in site A (56.88% and 22.9%) while in site B the flowering period is lengthy from May to mid June. The fruiting peak (65.49% and 32.96%) is highest by mid June in A and B and by May end in C. Maximum drying (29.5%, 19.2% and 47.37%) was observed in mid May.

The seedlings started appearing by end of June, end of July and mid July in site A, B & C respectively. The respective

peaks were during July end in site A (34.6/ sq.m) and B (22/ sq.m) and June end in C where the number of seedlings are very low. The survival of seedlings at the end of study period over the peak were 71.2%, 45.45% and 51% respectively. The survival of seedlings were maximum (75.5%) in the fringes of vegetative patches in site A.

Water salinity of A & C could only be studied as there was practically no inundation in site B. The salinity in A was higher compared to C and showed ranges of 29.1‰ - 7.3‰ and 17.6‰ - 2.89‰ respectively. The soil pH in the three sites ranged from 6.3 to 7.45 and did not show any drastic change over the period. Throughout the period the soil Eh is comparatively high in B followed by A & C. The initial Eh (April) is high as 247.66, 294.0 and 84.66 mVs and steadily falls down to a minimum value in June (-165, 0.5 and -223 mVs) respectively in the three sites. The electrical conductivity which is directly related to soil salinity showed peak (33.90 and 19.32 m mhos) in April in B & C and in mid May (8.89 m mhos) in A. Then it falls over the period to a minimum (5.63 and 1.445 m mhos) by August end in site A & B and in mid July in 3.1 m mhos in site C. The exchangeable Na^+ and chloride ions showed almost the same trend of the electrical conductivity. The exchangeable cations of K^+ and Ca^{++} also showed similar trend, but the fall during monsoon was not that drastic. The soil total nitrogen was high in A (0.59% - 0.8%) followed by B (0.5% to 0.7%) and C (0.29% to 0.75%). The range of available phosphorus in A & B was 0.009 - 0.103% and in C was 0.00436 - 0.0067%.

Some parameters, especially the electrical conductivity (EC) of the soil is related to many of the biological parameteras of the species. The change in biomass is in inverse relation to soil EC, though not proportionally. But in site B the effect is not very significant. The appearance of young clones (vegetative growth) and appearance of seedlings by germination is found to be related to the fall in EC of the soil in three habitats and is also found to be statistically significant.

For the particular spring tide peak of 13th and 15th June 1984 the tidal inundation in the three sites showed variation. The tidal inundation is found directly related to the shoot density and also found statistically significant.

The grain size analysis of soil in the three sites showed the following result.

	A (%)	B (%)	C(%)
Coarse sand (2-0.2 mm)	13.61	20.08	22.86
Fine sand (0.2-0.02 mm)	30.17	41.72	52.37
Silt (0.02-0.002 mm)	33.76	24.10	17.12
Clay (<0.002 mm)	22.46	14.12	7.65

The silt and clay percentage is higher in A followed by B and C.

Germination experiments

The effect of tidal inundation over germination in the field showed that the survival on the 40th day was maximum where tidal inundation of 8/12 days was available followed by condition of 12/14 days inundation and no inundation at all (72.91%, 64.58% and 8.33% respectively). Growth too was maximum in the condition of 8/14 inundation (12.7 cm at a growth rate of 0.382 cm/day).

In the laboratory experiments on inundation effects on germination the survival and growth on 22nd days was maximum in 1 + 1 hr treatment followed by 2 + 2 hr, 3 + 3 hr and moist soil. The germination was impeded in the 24 hr inundation treatment.

The salintity tolerance experiments show that in higher salinities of 50‰, 40‰ and 30‰ no germination occurred at all. Germination was not complete in 20‰. The survival and growth was maximum in 4‰ (91.66‰ and 5.22 cm on 19th day) followed by 0‰ (70.83‰ and 4.92 cm) and 10‰ (50‰ and 3.72 cm).

Statistical analysis (Analysis of variance) show that the relationship salinity vs growth and survival (lab) and inundation vs growth and survival (field) are significant.

Discussion

The parameters both biological and environmental studied, show variation over the period and from site based on which the differences in the habitat and periodical changes in the ecosystem can be explained.

The study shows that *A. ilicifolius* forms the dominant vegetation with highest spread in the Murukkampadam region, while it forms association with *Avicennia* in the southwest region of Vypeen and occurs in patches of single species on creek edges. Mudaliar and Kamath (1954, *J. Bombay nat. Hist. Soc.*, 52) had reported the species as forming large formation at the edge of backwaters of Chirakkal, Tellicherry, Kasargod and Udipi which is similar to the distribution in the first habitat. *A. ilicifolius* is also reported to form large formations in Chipulan where it dominates (Joshi and Jamale, 1975, *Bull. Dept. Mar. Sci., Cochin Univ.*, 7 (4) : 757-760; 1973, *Ecology of mangroves in progress of plant ecology in India*. Today and Tomorrow Printers and Publishers, New Delhi) and Bombay (Navalkar, 1956, *J. Bombay nat. Hist. Soc.*, 53 (3) : 335-345) where it exists along with *Avicennia alba*. Mukherji (1975, *J. Bombay nat. Hist. Soc.*, 72 (1) : 1-20) describes the species in reclaimed areas, embankments and edges of creeks.

In all cases the young clones appear only when EC of soil falls below 11 m mhos and the appearance of seedlings are on fall of EC below 9 mm mhos. This clearly shows that the sexual and vegetative means of reproduction *A. ilicifolius* is triggered by lowering of salinity during monsoon. The laboratory experiments also support the factor and germination and survival was maximum when inundation was given with water of 4‰ and no germination taking place above 20‰. Teas (1979, *Silviculture in Saline Water*. Plenum Press, New York, pp. 117-163) observed that too high interstitial salinity can inhibit seedling development in Rhizophora.

Similarly the influence of tidal inundation is clear and in the field germination and survival was maximum on an average inundation of 8/14 days. Eventhough survival is less in the canal side area where inundztion is more growth is more. In the laboratory experiment on influence of inundation, clear cut trends couldn't be made out, but found that a 1+1 hour diurnal inundation was ideal. This shows that a moderate tidal inundation is ideal for the colonization. such a case has been reported by Wieche (1935, *J. Ecol.*, 23 : 323-333) where the salt marsh species *Salicornia* sp. seedling density in the spring tide zone is 2/6 times that of neap tide zone. In the laboratory experiments the treatment of 24 hours inundation showed no germination at all, which may be due to anaerobiosis of soil. Clarks and Hanon (1970, *J. Ecol.*, 58 : 351-369) in several salt marsh species showed retarded and reduced germination by constant coverage of 5 cm water.

The standing crop in different habitats were in the range of 8.4 - 13.2 kg/sq.m in A, 3.34 - 4.8 kg/sq.m in B and 8.3 - 9.3 kg/sq.m in C. The drying percentages are indicative of the organic matter contribution of these habitats.

The flowering season of the species is found to be in April and extending June or July. This is slightly earlier than the flowering season of the species in Cauvery delta which is from June to August (Blasco, 1975, *The mangroves of India*, **14** : 1-175) and disagrees with Joshi and Jamale (1975, *loc. cit.*) in the statement the *Exoecaria agalodha* is the only mangrove in western India which flowers in monsoon.

The range of pH in all the habitats is found to be 6.3 to 7.5 which is similar to that in Sunderbans (7-8) reported by Mukherjee (1975, *loc. cit.*). Water logging of soil in monsoon may be the reason for the drastic fall of Eh in the sites over the season.

Comparative features of the three habitats

It is found that the shoot density, biomass shoot length and diameter is higher and almost similar in A and C and values for A slightly higher. Moreover the total areas colonized is also more in A. Site B have a lower value for the above features.

A factor having significant correlation to the shoot densities of the plant is the tidal inundation which is high in A and C significantly poor over B. It can then be inferred that *A. ilicifolius* is less efficient in competing with *Avicennia* in regions of lesser inundation.

The water and soil salinities in C is comparatively low and the summer soil salinity in B is higher than A. The lower salinity in C may be due to the greater distance of the site from the barmouth. The higher summer salinity in B may be due to higher evaporation and less inundation of the area, leaving the soil more saline. This again may be retarding the growth of *A. ilicifolius*. In hyper saline conditions *Avicennia* sp. was observed to grow in association with *A. ilicifolius*. Navalkar (1967, *Proc. 54th Indian Sci. Congr.*, Part III, Abst. 164) reports that *A. ilicifolius* in Bombay occurs in higher grounds where salinity is lower. The reasons for the species occurring as small patches along creek edges in site C may be due to the terrestrial plants dominating over the mangrove species in lower saline areas.

There is notable difference in the Eh of soil in the three systems. The Eh is comparatively higher in B even during monsoon. Eh at site C is the lowest and that of A slightly higher. The reason is evidently the greater aeration of soil of site B as it is subjected to lesser inundation, while Eh is lower in other two sites where proper soil aeration is prevented by tidal inundation. It is seen that where Eh is high *i.e.* soil aeration is high the shoot density of *A. ilicifolius* is brought down by competition by *Avicennia* sp. whereas Eh is low in regions of high shoot density (site A & C). This shows that *A. ilicifolius* shows higher tolerance to anaerobic condition of soil and such condition does not affect its colonization. This resembles the salt marsh species *Puccinellia maritima* (Ranwell 1972, *Ecology of salt marshes and sand dunes*. Chapman and Hall, London, pp. 258) which prefers water logged soil with low redox potential.

It seems that *A. ilicifolius* prefers soil of fine grain size as in site A (Silt 33.76% to Clay 22.46%).

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GENETIC VARIATION IN THE FISH *LIZA PARSIA* (HAMILTON-BUCHANAN)

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Introduction

A rational and efficient use of biological resources requires a thorough knowledge of the amount and distribution of genetic variability within the species considered. To assess genetic variation based on morphological characters is difficult and tedious and more over many of the morphological differences between fish of different origin are caused by environmental factors. With the advent of biochemical genetic markers, extensive studies in genetics of fishes have been carried out over the last two decades. But only meagre information on biochemical genetics of Indian fishes is available. With this in mind the present study was initiated in the fish *Liza parsia*, to (i) standardise the optimum conditions for protein and enzyme methodology; (ii) study ontological and tissue expression of protein and selected isozymes, and (iii) study genetic polymorphism in the population of *Liza parsia* in Cochin Backwater.

Material and methods

Electrophoretic studies were carried out on tissue extracts from live specimen of *Liza parsia*. Polyacrylamide disc gel electrophoresis method as described by Davis (1964, *Annals, New York Academy of Sciences*; pp. 402-427) was adopted. Stain and staining methods for enzymes were those described by Redfield and Salini (1980, *Report 116, CSIRO, Melbourne;* p. 120) and Brewer (1970, *Introduction to Isoenzyme techniques*. Academic Press).

For standardisation of methodology, the extraction of protein was tested in three solvents to ascertain the better solvent giving maximum extraction without denaturation. Different amounts of muscle tissue (30, 60 and 80 mg/ml) were

tested to ascertain optimum quantity for good resolution without diffusion or trailing in gel. Various stains such as amidoblack, coomassie brilliant blue, Kenacid blue with varying staining and destaining times and solution were tested to obtain bands with distinct margin and clear background. A total of 12 combinations of acrylamide (7, 9, 10%) and bisacrylamide (2-5%) concentrations were tested to obtain optimum acrylamide-bisacrylamide ratio for maximum number of protein bands.

Ontological changes in protein expression were tested in juveniles (20-40 mm) and adults (120-170 mm). To test ontological changes in lactic dehydrogenase system, larvae (2.5 mm) were also included. The juveniles were collected from creeks near Vypeen Barmouth and the adults from local Chinease dipnets. The larvae were obtained from induced spawning of adults collected from the same location. The tissue expression of protein was tested in adult eye, liver, muscle and brain. Lactic dehydrogenase (Ldh) and esterase (Est) were analysed in eye, muscle, heart, liver, kidney, brain of adults. Genetic polymorphism at Ldh locus 2 was tested in 124 juvenile specimens. The designation for gene loci and allelic variants encocling the enzymes surveyed are in accordance with the system proposed by Allendorf and Utter (1979, *Fish Physiology*, 8 : 407-449). Allelic frequency and heterozygosity were calculated and these values were compared with the expected values obtained using Hardy-Weinberg model. The differences between observed and expected values of allelic frequency were tested with the Chi-square method.

Results and discussion

Among the three solvents tested, protein was found best resolved after extracting with double distilled water. The optimum sample quantity for resolving the protein system in to maximum number of distinguishable bands was 60 mg/ml; lower sample amounts resulted in less number of bands and higher amounts caused diffusion and merging of bands. Among the different staining and destaining procedures tested staining for half an hour in 0.25% Kenacid in methanol : water : acetic

acid (5:5:1) and destaining for 2 to 3 hours in 7% acetic acid resulted in clear and distinct bands of the 12 varying combinations of bisacrylamide and total acrylamide tested, 10% total acrylamide and 2.5 bisacrylamide was found to be the optimum ratio which resolved protein into more fractions.

A total of 13 bands in muscle, 17 bands in eye, 18 bands in liver and 16 bands in brain of *Liza parsia* was resolved in the present study. In five species of grey mullets, the number of muscle proteins resolved by Herzberg and Pasteur (1975, *Aquaculture*, 5 : 99-106) by using a 7.5% total acrylamide gel ranged from five to nine. The more number of bands resolved in the present study is mainly by using a optimum ratio of bisacrylamide and total acrylamide concentrations. No ontological difference in muscle protein was observed in the present study.

In all the tissues tested Est enzyme has been resolved into 7 bands exhibiting different electrophoretic mobilities and based on electrophoretic patterns and tissue specific expression the 7 different isozymes have been grouped into five groups and each group could represent the product of one or more loci. Earlier workers have identified 3 to 8 groups of Est isozymes in other fishes. As in their studies, in the present study too more strongly stained bands were observed in the liver.

The Ldh system present in the eye of *L. parsia* is composed of five bands which are the products of two loci (Ldh 1, Ldh 2), since Ldh is a tetramer. The first and fifth bands from anode are Band 1 and 5 are darkly stained being the heteropolymers of loci Ldh 1 and 2 respectively. In other fishes a tissue specific expression of Ldh loci have been observed with locus 1 strongly expressed in muscle and locus 2 in heart and a third locus active in eye. But in the present study, locus 1 is strongly expressed in muscle, heart and eye and locus 2 is weakly expressed in both muscle and heart and only strongly expressed in eye. A similar findings were made by Jorgensen and Mustaka (1980, *Comp. Biochem. Physiol.*, 67 B : 244-256) in the flounder *Platichthys flesus* where the Ldh in heart and muscle exhibited identical mobilities.

The Ldh system in the adult and juvenile muscle tissue of *L. parsia* was identical. But in larvae only the homopolymer of locus 1 was observed indicating ontological development of locus 2 in *L. parsia.*

From the analysis of data on 124 juveniles, it was observed that locus 1 was monomorphic and locus 2 polymorphic with two alleles. The most common slower one was termed 100 and the other 125. The different genotype observed were the two homozygotes (100/100) and (125/125) and the heterozygotes (100/125). The observed allelic frequencies of allele 100 was 0.649 and 0.351 for allele 125. The differences between the observed values and those expected according to Hardy Weinberg genetic model was found to be statistically not significant. The observed heterozygosity was 0.492. The polymorphic locus 2 can be used to quantify the genetic variation in natural populations.

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ELECTROPHORETIC STUDIES ON *PENAEUS MONODON* FABRICIUS

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Introduction

Monitoring of genetic characteristics is essential to preserve and utilize economically important genetic traits through controlled breeding programmes in Aquaculture. As genes control, protein expression, the variation in protein pattern provide genetic information at various levels of species organization. The technique of gel electrophoresis is widely used to separate and isolate proteins of different molecular sizes and charges from different tissues and body fluids.

Objectives

The present study was taken upto standardize a suitable electrophoretic methodology and collect basic information on biochemical genetic characteristics of *Penaeus monodon* from different localities.

Material and methods

Penaeus monodon belonging to different size groups were collected both from the open sea as well as from the marine prawn hatcheries located at Narakkal (Cochin) and Kovalam (Madras). The polyacrylamide gel electrophoretic method of Davis (1964, *Ann. N. Y. Acad. Sci.*, **121** : 404-427) was followed with necessary modifications.

Results and discussion

10% polyacrylamide gel showed the best resolution of the protein fractions of all the tissues (eye, hepatopancreas, muscle

and hemolymph). Double distilled water as solvent and a tissue weight of 55 mg/ml of solvent were ideal to get good resolution of bands. General proteins were stained better by Coomassie Brilliant Blue GR 250 than by Amido Black. Glycoproteins were stained best by incubation of the gel in 12% TCA for 30 minutes followed by a treatment with periodic acid Schiffs reagent. Incubation of the gel at 50°C for 3 hours in a saturated solution of oil red O in 50% methanol containing 10% TCA resulted good staining of Lipoproteins.

The total number of protein bands obtained from muscle, eye, hepatopancreas and serum were 16, 15, 23 and 17 respectively with variation in electrophoretic mobilities from tissue to tissue.

The muscle protein pattern in the different size groups also showed considerable variations. The number of bands observed in the abdominal muscle of postlarvae, juveniles, subadults/adults were 12, 14 and 16 respectively. Such ontogenic variations may be due to the different physiological and biochemical changes occurring during different developmental stages. The myogen pattern of other penaeid prawns as reported by earlier workers, were identical in male and females in the number and electrophoretic mobilities of bands thus emphasizing the species specific nature of these proteins.

The lipoproteins and glycoproteins were also found to be tissue specific and had the same electrophoretic mobility as that of general proteins which may be due to the homologous nature of the protein.

The muscle protein pattern were similar in postlarvae, juveniles and subadults/adults collected from Cochin and Madras except in case of 8 postlarvae collected from Kovalam hatchery. As the number of specimens is very small, the reason for the variation cannot be specified. Since the postlarvae did not belong to the same brood stock the probability that the larvae belonging to a different genetic population cannot be ruled out. Such changes may reflect unknown eco-physiological

differences developing gradually or suddenly in the hatchery population.

The electrophoretic methodology developed and standardised in the present study with special reference to *Penaeus monodon* and the preliminary information collected on the distribution pattern in different tissues can form a strong basis for future detailed investigations of various proteins and isoenzymes.

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STUDY ON THE PROTEIN BUDGET IN DIFFERENT SIZE GROUPS OF *PENAEUS INDICUS* H. MILNE EDWARDS*

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Introduction

Aquaculture is looked upon as a source to meet the increasing demand of protein for human consumption. In culture of animals, it is protein which need to be fed to increase their biomass and thus protein in the commercial diet becomes a major cost factor. Workers involved in the study of growth of organisms as a response to quantity and quality of food, especially with formulated diets have given consideration to two parameters *viz.* quantity consumed and to the increase in growth observed. For precise understanding, the fate of food consumed need to be followed entirely, wherein the quantity of food assimilated and partitioning of assimilated food into growth and metabolism are taken into account. This not only reveals assimilability of food used and that part used for growth also suggest the quality of assimilated feed used for metabolism. The balance equation proposed by Winberg (1956, *Fish. Res. Bd. Canada, Transl., Ser.* 194) is a trend setter in this regard. Fish nutritionists have applied the Winberg's postulation on partitioning of food consumed, while little studies have been carried out on prawns.

In the present investigation two size groups of juvenile *Penaeus indicus* were fed with five diets, each with varying quantities of protein for a period of 15 days. During the study they were given with known quantity of food, faecal matter collected promptly and growth studied in terms of moulting, increase in live weight, length, dry weight and protein addition.

*Prepared by the Editorial Committee.

Material and methods

Preparation of diets: Five test diets with varying percentages of protein were prepared using purified ingredients with slight modifications to the procedure suggested by Kanazawa *et al.* (1982, *CMFRI Spl. Publ.*, 8 : 90 -94). After the praparation of the diets, check was again made by chemical method to the actual percentage of protein. At the time of the preparation of the diet, the vitamins and minerals were mixed together at the rate of 1 : 5. The diets were fed in the from of dried pellets of 1 mm diameter.

Experiments : Juveniles of *Penaeus indicus* belonging to the same brood and size group were collected from Narakkal Prawn Hatchery and transported to the laboratory.

The first set of experiments were conducted with the size group of 25 mm to 35 mm. The wet weight and the length of the animals were noted down after observing necessary precautions. Ten replicates were used for each diet. Four to five hours after the animals were fed the leftover food and faecel matter were removed from each container. After washing they were transferred to pre-weighed and numbered petridish. To obtain the dry weight of prawns used, weight of 30 animals belonging to the same brood and size were taken. Protein content and moisture of the animals were also found out. The weight of moults if any, were also noted after drying. The second set of experiments was conducted with the size group 35 mm to 55 mm for 15 days.

Estimation of protein content : Biuret method was followed for the estimation of protein for arriving at protein estimates for prawn, faeces, moult and food. Appropriate statistical analysis and calculation of parameters of nutritional index were also carried out.

Results and discussion

Protein budgetting was studied in the juveniles (25-55 mm) of *Penaeus indicus* giving iso-calorific diet with variation (0% - 60%) in protein content. Carbohydrate and lipid were

uniform in all the diets being well above the optimum. Daily food consumption ranged from 11.38 to 3.29 in percentage body weight.

The study of efficiencies of assimilation for overall nutrients and of protein showed that when shrimps were fed with optimum levels of protein, the differences between both the assimilation efficiencies narrowed down. In prawns fed with very low quantity of protein, the animals consumed much food to assimilate more proteins whereby resulting in low value for overall assimilation and high values for protein assimilation. The animals provided with high percentage of protein in diet liked to consume more food, but where poorly assimilating both protein and overall nutrients. The capacity to assimilate proteins tend to improve in the higher sizes of juveniles studied.

It is known that crustaceans prefer protein over carbohydrate as staple for metabolic fuel. Thus while more proteins are consumed, the prawns instead of putting up more flesh, used them for metabolism. Based on the budgets obtained, the following theoretical propositions were evolved. That for prawns 40.50 mg and 145.06 mg dry weight size groups for 15 days period the minimum protein required for metabolism is 11.88 mg and 8.93 mg respectively. Thus larger juveniles have a better protein sparing mechanism. Carbohydrates and lipids cannot further reduce protein required for metabolism lower than these quantities. The optimum requirement of protein for metabolism for these size groups for 15 days period is around 55 mg and between 84.88 mg respectively for 40.50 mg 145.06 mg prawns in dry weight.

This study on protein budget in terms of weight in 35 - 55 mm group, has revealed that about 8.93 mg protein is the minimum requirement for metabolism, while 89.48 mg of protein is required for optional use for 15 days period. Herein too, it is clear that when more and more protein is given in the diet, prawns instead of utilising more protein for growth tends to use more for metabolism rather than for body building.

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RELATIONSHIP BETWEEN GROWTH RATE AND RNA, DNA PROTEIN RATIO IN *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Growth in the animals has traditionally been expressed as a change in either length or weight or increase in the dry weight body mass. Accompanying these changes are changes in biochemistry and chemical composition of the animal at molecular level. By measuring the ratio of ribonucleic acid to deoxyribonucleic acid (RNA/DNA), the growth rate in its exponential phases of the animal can be determined. As growth is accomplished by protein synthesis and ribonucleic acid is involved primarily in protein synthesis, the control over the rate of protein synthesis is exerted by the rates of RNA synthesis, transport to the cytoplasm and degradation. Hence RNA concentration has been related to growth rate. Though deoxyribonucleic acid (DNA) is a carrier of genetic information it has also been used as an indicator of biomass and cell number. Thus the RNA/DNA ratio is found to be good indicator of the amount of protein synthetic machinery in cell.

Objectives

To find out the correlation if any between RNA and DNA concentration, RNA/DNA ratio and RNA/DNA ratio to protein content and growth rate in different size length of penaeid prawn *Penaeus indicus*.

Materials and methods

The penaeid prawn *Penaeus indicus* of different size lengths ranging from 24 mm to 147 mm were used as a study material for estimating the RNA, DNA and protein content to

relate them to growth. The animals selected for the estimation are of intermoult stage. The effect of starvation and bilateral eyestalk extirpation on RNA, DNA level and protein content was also determined.

RNA content determined was as per the methods described by Schmidth and Jhannhaauser (1945, *J. Biol. Chem.*, **161** : 83-89) and further modified by Munro and Fleck (1966, *Analyst Lond.*, **91** : 78-88) using yeast RNA as the standard.

The DNA content was determined by Indole method (Cerriotti 1952, *J. Biol. Chem.*, **198** : 297-303) and highly polymerised calf thymus DNA was used as the standard.

Protein was analysed by the Biuret method (Gornall *et al.* 1949, *J. Biol. Chem.*, **177** : 751-766).

Results and discussion

The RNA concentration was found to be high in small size *P. indicus* (upto 42 mm), thereafter a steady decline or an inverse relationship was noticed with increase in protein content, reaching lowest values of 3.2 g/mg in adult size of 147 mm. These results are similar to those observed by Dagg and Littlepage (1972, *Mar. Biol.*, **18** : 162-170) in *E. elongata*. Though DNA value also showed a slight decline with increase in size, among large sized prawns there was a tendancy to conserve the DNA content. These results are in correlation with those observed by Bulow (1970, *J. Fish. Res. Bd. Canada*, **27** : 2343- 2349) and Dagg and Littlepage (1972, *loc. cit.*), but no correlation was seen with increased protein level like RNA as there was much fluctuation in the DNA values.

In the present study the RNA/DNA ratio has been found to be useful in correlating growth (in terms of protein increase) in small sized prawns. But as the animal grows to a larger size, such a correlation was not possible due to greater variations in the values. The findings reported for smaller prawns here are similar to those observed by Bulow (1970, *loc. cit.*).

The RNA, DNA and protein value increased from 5.03 µg/mg to 8.28 µg/mg (control 4.85 µg/mg), 0.71 µg/mg to 0.87 µg/mg (control 0.37 µg/mg) and 244.62 µg/mg to 321.18 µg/mg (control 231.14 µg/mg) respectively after bilateral eyestalk ablation from third day to tenth day of experiment. In the case of RNA/DNA ratio, the values remained more or less steady between 9.3 to 9.55 (control 13.15) during the same period.

The pattern of change in RNA, DNA content, RNA/DNA ratios and protein levels in *P. indicus* after starvation was similar to the observation of these workers. In the present study there was a significant decline in RNA content and protein levels during a ten day period of starvation. But the DNA content remained unchanged. Therefore a sharp decrease in RNA/DNA ratio was also noticed.

Publication

GEORGE THOMAS AND A. D. DIWAN 1990. Changes in nucleic acids and protein content in relation to body size in the prawn *Penaeus indicus* H. milne Edwards. *Proc. Indian Acad. Sci. (Anim. Sci.)*, 99 (2) : 125-130.

CMFRI Spl. Publ., 1993; 53 : 61 - 63

CALCIUM EXCHANGES BETWEEN SEDIMENTS AND WATER IN SOME CULTURE PONDS WITH STRESS ON CARBONATE AND BICARBONATE ALKALINITIES

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Introduction

Calcium element should be present in the environment for the activities of organisms. Marine decapods lose their 90% of body calcium during ecdysis and calcification of new exoskeleton is achieved predominantly with calcium absorbed from sea water (Greenway Peter 1983, *Comp. Biochem. Physiol.*, **75** (A2) : 181-184). The calcium content of the integument of the crustaceans was found to be decreased appreciably with drops in calcium content in the surrounding (Huner V. Jay 1976, *Crustaceana*, **34** (3)). The pond with less concentration of available calcium both in soil and water will be less productive than others where calcium is abundant. The alkalinity of water is a very important factor which influences the exchange of nutrients between sediments and the water, the water with more than 50 ppt $CaCO_3$ alkalinity is found more productive than other with lesser alkalinities (Banerjee 1967, *Indian J. Fish.*, **14** (1&2) : 115-143.

Objectives

To study the calcium and nutrient contents in the bottom sediments and overlying water in three sites where fish culture operations are carried out.

Materials and methods

Two brackish water ponds were selected and water and soil samples were collected from three sites for oxygen, calcium, phosphate and other nutrients estimation. The following parameters *viz.* temperature, pH, salinity, dissolved oxygen,

nitrite nitrogen, nitrate nitrogen, reactive phosphorous carbonate and bicarbonate alkalinity and total alkalinity and dissolved calcium from water samples were determined. From the sediment samples the available phosphorus, nitrate, exchangeable calcium and grain size were determined. All the analyses were carried out employing standard methods.

Results and discussion

The range of temperature was more or less same in all three sites. Salinity considerations were comparable in all the three sites, but the abrupt decrease in salinity oxygen contents were uniformly high ranging from 3 to 5.5 ml/1, the lowest being during September. The general value for bicarbonate and carbonate alkalinities were 50-100 ppm and 0-30 ppm respectively. The pH values ranged from 7.5 to 8.5 in general. At site 5 the bicarbonate alkalinity was higher than site 2 and likewise the pH values. At site 3 the carbonate alkalinity was uniformly high (30-40 ppm). This showed that the tidal influence was maximum at site 1 and the carbonate dissolution was also more there. The more or less stagnat condition of the site 3 gave rise to maximum amount of carbonates and minimum bicarbonates.

The maximum soil calcium content was in site 2 which received minimum tidal influence. Calcium content of overlying water was much lower and the distribution was similar in three sites. There was drastic decrease in both soil and water calcium contents during monsoon and the general trend was of a fluctuating nature for the soil calcium. The calcium content of water showed an inverse relationship with that of the soil in site 1 by the onset of monsoon whereas during the peak monsoon a more or less a direct relationship was conspicuous. More or less the same features prevailed in site 3 also.

The phosphorus contents in the bottom sediments and overlying water showed conspicuous fluctuations during the investigational period. Maximum phosphate content occurred in site 1 where the tidal fluctuation were high. In site 1 when the phosphate content of the water was high the corresponding

soil phosphorus was low and vice versa. However a more or less a direct relationship existed in site 2 and 3. Of all the three sites, site 3 had the maximum soil phosphorus.

Nutrients are produced by the decomposition of precipated organic matter. Under oxidising conditions the ions except nitrite and nitrate are absorbed on soil colloids. Thus the retention capacity of the sediments with respect to nitrite and nitrate seems to be small which was observed in the present case also. Throughout the investigational period the nitrate contents of the bottom sediment were below 5 μg at/g whereas in the overlying water a maximum of 60 μg at/g during the premonsoon and another high peak during the monsoon period were observed. The nitrite contents also were high during this period.

Publication

RAMAMIRTHAM, C. P. AND A. P. DINESH BABU 1988. Calcium exchange between sediments and water in some culture ponds with stress on carbonate and bicarbonate alkalinities. *Indian J. Fish.*, 35 (1) : 37-40.

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STUDIES ON THE THELYCUM AND SPERMATOPHORE OF THE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS*

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Introduction

The culture of penaeid prawns has generated interest in their reproductive biology for breeding them in captivity for production of eggs on a large scale. Mating and spawning activities in prawns unlike fishes are two different processes separated by time interval. During mating the non-motile sperms in sperm bags called Spermatophores are transferred by the male to the female thelycum where the sperms are stored till the female spawns. During spawning both ova from ovary and sperms from thelycum are released into the sea water where fertilization takes place. The present study deals with the morphology, histology and histochemistry of the spermatophore and the thelycum of *Penaeus indicus* to understand the function of these two structures to solve the various problems encountered in induced breeding in hatcheries.

Materials and methods

Live specimens of *Penaeus indicus* were collected from Prawn Hatchery Laboratory, Narakkal. The spermatophore situated within the terminal ampoule at the base of the fifth walking leg was obtained by electro-ejaculation from male specimens. In female of different size groups in the intermoult stage and impregnated females, the thelycum located between the fifth to eighth sternal plates was cut and removed carefully. The structure of spermatophore and thelycum was studied using the steroscopic microscope. The histological and histochemical studies were also conducted by employing appropriate staining techniques.

*Prepared by the Editorial Committee.

Results and discussion

The spermatophore of *P. indicus* consists of a chitinous sperm bag and wings with a sticking mass of granules rich in sulphated AMP at the anteriomedial corner of the sperm bag. The sticking substance serves to cement the two spermatophores as they issue out of the male genital openings into one unit. Inside the sperm bag, the sperm mass is embedded in a spongy matrix rich in carboxylated AMP. The spermatozoa appear to have a glycogen store for endogenous energy metabolism.

The thelycum of *P. indicus* is very complex. The lateral plates hide the posterior projections of the folds of the sternite XIII which form a 'trident' consisting of a conical median process having a median ventral keel and two lateral horns covered with minute setae on the ventral side. The lateral plates meet in the centre to form the raised lips, the median surfaces of which are covered with numerous villi. The ventral surface of the conical median process of the trident also bears short villi in adult females. The dorsal margin of the lips bear a row of stiff rounded teeth which guard the entrnace to the seminal receptacle which is single chambered. On the ventral margin of the lips numerous minute cuticular pores are seen. The secretions produced by the thelycal epithelium appear to come through these pores. In transverse sections of the impregnated thelycum, dark staining patches are observed inside the lips and at the base of the keel. They appear to be accumulations of the epithelial secretions which are rich in carboxylated AMP and protect the stored spermatozoa from bacterial and fungal infections. These secretions could also effectively block the entrance to the seminal receptacle and prevent the loss of spermatozoa from the thelycum.

Though this study has thrown some light on the possibility of one of the thelycal secretions acts on the wall of the spermatophore and dissolves to release of the sperms, the mechanism which triggers the release the spermatozoa, first from the sperm bag and then from the thelycum needs further study.

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STUDIES ON HETEROTROPHIC BACTERIA IN THE MANGROVE ECOSYSTEM NEAR COCHIN

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V. CHANDRIKA
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Introduction

Importance of heterotrophic bacteria in the chemistry and fertility of the marine environment is well established. The relative abundance of heterotrophic bacteria and their activities reflect the hydrological structure and nutrient levels in the marine environment. The knowledge of microbial biomass, distribution, productivity and *in situ* metabolism in the mangrove ecosystem is scanty, when compared to all other marine ecosystems. The regeneration and recycling of nutrients in the mangrove environment is influenced by the existing heterotrophic bacterial population which in turn enhances the productivity of the ecosystem, thus making this ecosystem a potential area for mariculture.

Objectives

The investigations are aimed at quantitative estimation of bacterial population, isolation and identification of bacteria from mangrove area. Investigations are also made in the seasonal variations of some of the physicochemical parameter along with bacterial population and statistically analysed to know how far the environmental factors influence the distribution of heterotrophic bacteria in this biotope.

Material and methods

Study area : The studies were conducted at 3 stations for a period of 6 months during March-August 1985, around Murukkumpadam area in Vypeen Island near Cochin. Station A was an area which was always influenced with tidal

inundation and dominated by a single species of mangrove flora *Acanthus ilicifolius* and Station B was dominated by two genera namely *Acanthus* and *Avicennia*. Station C was a shallow prawn filtering pond situated in the mangrove ecosystem.

Parameters of study : The bacterial population was analysed quantitatively and qualitatively. Seasonal variations in rainfall, soil temperature, soil pH, soil Eh, electrical conductivity of soil, organic carbon, available nitrate and available phosphorus present in the soil were also investigated. Rainfall data was obtained from the Naval Meteorological office.

Collection of samples : Fortnightly sampling of soil from surface upto 5 cm depth, from the above 3 stations was done between 0700 and 0900 hrs using a plexiglass mud corer aseptically into sterile polythene bags. Care was taken not to contaminate the soil samples by any means. The samples were subjected to bacteriological investigations within 3 hrs of sampling.

Results and discussion

Bacteriological observations

Quantitative analysis of total heterotrophs : Total heterotroph did not show much variations in all stations. Comparatively higher average counts have been obtained in Station A (13.6×10^6/g) and Station B (9.8×10^6/g) than in Station C (8.9×10^6/g).

Seasonal variation in total heterotrophic bacterial count could be observed in all the three stations. The count was maximum during summer months and gradually showed a decline and became minimum during peak monsoon. A gradual increase in bacterial count was noted after the peak monsoon (till August). In all the stations, the minimum count was recorded during June and maximum during March. In station A, total heterotrophs varied from 3.33×10^5/g to 52.00×10^6/g. In Station B, it varied from 6.59×10^5/g to $29.23 \times$

10^6/g. In Station C, the minimum count observed was 10.00 X 10^5/g and the maximum was 30.75 X 10^6/g.

Qualitative analysis : A total of 108 strains were isolated and identified upto genera level using the scheme of Usio Simidu and Kayuyoshi Aiso (1962, *Bull. Jap. Soc. Sci. Fish.*, **28** : 1133-1141). The analyses were carried out both in premonsoon and monsoon period in all the three stations.

Biochemical test : In station A, 2.8% of the isolates turned to be gram positive and 97.2% were gram negative strains. Pigmented forms were recorded as 36.1%. 11.1% of the total isolates produced indole. The saccharolytic activity of all the isolates was observed to be very poor in all the three stations. Hugh and Leitfson's test employed to distinguish between oxidative and fermentative utilisation of carbohydrates, revealed that oxidative metabolism was more common among the isolates in station A (52.8%). 13.9% of the isolates were fermentative and 33.3% showed no reaction for this test. None of the isolates were found to be alkaline. All the isolates were catalase positive. 72.2% oxidase positive forms and 61.1% penicillin sensitive forms were also recorded in this station.

In station B, 5.6% of the isolates were gram positive and 94.4% were gram negative. Motile forms were recorded as 94.4% and pigmented forms as 36.1%. Biochemical tests showed that 80.6% were nitrate reducers, 61.1% gelatin liquifiers, 55.6% starch hydrolysers, 19.4% indole producers and 58.3% H_2S producers. 37.9 were oxidative and 25% were fermentative and 36.1% were non-reactive. In station B also, none of the isolates turned to be alkaline in their reaction to Hugh and Leifson's test. 77.8% were oxidase positive and 50% of the isolates were penicillin sensitive.

Similar results were obtained in station C also. 2.8% of the isolates were gram-positive and 97.2 were gram-negative. 97.2% were motile and 41.7% were pigmented forms. Of all the isolates 58.3% were nitrate reducers, 61.1% gelatin liquifiers and 91.7% starch hydrolysers. 72.2% of the total isolates produced

H_2S. 58.3% were oxidative in glucose metabolism, 2.8% were fermentative and 38.9% gave no reaction to glucose. 66.7% were oxidase positive and 88.9% of the isolates were sensitive to penicillin.

Relative abundance of taxonomic groups : The relative abundance of all these genera varied seasonally in the three stations.

Alcaligenes was predominant in Station A during pre-monsoon period (33.3%) followed by *Pseudomonas* (25%), *Vibrio* (25%), *Flavobacterium* (8.3%) and *Micrococcus* (8.3%). The genus *Cytophaga* was not recorded. During monsoon *Alcaligenes* (33.3%) remained as the predominant group followed by *Pseudomonas* (29.2%), *Cytophaga* (25%), *Flavobacterium* (8.3%) and *Vibrio* (4.2%). *Micrococci* was not recorded during monsoon.

In Station B, the predominant group recorded in pre-monsoon period was *Pseudomonas* (33.3%) followed by *Alcaligenes* (25%), *Flavobacterium* (16.7%) and Cytophaga. *Vibrio* and *Micrococcus* were recorded in 8.3%. During monsoon *Alcaligenes* was dominant (33.3%) followed by *Pseudomonas* (29.2%), *Vibrio* (25%), *Flavobacterium* (4.2%), *Cytophaga* (4.2%) and *Micrococcus* (4.2%).

Like in Station A, *Alcaligenes* (58.3%) was the dominant heterotroph in Station C also during premonsoon. It was followed by *Flavobacterium* (25%), *Cytophaga* (8.3%) and *Pseudomonas* (8.3%). *Vibrio* and *Micrococcus* were not encountered from the soil samples in the premonsoon period. *Alcaligenes* (54.2%) remained predominant during monsoon also. This was followed by *Cytophaga* (20.8%), *Flavobacterium* and *Pseudomonas* in 8.3%, *Vibrio* and *Micrococcus* in 4.2%.

The relative abundance of different genera are as follows (taken by the average from three stations) *Alcaligenes* (39.6%), *Pseudomonas* (22.2%), *Flavobacterium* (11.8%), *Cytophaga* (11.1%) *Vibrio* (11.1%) and *Micrococcus* (4.2%).

Biochemical characteristics of the isolates were investigated and the results are given in Table 1.

TABLE 1. *Morphological and biochemical characteristics of heterotrophic bacteria isolated from the three stations (A, B & C) in the mangrove ecosystem*

Characteristics	Frequency of occurrence (%)		
	Station - A	Station - B	Station - C
Gram			
Positive	2.8	5.6	2.8
Negative	97.2	94.4	97.2
Motility	97.2	94.4	97.2
Pigmentation	36.1	36.1	41.7
Nitrate reduction	80.6	80.6	58.3
Gelatin liquifaction	72.2	61.1	61.1
Starch hydrolysis	63.9	55.6	91.7
Indole production	11.1	19.4	2.8
H_2S production	69.4	58.3	72.2
Carbohydrate fermentation			
Glucose	2.8	16.7	5.6
Lactose	2.8	0	5.6
Maltose	2.8	16.7	5.6
Mannitol	2.8	16.7	2.8
Sucrose	2.8	16.7	2.8
Hugh & Leifson's test			
Oxidative	52.8	38.9	58.3
Fermentative	13.9	25	2.8
Alkaline	0	0	0
No reaction	33.3	36.1	38.9
Oxidase test	72.2	77.8	66.7
Catalase test	100	100	100
Penicillin sensitivity	61.1	50	88.9

Conclusions

The correlation between different environmental parameters and total heterotrophic bacterial count was statistically analysed and only available phosphorus showed significant relationship with total heterotrophs in all the three stations. However, in Station C apart from available phosphorus, soil temperature, electrical conductivity and available nitrate are also found to have significant relationship with total heterotrophs. The Z-test carried out to know the homogenity of relationship between the environmental parameters and bacterial count among the 3 stations gave values well below 1.96 in all the three stations which indicated that the Stations A, B and C are more or less homogenous.

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ELECTROPHORETIC STUDIES ON *MUGIL CEPHALUS* AND *LIZA PARSIA*

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Introduction

Understanding of the present genetic make up of wild fish population has a significant practical value particularly in the field of aquaculture. The use of multiple buffer-systems, multiple tissues and a wide variety of staining procedures are necessary to maximise the amount of genetic data that can be detected in a given species. A study on biochemical genetics using electrophoretic methods had been initiated by Karia and Ponniah (1993, *CMFRI Spl. Publ.*, 53 : 48-51) in the fish *Liza parsia*. They had carried out standardisation of protein methodology, analysed the tissue expression of lactic dehydrogenase and esterase system and studied the ontological changes and genetic polymorphism in *Liza parsia*. The present study on *Mugil cephalus* and *L. parsia* is an extension of the earlier study to cover more enzymes and to (i) standardize the experimental condition such as type of buffer and current strength to be used for different enzymes; (ii) find out the tissue expression of acid phosphatase (Acph), Malate dehydrogenase (Mdh), tetrazolium oxidase (To), alcohol dehydrogenase (Adh), Maleic enzyme (Me) and lactic dehydrogenase (Ldh) in the muscle, liver, eye, heart, brain, stomach and kidney; and (iii) to understand genetic polymorphism of Ldh system in *Mugil cephalus*.

Material and methods

Live specimens were used for electrophoretic studies as far as possible. Fingerlings (20-40 mm) and adults (200-250 mm) of *Mugil cephalus* and fingerlings (20-30 mm) of *Liza parsia* were part of the collections of Fisheries Stations of the Kerala Agricultural University. The adult specimens of *L. parsia* (150-

170 mm) were collected from Chinease dip nets. Both the disc gel electrophoretic method as described by Davis (1964, *Annals,New York Academy of Science,* **121** : 404-427) and vertical slab gel method as described by Ackroyd (1967, *Anal. Biochem.,* **19** : 399) were used. The staining procedure described by Guyornard (1981, *Can. J. Genet. cytol.,* **23** : 83-47) was used for enzymes. Different enzyme systems such as Aeph, To, Adn, Me, Mdh and Ldh were studied in different adult tissues such as muscle, liver, eye, heart, kidney, stomach and brain. The resolution and the preparation of the different enzymes were tested using eight different buffers. The genetic polymorphism in 85 fingerlings of *M. cephalus* was studied on the vertical slab gel electrophoretic system for enzyme lactic dehydrogenase. Unlike in the earlier study on genetic polymorphism in *L. parsia* by Karia and Ponniah (1993, *loc. cit.*) where the elctrophoresis was carried out only on disc, in the present study slab gel was used since allele scoring is more accurate.

Results and discussion

The voltage used on the slab gel had to be standardised to obtain maximum migration without denaturation and 70 V with a current strength of 42 mA was found optimum. Lower voltage resulted in less migration and higher voltage resulted in trailing of bands. The enzyme acid phosphatase was resolved in muscle, liver and eye in eight buffers and from a comparative point of view, Tris-citric Boric-lethium hydroxide (pH 8.1) buffer of Ferguson and Wallace (1961, *Nature (Lond.),* **190** : 629-630) was better. Since for To and Ldh Tris-Glycine HCl pH 8.3 buffer of Davis (1964, *loc. cit.*) was found suitable, for the other enzymes same buffer was tested and the resolution was found to be adequate.

The enzyme Acph of *L. parsia* resolved into five bands in the muscle tissue. In the liver and eye the second and fourth bands were absent. The expression of the Acph enzyme in the eye tissue of the fingerlings of *L. parsia* differed from the adult eye expression in the absence of first, second and fifth band. The To enzyme of *L. parsia* was studied in muscle, liver and eye and

a total of five bands having different electrophoretic mobilities were observed. Of these four bands of varying intensities were seen in the muscle and the liver and the three bands in the eye tissue. The first three bands were found common in all tissues.

The Adh system in the tissues of muscle, liver, eye and brain of the adult *M. cephalus* revealed 5 bands. The fifth band was the darkest in the liver tissue while in the muscle tissue, the first band appeared to be darkest. The second and third bands were common in all tissues indicating that the loci controlling these bands were equally expressed in all the tissues. The Mdh enzyme system was studied in the muscle, liver, eye, kidney, heart and stomach of the adult *M. cephalus*. Seven different bands could be darkest in their intensities in the tissues of the muscle and kidney, whereas in the heart and liver tissue, these bands were not present at all. The Me enzyme was resolved in liver, heart, kidney of the adult *M. cephalus*. Of the five bands resolved in all tissues put together, the fifth band was absent in heart and kidney. Bands one and two common in all the tissues. The Ldh system was tested in both *L. parsia* and *M. cephalus*. The expression in muscle, liver and eye of *L. parsia* was similar to that observed by Karia and Ponniah (1993, *loc. cit.*). In *M. cephalus* the expression of Ldh in liver, eye, stomach and brain was studied. Like in *L. parsia*, five isoenzymes were observed in the eye tissue of *M. cephalus* which were the product of two loci. The expression of homotetramer of Ldh 2 was absent in stomach tissue. While in liver, band 3 was absent in brain and bands 3 and 4 were absent. Like in *L. parsia*, in *M. cephalus* too there was no ontological difference in Ldh system between the fingerlings and adult. Since no fry of *M. cephalus* were available the ontological differences observed in *L. parsia* fry could not be verified here. A total of five muscle protein bands were resolved in *M. cephalus*. Though Tris-Glycine-HCl buffer was suitable for Ldh, due to increased resolution with Tris-boric EDTA buffer it was used in the vertical slab electrophoretic system. Seven alleles were identified at Ldh-1 loci and termed as A, B, C, D, E, F and G and the homozygotes had rf value of 0.38, 0.48, 0.56 0.6, 0.62, 0.72 and 0.8 respectively. The

phenotypes were assigned to each individual based on rf values and the presence of single or double band. Only 13 phenotypes were present. The allele F was the common one and with a allelic frequency of 0.880. Since the number of allele observed at Ldh-1 were more than the normal seen in other fishes, the Hardy-Weinberg genetic model was not used to calculate the allelic frequency. Though Ldh-2 exhibited two distinct types of bands position, the heterozygotes could not be identified. In the present study in *M. cephalus*, seven alleles have been identified while Karia and Ponniah (1993, *loc. cit.*) have identified two alleles in *L. parsia* indicating that Ldh is a highly polymorphic enzyme in mullets and can be used for quantifying genetic variation in natural population.

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METABOLIC EFFECTS OF EYESTALK REMOVAL IN *PENAEUS INDICUS* H. MILNE EDWARDS*

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Introduction

Metabolic studies of cultivable species of fishes and prawns have wide implications in aquaculture. Maintaining the optimum level of oxygen and ammonia concentrations in the culture systems can lead to a maximum production at minimum cost. The energy requirement obtained by measuring the oxygen concentration, the ammonia excretion by estimating the protein degradation in ammonotelic animals, the behavioural changes indicated by random (spontaneous) activity to findout the energy utilization, the assessment of the metabolic effects of the carbohydrate levels in different tissues and the calculation of the ammonia quotient, etc. can all be utilized towards scientifically improving the aquaculture technology to increase the production. The penaeid prawns, especially the Indian white prawn *Penaeus indicus* is the most important species among the cultivable animals. The eyestalk ablation is used as a major technique to induce maturation of these prawns in culture systems. The eyestalk 'X organ sinus gland complex' extirpation though induces maturation also secretes neurohormones that regulates the various physiological and metabolic reactions of the animals. Therefore, the present study attempts to understand the metabolic effects of the eyestalk ablation in *P. indicus* exposed to different temperatures and salinities.

Materials and methods

Males and intermoult females of *P. indicus* of 120-140 mm TL were collected, maintained and acclimated in the best envi-

*Prepared by the Editorial Committee.

ronment under captivity using a water recirculation system for the study. The eyestalk ablation was done by using an electrocautery apparatus. The oxygen consumption, ammonia excretion and the random activity were measured from unablated, unilaterally and bilaterally ablated *P. indicus* male and female prawns acclimated at ambient water temperature and full strength sea water, using a modified annular "Frys respirometer" having an electronic counter. These animals served as the control.

The experiments were performed at three different temperatures of 27°C, 30°C and 33°C and five different salinities of 2, 8, 17.7, 25.7 and 32.4 ppt. The ammonia quotient was calculated for all the experiments from

$$\frac{\text{Volume of ammonia excreted}}{\text{Volume of oxygen consumed}}$$

The unablated, unilaterally and bilaterally ablated female prawns at intermoult stage acclimated as controls were sacrificed at 24, 48, 72, 96 and 120 hours and the muscle and hepatopancreas tissues were dissected out for the analysis of carbohydate content.

The dissolved oxygen content of the water was estimated by Winkler method and the ammonia by modified phenol-hypochlorite spectrophotometric method at 640 mμ. The salinity of the water was assessed by standard Argentotitric method. The carbohydrate content in tissues was estimated by Dubois phenol sulphuric acid method. The mean values were represented with standard deviation. The analysis of variance was calculated directly from the original values which were transformed using log transformation.

Results and discussion

The oxygen consumption is an index of the amount of energy utilized by the organism. The non-ablated control female prawns had an oxygen consumption rate of 234.64 ml/kg/hr and males 220.98 ml/kg/hr. The rates increased in the unilaterally ablated males and females and further increased to

313.03 ml/kg/hr and 308.87 ml/kg/hr in the bitaterally ablated females and males respectively. The oxygen consumption tend to increase with the increase in temperature and tend to decrease with the increase in salinity. But the rate was a minimum of 200.3 ml/kg/hr at 25.7 ppt salinity and maximum at 2 ppt salinity. However, in all the groups tested the increase in oxygen consumption rate 1 hour after ablation had a tendency to reach the normal value at 5 hours in the unilaterally ablated group and 7 hrs in the bilaterally ablated group. In any case the oxygen consumption of male prawns is always lower than in the females, in all the experiments. The increase in oxygen consumption along with the increase in temperature in ablated animals may be due to the removal of some factor which regulates the oxygen consumption in response to thermal acclimation as suggested earlier of the presence of respiration depressing hormone in the eyestalk of a crayfish when acclimated to warm conditions. The minimum oxygen consumption at 25.7 ppt of the ablated prawn suggests that after eyestalk ablation the *P. indicus* expends least energy in this salinity.

The rate of ammonia excretion increased from 7.26 ml/kg/hr to 11.01 ml/kg/hr in control through unilaterally ablated animals to bilaterally ablated female prawns. The rate of ammonia excretion increased with increase in temperature and decreased with an increase in salinity. The minimum value of ammonia excretion were 8.53 ml/kg/hr at 27°C and 8.53 ml/kg/hr at 32.4 ppt salinity which means that the relation of proteins is higher at 27°C and 32.4 ppt. The other observations show the same trend as for the oxygen consumption. The females always exhibited a higher value than males. The sudden increase or decrease observed one hour after ablation tries to attain the normal level at 5 hours and 7 hours respectively for the unilaterally and bilaterally ablated animals. The ammonia quotient calculated showed a decrease from 0.36 to 0.032 immediately after the eyestalk ablation. The ammonia quotient increased with increase in temperature and decreased with the increase in salinity in all experiments.

The random activity in control females was 28.1 counts/hr and 32.9 counts/hr in the males. The activity increased after unilateral and bilateral ablations. The activity decreased with increase in temperature and increased with the increase in salinity. The activity was minimum at 25.7 ppt where the oxygen consumption was also minimum. Unlike in the oxygen consumption and ammonia excretion rates, the random activity was always higher in the males than in the females.

In the muscle tissue of the control prawns the zero reading of carbohydrate content was 2.78% which decreased to 2.26% at 24 hrs and then increased to 2.49% at 48 hrs. The unilaterally ablated prawns also showed the same trend. But the carbohydrate content in muscle tissue of bilaterally ablated prawns increased after 24 hrs and descend towards the normal value at 48 hrs. The hepatopancreatic carbohydrate content of 8.52% at zero hour decreased to 7.54% at 24 hrs and 5.05% at 48 hrs in the control prawns. The carbohydrate percentage of 8.51 increased to 8.95% at 24 hrs and decreased to 6.93% at 48 hrs in unilateral as well as bilaterally ablated prawns.

In brief, the present study indicated that in the bilaterally ablated *P. indicus*, there was a remarkable increase in carbohydrate content in muscle. In the hepatopancreas there was a decrease in the carbohydrate content. These observations are in agreement with the earlier observation on crustaceans. It is possible that eyestalk removal in *P. indicus* accelerates the mobilization of the carbohydrates (glycogen) from the hepatopancreas to the integument and as a result there is a depletion of this metabolite in the hepatopancreas. It may be possible that excess of sugar present in the blood which is obtained through the process of glycogenolysis in the hepatopancreas is quickly mobilized for the synthesis of glycogen in the epidermis and muscle tissue.

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OBSERVATIONS ON THE GERMINATION AND GROWTH OF *AVICENNIA OFFICINALIS* LINNAEUS*

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Introduction

Mangroves are specialized flora adopted for growing in saline soils. The importance of mangrove ecosystem to coastal fisheries and aquaculture is fast gaining momentum. A proper understanding and appreciation of the ecosystem especially in the developing countries of the tropics will confer immense economic benefits to the coastal rural population who are engaged in fishing and aquaculture activities. With increasing demand in land for farming and housing, and firewood almost the whole mangrove ecosystem is under progressive destruction. The problem of sea erosion can be taken as an outcome of such drastic steps.

With these considerations in mind, the present study was carried out on *Avicennia officinalis* the most dominant mangrove plant in this region. Investigations were made in the present work on the growth of *Avicennia* in nature, the influence of salinity and tidal inundations on germination *in situ* and under laboratory conditions, and the biochemical changes associated with germination and early growth.

Material and methods

The dominant mangrove vegetation of *Avicennia officinalis* at Cochin (Thoosath) and Narakkal was selected for the study. Water and soil samples were collected fortnightly from the sites for the study of physico-chemical parameters such as salinity, pH, Eh, exchangeable cations - sodium, potassium and calcium

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ions; available magnesium, phosphorus, nitrate, organic carbon and organic matter.

The chemical analysis of water was carried out according to Strickland and Parsons (1968, *Bull. Fish. Res. Bd. Canada*, 167). Proximate composition analysis was carried out in radicle, root developed and plumule stages of development in two leaved sapling and in seed, following modified Weendee method of analysis. The granulometry of the soil and its percentage composition of organic matter comprising of both plant and animal origin was studied by visual enumeration.

Germination of *Avicennia* seeds in different salinities with a difference of 5‰ from 0 to 50‰ and the influence of duration of immersion of tidal water (inundation) was studied. Germination experiments in two different media namely moist beach sand and moist silica gel were conducted. Field experiments on germination with regard to tide were done at Thoosath. The natural growth of *Avicennia* was studied by noting the progressive changes in leaf length and breath, internodal length, shoot diameter and the length from the top of the first node. The changes in the composition of major biochemical constituents - protein, lipid, carbohydrate, fibre and ash - with germination; apart from ripe seed and two leaved sapling, radicle, plumule and root developed stages growing in nature were estimated. Linear regression analysis and test of significance was calculated for the data.

Results and discussion

The study on chemical parameters of the water and soil showed wide variation. The range of fluctuations in the parameters studied at the two stations is given in Table 1. Granulometric studies on the soil showed 46.4% and 47.2% of sand at Thoosath and Narakkal. Silt content was 43.5% and 44.7% respectively at both the places and the rest was composed by clay.

The analysis of detritus made by visual estimation revealed that the major organic components are decayed plant material comprising nearly 40-45%.

TABLE 1. *Fluctuations (range) in the chemical parameters at the two stations*

Station	Water		Soil								
	pH	Salinity (‰)	Eh (mv)	Exch. Sodium (ppm)	Exch. Potassium (ppm)	Exch. Calcium (ppm)	Magnesium (mg/l)	Chloride (ppm)	Available nitrate (µg at NO_3-N/g)	Available phosphorus (µg at PO_4-P/g)	Oranic carbon (%)
Thoosath	7.0 to 7.8	1.2 to 5.0	-200 to -120	920 to 6699	100 to 700	200 to 686	112 to 320	106 to 510	1.6 to 5.3	9.7 to 22.1	0.9 to 2.1
Narakkal	7.6 to 8.3	1.9 to 8.3	-235 to -130	650 to 3500	120 to 620	154 to 726	84 to 290	106 to 490	1.0 to 3.9	15.1 to 21.5	1.2 to 2.3

It was found out that *Avicennia* germinate best in salinities lesser than 15‰. The seeds failed to grow while kept immersed through out the day. Though the seeds germinate in low and high tide levels, 45% germinated in low tide levels while in high tide level (wherein the seeds are kept wet, but remain immersed only for a short duration per day) 60% germinated. The studies conducted in the laboratory and as well as in the field indicated the same conclusion.

Of seeds allowed to germinate in darkness in an inert medium 20% grew to the 2 leaved stage in 40 days. Whereby showing that the food reserved in the seed is sufficient for the plant to grow to the 2 leaved stage. The offer of minerals by way of growing the seeds in beach sand indicated that this could give better germination. Herein 60% germinated.

The biochemical composition studies conducted with the seed at the radicle stage, root developed stage, plumule stage and 2 leaved stage indicate that with the early development there is an increase of lipid, ash and fibre content, while protein and carbohydrate show reduction in percentage.

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A COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION OF SOILS FROM AQUACULTURE SYSTEMS IN THE COCHIN ESTUARINE AREA

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Introduction

This study deals with physical and chemical properties of soils in aquacultural systems located around the Cochin Bbackwater, covering an area of about 250 sq. km during the pre-monsoon and monsoon periods of 1985. The stations were selected taking into consideration the potential areas to be developed for brackishwater aquaculture in this area.

Material and methods

Soil samples were collected from fifty different seasonal and perennial prawn culture fields located in the Cochin estuarine system including the Vembanad Lake. Analyses were done for soil pH, exchangeable cations *viz.* Sodium, Potassium and Calcium and total Phosphorus, grain size distribution of the sediment was determined and lime requirement rates of the soil were also estimated.

Results and discussion

Among the cations studied, sodium was the highest in concentration in the soil followed by calcium and potassium. The distribution of these cations did not show any specific pattern of variation during the present investigation. However, the heavy rains during the monsoon period had a sequential effect on the concentration of the cations registering considerably lower values during the period. All cations showed appreciable amount of leaching during the monsoon period, with calcium displaying the maximum variation.

Significant relationships could be established by statistical analysis of the data between the exchangeable cations and the grain size distribution of the soil. The concentrations of the cations showed inverse relationship with the dominance of sand fraction during both the periods which indicated that higher concentrations were associated with lower fraction of sand in the soil. The cations also showed direct relationship with silt fraction of the sediment while clay did not show any relation. A direct relation was also observed between the different cations, as higher concentration of one cation was indicative of greater concentration of the other and *vice versa*. Ponds with higher rate of water exchange had lower concentration of the cations in the soil and also the amount of leaching was more in such ponds. Thus it is evident that both the rate of water exchange as well as soil texture play significant roles in the concentration of the cation in the soil.

The important role of the soil phosphorus in the productivity of fish ponds is well recognised. It is generally considered as the key nutrient in the fertility of fish culture ponds. This nutrient frequently limits plant production and ultimately influences fish production.

Total phosphorus showed direct relationship with silt and clay fraction of the soil. It also showed a definite pattern of spatial distribution. The northern and north central regions of the Cochin Backwater recorded higher concentrations of phosphorus, while the southern areas showed relatively lesser values. The declining trend in phosphorus concentration in the soil from the marine to the fresh water zone has been reported by the earlier workers from this area. The same trend was noticed in the present investigation also including the decline in phosphorus during the monsoon season may be due to the leaching of phosphorus both from the interstitial and absorbed forms from the mud to the overlying water.

The soil pH is dependent on various factors and it influences inorganic transportation of soluble phosphate and control the absorption and release of ions of essential nutrients at soil water interface. In the present study, wet pH values

ranged from 6.2 to 8.0 in the premonsoon and from 6.0 to 8.4 during monsoon. The dry pH of the soil showed an acidic trend during both the periods, with lower values during monsoon.

Usually lime requirement for fish pond soils are determined on the basis of estimation of exchange acidity. In countries like India, Philippines, etc. where brackishwater regions are prevalent with acid sulfate soils, in order to estimate the correct lime requirement rates, the potential acidity of the soil is also to be taken into consideration. The soils of some culture ponds covered in the present study especially in the southern region are of acid sulfate in nature.

Potter (1977, *Jt. FAO/UNDP/SCSP and SEAFDEC Regional Workshop on Aquacult. Engg.*) opined that acid sulfate soils require large quantity of lime, between 20 and 100 tonnes of $CaCO_3$/ha due to their low pH values. The lime requirement estimated in the present study ranged from 10 to 95 tonnes/ha. But most of the samples recorded a pH value of above 6.0.

From the present investigation it was observed that in the Cochin area the ponds with soil predominantly composed of the finer fragments of silt and clay were rich in nutrient and hence ranks high in fertility. In soils with higher proportion of sand, these nutrients were found to leach out particularly during the heavy rains and correspondingly reducing the soil fertility which in turn can adversely affect the productivity of the area. Gopinathan *et al.* (1982, *Proc. Symp. Coastal Aquacul.*, 1 : 369- 382) reported that the north central regions of the Cochin Backwater were the most productive areas in the system. The seasonal and perennial fields located in the north central part of the Cochin Backwater, between Cochin Barmouth and Azhicode, have higher contents of exchangeable cations and phosphorus in their soils. The soils in this region also have predominance of silt and clay. The two openings to the sea at a short distance allows for greater water circulation and nutrient exchange in this part of the estuary. The River Periyar deposits a large amount of silt and clay in this region annually and this attributes to the relatively higher rate of production in the culture systems situated in this area.

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EVALUATION OF THE NUTRITIVE VALUE OF MANGROVE LEAVES AS A FEED COMPONENT FOR JUVENILES OF *PENAEUS INDICUS*

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Introduction

Feed forms the major operational input and greatly influences the running expenditure of prawn culture operations. Therefore, formulation and supply of nutritionally adequate practical feeds, either complete or supplementary, become essential for the success of prawn culture. A number of compounded feeds have been developed for juveniles of Indian penaeid prawns with a variety of conventional feed ingredients. However, with the growth and expansion of aquaculture industry in India, shortage of these conventional feed ingredients can be expected as most of these ingredients are already being used extensively for the formulation of feeds for animal husbandry. Keeping this in view, research has been intensified at the Centre of Advanced studies in Mariculture, CMFRI, Cochin to develop practical feed formulation using cheap non-conventional feed ingredients for extensive prawn culture.

Most species of penaeid prawns have been considered as omnivorous and this factor has been viewed as an advantage in developing compounded feeds incorporating several percentages of plant matter, without significantly affecting their growth. Very recently, algae and fresh and decomposed leaves of salt-marsh plants have been successfully used in prawn feed formulations in other countries. Studies on the gut content of prawns from mangrove swamps, which form some of the most productive ecohabitats, have clearly shown that fresh and decomposed mangrove plant matter constitute a significant

percentage of the food ingested by prawns. Encouraged by the foregoing observations the present investigation was made to study the suitability of mangrove leaves as a feed component for the juveniles of the prawn *Penaeus indicus*.

Objectives

Biochemical composition of the fresh leaves of four species of mangrove plants found in Cochin area and the changes in biochemical composition during the decomposition of mature green mangrove leaves, were studied. The nutritional value of fresh and decomposed leaves of mangrove plants in diets for juveniles of *Penaeus indicus* was evaluated.

Materials and methods

Leaves of four species of mangrove plants *Rhizophora mucronata, Avicennia officianalis, Acanthus ilicifolius* and *Bruguiera gymnorhiza* from Cochin area, were selected for the present study. Actively photosynthesizing green leaves, mature or yellow leaves from plants and fallen (withered) leaves were collected from all the four species. The moisture, total nitrogen, non-protein nitrogen, crude protein, total lipids, crude fibre, ash, nitrogen-free extract, phosphorus, calcium. sodium and potassium contents of the selected mangrove leaves were determined.

Since fresh green leaves had relatively higher protein percentage, they were selected for studying the biochemical changes associated with decomposition. The leaf material was kept for decomposition for a period of thirty days in plastic bins in water of salinity 14-16‰. The biochemical constituents were determined in samples taken from each of the bins on the first, fifteenth and thirtieth days using standard analytical procedures.

Two sets of laboratory experiments were also conducted in juveniles of *Penaeus indicus* using fresh and decomposed mangrove leaves as one of the ingredients in diets. In the first experiment green leaves of *R. mucronata* were selected as one of

the ingredients, as its crude protein content was relatively higher than all other species. Green leaves of the species was included in diets at levels of 5, 10, 15, 20, 25 and 30% by replacing rice bran and fed to the prawns.

In the second experiment decomposed leaves of *R. mucronata*, *A. officianalis* and *A. ilicifolius* were included at 15 and 25% levels in compounded feeds, replacing groundnut oilcake. A control diet was also prepared without mangrove leaf for both the experiments.

Data on apparent digestibility coefficient, food conversion ratio, gross conversion efficiency and specific growth were recorded and the proximate composition of prawns were determined for both the set of experiments.

Initially, the mean, standard deviation and standard error of the data were computed. Then analysis of variance (ANOVA) was applied to find out the statistical significance between the values for biochemical constituents, recorded from various leaf stages of different species as well as during decomposition. In the case of feeding experiments, variance analysis was performed to test the significance between treatments with reference to the inclusion of mangrove leaf on the food intake, growth, apparent digestibility coefficient, protein efficiency ratio, etc. Least Significance Difference Test (LSD) was applied to evaluate the difference between each of the observed parameters with reference to treatments.

Results and discussion

The leaves of *A. ilicifolius* had the highest moisture content (75.37%) among the green leaves, while that of *Avicennia officianalis* (70.62%) and *R. mucronata* (70.87%) had relatively low moisture contents. Significant differences ($P<0.05$) in moisture content were observed between the green and yellow, and the green and withered leaves of all the three species.

Among the four species, green leaves of *R. mucronata* had the highest total nitrogen (2.52%) and crude protein (15.71%)

contents. The lowest levels of total nitrogen and crude protein (11.26%) were recorded for *B. gymnorhiza*. In all the four species, total nitrogen showed a decrease as the leaves grew into yellow mature leaves, but the reduction was maximum in the case of *A. officianalis* and minimum in *B. gymnorhiza*. Green leaves of all the four species had significantly ($P<0.05$) higher total nitrogen and crude protein than yellow leaves.

The non-protein nitrogen content also followed a similar pattern as that of the total nitrogen, with the values decreasing as the leaves turned yellow and then again increasing in the withered leaves. *A. officianalis* had the highest non-protein nitrogen content in all the three stages of leaves. All the three types of leaves of *R. mucronata* and *A. ilicifolius* had relatively less non-protein nitrogen than those of *A. officianalis* and *B. gymnorhiza*. It is also apparent from the data that non-protein nitrogen forms a significant percentage of the total nitrogen content of *A. officianalis*.

Green leaves had relatively higher total lipid than yellow and fallen leaves, with green leaves of *A. ilicifolius* having the highest (13%) value and the lowest in *B. gymnorhiza* (9.27%). The total lipid content showed a general decreasing trend from green to fallen stages. The decrease was found to be maximum in *B. gymnorhiza* (3%) and minimum in *R. mucronata* (1.1%).

The highest nitrogen-free extract content, for all the three stages of leaves, was obtained for *B. gymnorhiza* with 41.75% for the green, 41.185% for yellow and 36.514% for fallen leaves; whereas the lowest values were recorded in *A. ilicifolius*. Among the four species crude fibre content of green leaves was maximum in *A. ilicifolius* (30.17%) and minimum in *R. mucronata* (24.2%), with the other two species, having intermediate values.

The ash content of green leaves in all the four species ranged from 10-12.3%. It showed a gradual increase in the yellow leaf stage, but showed a marked increase in the withered stage. The phosphorus content in the mangrove leaves seems to be very low. All the three stages of leaves of *R. mucronata*

and *A. ilicifolius* had relatively higher percentage of phosphorus when compared to the other two species. In all the four species, yellow leaves had relatively higher phosphorus than green and withered leaves. In all the four species, green leaves had relatively higher percentage of calcium when compared to yellow and withered leaves.

The total nitrogen, non-protein nitrogen and crude protein contents of all the four species showed significant ($P<0.05$) increases after 30 days of decomposition. The overall increase in percentage crude protein was 71.11% for *R. mucronata*, 56.47% for *A. officianalis*, 48.27% for *A. ilicifolius* and 75.48% for *B. gymnorhiza*. Although the decomposed leaves of *B. gymnorhiza* showed more percentage increase in protein content, the actual protein content was less than the other species.

The total lipid, crude fibre and nitrogen-free extract showed significantly decreased levels after decomposition. The overall percentage decrease in lipid levels was 60.82% for *R. mucronata*, 51.16% for *A. officianalis*, 41.24% for *A. ilicifolius* and 65.72% for *B. gymnorhiza*. The decrease in nitrogen free extract was 49% for *R. mucronata*, 66.4% for *A. officianalis*, 37.6% for *A. ilicifolius* and 33.48% for *B. gymnorhiza*. The gross percentage loss of crude fibre after thirty days of decomposition was maximum for *R. mucronata* (27.5%), which was followed by *A. ilicifolius* (26.5%) and *A. officianalis* (17.6%) and the minimum in *B. gymnorhiza* (15.8%).

Conversly, the ash content increased steadily as the duration of decomposition increased. The gross percentage increase in ash was 182% for *R. mucronata*, 238% for *A. officianalis*, 137% for *A. ilicifolius* and 129% for *B. gymnorhiza*.

Sumitra Vijayaraghavan *et al.* (1980, *Indian J. Mar. Sci.*, 9 (2) : 120-122) obtained a gain of 66.43% in the crude protein content of dead leaves of *R. mucronata* after ten weeks of decomposition. In the present study the gain in protein content obtained for the green leaves of the same species after 30 days

of decomposition was 71.11%. According to Harrison and Mann (1975, *Limnol. Oceanogr.*, 19 (6) : 924-927) leaves of various ages differ in total organic matter and nitrogen contents and therfore can be expected to decompose at different rates. They showed that the green leaves decomposed more quickly than dead leaves.

The increase in nitrogen and protein content during decomposition may be due to the colonisation of the microbial flora and the decrease in particle size. Studies by various other authors have shown that the increase in nitrogen content is because of the increase in micro-organisms which leads to the formation of a rich protozoa-bacteria-fungi-detritus complex with much potential food value. The decrease in the crude fibre, total lipids and nitrogen free extract during the decomposition of leaves of all the four species indicate their utilisation by the microbes as sources of energy. Microorganisms convert the substances like cellulose and lignin present in the mangrove leaves into digestible matter which is utilised by animal communities (Odum and Heald 1972, *Bull. Mar. Sci.*, 22 : 671-738). The increase in ash content may be due to the reduction in the total organic matter, microbial mineralisation and the accumulation of minerals from the water.

When juvenile *Penaeus indicus* were fed on a diet containing the green leaves of *Rhizophora mucronata*, fairly good survival rates, ranging from 83.33% to 90% were obtained. Growth of prawns was found to increase with mangrove leaf content in diets upto 15% and thereafter showed a decline with further increase in the mangrove leaf content. The food intake of the prawn was not significantly affected by the level of mangrove leaf in the feed, although prawn groups fed on feeds with 10% and 15% mangrove leaf ingested relatively higher amount of food. The gross apparent digestibility coefficient of feeds was also not significantly influenced by the level of mangrove leaf in the feeds and it ranged from 85% for feed with 30% mangrove leaf to 89.85% for the control. The food conversion ratio, gross conversion efficiency and protein

efficiency ratio were significantly influenced by the mangrove leaf content of feeds. Moisture, protein, lipid and carbohydrate content of prawns did not show any significant differences between treatments.

The diets, containing fresh and decomposed mangrove leaf meal, fed to the juvenile prawns were found to be well accepted. There were also no significant differences in survival rates showing that fresh and decomposed mangrove leaf-meals in diets at the given levels do not have any appreciable effect on survival of juvenile prawns.

In the second experiment, decomposed mangrove leaves of *R. mucronata, A. officianalis* and *A. illicifolius* were included at 15 and 25% levels in the diets of juvenile *P. indicus*. The percent survival of prawns ranged from 80 to 83.33%. Inclusion of decomposed leaf-meal from all species of mangrove plants at 15% in the feeds resulted in relatively higher growth than that of 25% decomposed leaf-meal. Diets with 15% decomposed *R. mucronata* leaf-meal gave significantly ($P<0.05$) higher growth than all other feeds. The inclusion of decomposed mangrove leaves even at 25% level in the diets of prawns gave gains in length, live-weight and dry weight which were comparable to that obtained with the control diet. These results indicate that though growth of juvenile prawns is significantly enhanced by diets containing 15% decomposed mangrove leaf-meals, diets containing 25% do not affect growth showing that decomposed mangrove leaves of the above three species can be included at a level of 25% in the feed of juvenile *P. indicus* by replacing groundnut oil cake, without any significant effect or alterations in growth.

The gross food intake by the prawns also ranged from a minimum of 27.54 to 36.7 for groups of prawns fed on diet with 15% *Acanthus* leaf-meal. Gross apparent digestibility coefficients ranged from 81.95 to 90.37% among the groups of prawns fed on different diets. The value obtained was highest for the control feed. The highest food conversion ratio indicating the lowest food conversion efficiency was observed for the control diet

which was closely followed by diet with 25% *Avicennia* and 25% *Acanthus* leaf-meals. Diets with 15% and 25% decomposed *Rhizophora* leaf-meals gave significantly ($P<0.05$) lower food conversion values, when compared to other feeds. Feeds containing 15% leaf-meals gave relatively lower food conversion values, when compared to the food conversion values obtained with feeds containing 25% leaf-meals.

The gross conversion efficiency also was significantly ($P<0.05$) higher in feeds containing 15 and 25% of decomposed *Rhizophora* leaf-meal as feed component, when compared to all other feeds. The protein efficiency ratios recorded for the various treatments ranged from 0.8224 for the control feed to 1.2918 for the feed with 25% *Rhizophora* leaf-meal. Both the feeds with decomposed *Rhizophora* leaf-meal gave slightly higher PER values, when compared to other feeds.

There were no significant ($P<0.05$) differences in the moisture, protein, lipids and carbohydrate contents among the prawns fed on the various feeds indicating that the biochemical constituents are not significantly altered by the inclusion of decomposed leaf-meals.

In both the experiments all the diets fed to the juvenile prawns were found to be well accepted as can be seen from the gross food consumption. It is also evident that the test feeds contained adequate nutrient levels as they promoted good growth and survival rates equal to or better than that of the control feed. There were also no significant differences among the survival rates of prawns fed on diets with different levels of either fresh or decomposed mangrove leaf-meals indicating that inclusion of leaf-meals in diets at the given levels do not have any appreciable effect on the survival of juvenile prawns.

Although growth was found to be significantly enhanced by inclusion of decomposed mangrove leaf-meals at a level of 15%, there was no proportional increase in growth by inclusion of 25% leaf-meal, but the growth was more than that of control feed. This may be due to the relatively higher fibre content in

diet which may lead to reduced assimilation rates as cellulose is reported to be poorly assimilated by prawns.

Conclusion

The present study clearly indicates that fresh and decomposed leaf-meals of mangrove plants can be included at 15-25% in the diet of juvenile *Penaeus indicus*, without affecting growth, survival, feed efficiency and proximate composition of prawns. The study further demonstrates the superiority of decomposed leaf-meals, with reference to higher protein content, lower crude fibre, nitrogen free extract and total lipids over that of fresh leaves.

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EFFECT OF SOME PESTICIDES ON *PENAEUS INDICUS* H. MILNE EDWARDS*

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Introduction

The increase in the quantity of wastes that are being poured into the sea is directly related to the industrial development and the related human activities. The use of chemicals for controlling insect pests and the ecological influence of these chemicals in aquatic pollution is a subject of great concern. This results in the deterioration of the ecological niche of the marine organisms. Synthetic pesticides, especially organochlorine and organophosphorus have become increasingly important additions to the chemical wastes polluting the natural aquatic communities. The most striking feature of insecticide is that they are relatively more toxic to crustaceans than to any other marine organisms; as the insecticides were developed specifically for killing the arthropods. The persistent pesticides like organochlorine, seem to bio-concentrate in crustaceans and cause long term effects. Due to their low persistence, the organophosphorus compounds are being substituted for chlorinated biocarbons. But beacuse of their greater toxicity they are liable to cause short term acute effects on all aquatic animals.

Malathion and Ekalux are organophosphorus pesticides and are widely used to control insect pests, especially, their larval forms. Hence the cultured crustacean larvae should be considered very sensitive to these toxic substances. Moreover, the postlarvae and juveniles of the *Penaeus indicus* inhabits the estuarine and brackishwater area which are subjected to these compounds compared to the coastal zones. Thus the aquatic

*Prepared by the Editorial Committee.

organisms, especially the early stages of the prawns become the non-target organisms for the secondary effects of these pesticides. Though long term tests using suitable parameters such as growth and reproduction are efficient to study the effects of organochlorine compounds, short term or acute single species tests using lethality as an end point is also significant for estimating the hazards of the low persistent high toxicant organophosphorus pollutants.

In southern and eastern parts of India, where aquaculture was a traditional practice, the major aquaculture systems are closely linked with the agriculture fields through backwaters and chances are likely that the pesticides will eventually reach the culture ponds and may cause serious effects on the juveniles of the most important species, the Indian white prawn *P. indicus*.

Hence, an attempt was made in the present study to find out the lethal effects of malathion and ekalux on the larval and postlarval stages of *P. indicus*, to estimate the lethal and sublethal effects of malathion on juveniles and to quantity the sublethal effects of malathion in oxygen consumption, annomia excretion and random activity on large size juveniles of *P. indicus*.

Materials and methods

The methodology employed to study the lethal and sublethal effects of malathion and ekalux on *P. indicus* were based on already accepted standardised procedures followed for measurement of pollutant toxicity on aquatic organisms.

The mysis stage, postlarva 1, juveniles of 20-40 mm TL and 70±5 mm TL of *P. indicus* were selected as the test animals. Sea water collected from off Narakkal were filtered and brought down to 15 ppt salinity by dilution and used as the test medium. 1 ppt stock solution of malathion-50 and ekalux EC-25 were prepared and measured quantity were added to the test medium to obtain necessary concentrations of test solutions for the experiments.

Lethal toxicity tests : Lethal toxicity tests were conducted in mysis stage and postlarva I of *P. indicus* with malathion and ekalux.

The test animals were fed with a mixed culture of phytoplankton. The tested concentrations of the pesticides ranged between 1 ppb and 10 ppb. The observations were continued for 48 hrs with periodical monitoring at every 12 hrs The experiments were conducted in triplicates.

The lethal bioassay for juveniles (size 20-40 mm TL) were conducted at 20 ppb, 30 ppb, 50 ppb, 60 ppb and 70 ppb of malathion. The observations were continued for 96 hrs.

Sublethal tests : *P. indicus* juveniles of 20-40 mm TL were used to study the changes in oxygen consumption to see the effect of 5 sublethal concentrations of malathion, like 0.5 ppb, 1 ppb, 2 ppb, 3 ppb and 4 ppb. Experiments were done in triplicates. The duration of the experiment was for 6 hrs and the water was collected for oxygen estimation at every one hour.

Activity studies : Juvenile *P. indicus* of 70 mm TL were exposed to two sublethal concentrations, 0.5 ppb and 4.0 ppb of malathion. Each experiment lasted for 12 hrs and made in triplicates. The apparatus used was a modified version of Fry's respirometer in which simultaneous measurements of oxygen consumption, ammonia excretion and random activity were made.

Water analysis : Dissolved oxygen was estimated using unmodified Winkler method, ammonia by phenol-hypochlorite spectrophotometeric method and salinity by using standard argentotitric method. LC_{50} values for malathion and ekalux were estimated by weighted probit analysis. The effect of different concentrations of the pesticides on oxygen consumption, ammonia excretion, ammonia quotient and random activity were treated statistically by analysis of variance.

Results and discussion

The effects of malathion and ekalux on *P. indicus* young ones are discussed under the lethal toxicity studies and sublethal toxicity studies in the present investigation. The median lethal concentration of malathion on mysis stage was 2.97 ppb at 36 hr. It increased to 4.8 ppb at 24 hr and 7.2 ppb at 12 h, whereas

the median lethal concentration of ekalux on mysis stage was 2.62 ppb at 24 hr which increased to 5.4 ppb at 12 hr. The LC_{50} of malathion on postlarvae was 2.25 ppb at 48 hr which increased to 2.84 ppb at 36 ppb and 3.55 ppb at 24 hr and the LC_{50} for ekalux was 2.77 ppb at 24 hr which increased to 4.53 ppb at 12 h. On comparison, the larval and postlarval stages were more sensitive to ekalux than malathion.

The median lethal concentration of malathion in smaller juveniles was 43.42 ppb which increased to 53.16 ppb at 84 hr and 59.59 ppb at 96 hr. The results revealed that there was an obvious relationship between the concentration of the pesticides and the survival of the larvae even at very low concentrations. A cent percent mortality was observed within 48 hr in concentrations below 5 ppb in case of both the pesticides.

The oxygen consumption was reduced when juveniles were pre-exposed to sublethal concentrations of malathion, whereas the oxygen consumption increased in large juveniles at sublethal concentrations of malathion. The ammonia excretion and the ammonia quotient calculated were both reduced when large juveniles were exposed to sublethal concentrations of malathion, whereas the random activity increased in the same animals when exposed to sublethal concentrations of malathion. The study indicated that *P. indicus* were susceptible to the pesticides tested and the susceptibility varied with its stages in the life cycle.

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STUDIES ON RHIZOSPHERE MICROFLORA OF *ACANTHUS ILICIFOLIUS*

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Introduction

The rhizosphere which occupies an important niche in the mangrove ecosystem, consists mainly of the mangrove plant root surfaces and the surrounding soil. They harbour a number of beneficial micro-organisms which enhance the productivity of the area. The mangrove plant *Acanthus ilicifolius* found abundantly in the swampy areas along Cochin Coast was studied to estimate quantitatively and qualitatively the micro-organisms inhabiting the rhizosphere and their role in enhancing productivity.

Material and methods

The plant *Acanthus ilicifolius* along with its root system was collected from two centres namely Murukkampadam area in Vypeen Island and near Cochin Backwater in front of CMFRI. Collections were made at fortnightly intervals for five months. The samples were transported in sterlised polythene bags and analysed for the different microflora present. Viable counts of bacteria, fungi, actinomycetes and zymogenous populations were estimated by the Pour plate method using selective media. The different strains were isolated, identified and examined for colony characteristics, cell morphology and gram stain. The effect of certain environmental parameters such as soil temperature, pH, Eh, salinity, organic carbon, available nitrate and available phosphorus on the microfloral population were also studied.

Results and discussion

The total bacterial population in the root of *Acanthus ilicifolius* ranged from 71.42 x 10^4/g to 262.62 x 10^6/g. In soil,

it ranged from 72.5 x 10^4/g to 226.53 x 10^6/g. The heterotrophic bacteria identifed, belonged to the following five genera *Alcaligenes, Flavobacterium, Cytophaga, Vibrio, Aeromonas* and family *Enterobacteriaceae.*

The total count of fungi in the rhizosphere ranged from 25.71 x 10^4/g (August) to 4 x 10^6/g (September). In soil the fungal population ranged from 15 x 10^4/g (July) to 10 x 10^6/g (October). The fungi isolated were mainly species of *Fusarium, Pencillium, Aspergillus* and *Rhizopus.*

The total actinomycetes population ranged from 0.98 x 10^4/g to 21.42 x 10^5/g in the rhizosphere and from 7.05 x 10^5/g to 0.46 x 10^4/g in the rhizosphere soil.

The total microflora showed a seasonal cycle in their counts. While bacteria recorded maximum counts during the postmonsoon months, the fungi and actinomycetes recorded maximum counts during the monsoon months. The maximum B:F and B:A ratio observed during October further confirmed the dominance of bacteria over fungi and actinomycetes in the post-monsoon season. The zymogenous population (proteolytic, caseinolytic and lipolytic) also showed seasonal variations. The number decreased during August and then gradually increased during September and October.

The decrease in the zymogenous population during August may be attributed to the high rainfall and low temperature. Lowering of salinity had little or no influence on the fungal and actinomycetes populations. From this it may be concluded that the fungi and actinomycetes species are extotic or terrestrial forms acclimatised to the marine environment. Statistical analysis did not reveal any significant relationship between the physico-chemical factors and the microflora. This may be due to the limited number of observations during the present study.

The morphological and physiological characteristics of 30 bacterial strains isolated from the rhizosphere of *Acanthus ilicifolius* is given in Table 1. The predominance of fermentative

bacteria in the rhizosphere indicated that they are mostly facultative anaerobes. Furthermore, the high degradative potential in the mangrove swamps of Murukkampadam and Cochin area, reflect extensive microbial activities and continuous decomposition of foliage and detritus and the turn over of nutrients.

TABLE 1. *Morphological and physiological characteristics of 30 bacterial strains isolated from the rhizosphere of* Acanthus ilicifolius

Characteristic	Frequency of occurrence (%)
Gram	
Positive	3.34
Negative	96.66
Motility	96.66
O/F Media	—
Oxidative	—
Alkaline	
Fermentative	98.2
Gelatin Hydrolysers	60
Starch Hydrolysers	80
Nitrate Reducers	70
H_2S Producers	73.33
Oxidase	90
Catalase	100
Pencillin resistance Test	30
Pigmentation	26.4

The biochemical activity of the rhizosphere microflora of *Acanthus ilicifolius* has revealed that (i) the microflora have great ability to effect rapid biochemical changes, (ii) the combined respiratory activity of microorganisms and roots of *A. ilicifolius* results in greater carbondioxide production which dissolves in soil water to form carbonic acid that leads to increased solubility of primary minerals and (iii) the loss of photosynthate as root exudate enhances growth of chelate producing microbes. The enzymes of chelate producing microbes facilitate the solubilisation of primary minerals which act through a positive feed back mechanism to increase the productivity of this ecosystem.

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A COMPARATIVE STUDY OF SEDIMENT NUTRIENTS IN SEASONAL AND PERENNIAL PRAWN CULTURE PONDS DURING THE SOUTHWEST AND IMMEDIATE POSTMONSOON MONTHS

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Introduction

Prawn, a benthic feeder and an omnivore, feeds on the sediment heterotrophic organisms such as polychaetes, molluscs, etc. The prawn production in ponds, therefore depends mainly on the abundance of these food organisms. Sediment being the source of nutrients for the heterotrophic community, its quality determines the population density of these organisms. In order to evaluate the potential of seasonal and perennial ponds one each at Narakkal and Edavanakad in Vypeen Island for prawn production, observations were made on the organic and inorganic nutrients of pond sediments between June and October 1986.

Materials and methods

Sediments were collected from different areas of the pond using a core. Around 2 cm of the upper portion of the core sample was removed, dried, powdered and analysed. Organic carbon was determined by the method of Walkley and Black (1934, *Soil. Sci.*, 37 : 29). Protein was analysed by Biuret method (Subhashini and Ravindranath, 1981, *CMFRI Spl. Publ.*, 7 : 31-41) and carbohydrate by Anthrone method (Sarvanan and Ravindranath, 1981, *CMFRI Spl. Publ.*, 7 : 17-21). Lipid was estimated by the Bligh and Dyer method (Kanazawa, 1982, *CMFRI Spl. Publ.*, 8 : 52-67). Reactive and total phosphorus, nitrate and nitrite were extracted from the sediments into solution by the methods of Jackson (1973, *Soil Chemical Analysis*. Prentice Hall, 498 pp). The solution was then tested for the

nutrients by the procedures of Strickland and Parsons (1968, *Bull. Fish. Res. Bd. Canada*, 167, 311 pp). Total nitrogen was estimated using a microkjeldhal assembly.

Results and discussion

Environmental background : Difference in values of salinity in seasonal and perennial ponds were only a few ppt even though the water level in the latter changed with every successive tide and that of the former remained static except for minor changes caused by the monsoonal rains. This constant flow of water in and out of the perennial ponds could account for high concentrations of oxygen especially when the salinity is low. The demand for oxygen by the decaying vegetation and hay, on the other hand, perhaps reduced the dissolved oxygen in the seasonal ponds. The sediment temperature varied between 26° and 33°C in the seasonal ponds and 27° and 31°C in the perennial ponds. The low pH in seasonal ponds may indicate an increase in the products of the decaying organic matter. Gopinathan *et al.* (1982, *Proc. Symp. Coastal Aquaculture*, **1** : 369-382) attributed similar observation to the effect of large scale decomposition of weed deposits.

Organic sediment nutrients : Concentrations of organic carbon in seasonal ponds were greater than that of perennial ponds. There appeared to be a negative relationship between salinity and organic carbon, which was assigned to a higher sedimentation of suspended organic detritus owing to the change in salinity (Nagarajaiah and Gupta 1983, *Indian J. Mar. Sci.*, **12** : 81-84). It is significant that the enhanced organic carbon leads to greater availability of nutrients for the benthic organisms which form food of prawns. Therefore it is likely that the greater concentration of organic carbon/nutrients in the seasonal ponds would have led to the enhanced prawn production in these ponds as observed by George (1974, *Indian J. Fish.*, **21** : 1-19). High molecular protein compounds are very rapidly hydrolysed and decomposed by microorganisms on account of their small size (Krey 1961, *Kieler Meeresforsch*, **17** : 163-175). The detritus formed from plant debris, although a poor source of protein,

when converted into bacterial biomass serve as a good source of protein. According to Mann (1982, *Ecology of Coastal Waters: A Systems Approach*. Blackwell Scientific Publ., pp. 183-209) as a consequence of increase in microbial biomass the protein content of detritus also increases. The increase in lipid content corresponded to an increase in organic carbon indicating a higher input of organic matter.

Inorganic sediment nutrients : The values of reactive phosphorus observed in seasonal ponds were greater than those of the perennial ponds. Peaks of reactive phosphorus matched a drop in dissolved oxygen content. Mortimer (1971, *Limnol. Oceanogr.*, **16** : 387-404) noticed that a progressive decline in dissolved oxygen at the water/sediment interface was accompanied by the transfer of substantial quantities of phosphorus into the water. Total nitrogen decreased in sediments of seasonal ponds during flowering of paddy. It is also likely that the estuarine production within the ponds are consumed by detritivores and other fauna leading to lower level of nitrogen in the sediment. Valiela and Teal (1977, *Ecological Processes in Coastal Environments*. Blackwell Scientific Pub., pp. 339-414) lists ground and rain water and nitrogen fixation by bacteria as sources of nitrogen and denitrification as a major loss of nitrogen.

Seasonal ponds maintain a high nutrient load due to the increased organic matter available while perennial ponds depend on the dynamics of water and input from external sources. The variations and abundance of sediment nutrients in these ponds indicate that they are capable of suporting a rich benthic fauna. This, in turn, would sustain prawn production provided drastic changes in flow pattern and organic matter do not occur.

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PHOTOSYNTHESIS IN RELATION TO SOME SELECTED ENVIRONMENTAL PARAMETERS IN PRAWN CULTURE FIELDS

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Introduction

The ever increasing demand for prawns has stimulated countrywide interest in scientific prawn culture and entrepreneurs are coming forward to establish prawn farms. If the farm is to be economically viable, various factors have to be taken into consideration before selecting a suitable site. The carrying capa- city of any culture system depends mainly on its primary productivity. Therefore it is very essential to assess the biogenic capacity of water to determine the stocking strategies, growth and production of all culture organisms in a pond very according to the level of primary production. The primary productivity is the basic link in the chain of events leading to the tertiary production in the ecosystem. In order to attain the requisite productivity, the rate of photosynthesis has to be increased by manipulating the different environmental parameters. Hence an attempt has been made in the present study to investigate the role of some selected environmental factors on the photosynthesis and their effect on the productivity.

Material and methods

Studies were carried out in some brackishwater ponds at Narakkal in the Vypeen Island (10°01'N-75°16'E).

The samples were collected from two stocking ponds at MPHL (Marine Prawn Hatchery Laboratory) and a coconut groove located approximately 75 m east of MPHL. All the three sampling sites were separated from sea by a narrow stretch of

land and were connected to the brackishwater through a channel.

The description of the three sites is as follows :

Site I : Stocking pond of 0.2 ha area with an average depth 0.95 ± 0.25 m having muddy substratum.

Site II : Area 0.2 ha adjacent to the first pond with an average depth 1.05 ± 0.2 m and clay substratum.

Site III : This is a coconut groove characterised by very shallow water (0.5 ± 0.25 m) having plenty of vegetation and water exchange.

Sampling procedure

Weekly sampling was carried out from late June to early October, during the early hours between 0600 hrs and 0700 hrs. From site I and II, surface and bottom samples were collected. But from site III, only one sample was collected as the depth was very low to differentiate surface and bottom significantly.

Water samples for nutrient analysis, were collected in narrow mouthed air tight plastic bottles of 500 ml capacity and analysed by following the method of Strickland and Parsons (1968, *Bull. Fish. Res. Bd. Canada*, **167** : 127).

The *in situ* temperature was noted everytime and salinity was calculated by Mohr - Knudsen method, while pH was measured using pH paper.

Ammonia in water was estimated by phenol-hypochlorite method, chlorophyll *a* by the method of Timothy. Productivity studies were made by light and dark bottle technique introduced.

Results

The study period was mainly restricted to the monsoon period from June to October. The monsoon has a direct effect on the environmental parameters which in turn effect the photosynthetic process.

Temperature : *Site I* : The water temperature varied from 27°C to 31.5°C. During August the temperature fell to 27°C. Thereafter a peak was observed in September, the temperature fluctuated within a range of 30°C and 31°C.

Site II : The water temperature at site II did not show much variation from that of site I. The temperature values ranged between 27.5°C and 31.5°C. The trend in temperature fluctuation was quite similar to that of site 1.

Site III : The water temperature at site III ranged between 25°C and 29°C. The trend in temperature fluctuation was similar to that of other two sites with a steep fall in temperature in mid July.

pH : The water pH of all the sites ranged between 6.5 and 8.5.

Salinity : The salinity showed a decreasing trend till August and then an increasing trend upto the last week of September with a fall by October first week. The maximum salinity value was recorded in late June. The bottom water salinity has shown a similar trend as that of the surface water with slightly higher values.

Dissolved oxygen : In site *I*, the dissolved oxygen values varied throughout the period of study and ranged between 0.95 and 5.65 ml/l at the surface and at the bottom it was from 0.95 to 7.8 ml/l while in site 2 the oxygen values ranged between 0.018 and 6.2 ml/l in the surface waters and 0.7 and 7.0 ml/l in bottom waters. In site 3 the variation was between 0.75 and 4.62 ml/l.

Ammonia : Wide variation in the ammonia content of surface water and bottom water was noted. At site II, a maximum of 54.6 and a minimum of 1.8 µg at/l at surface waters and at the bottom a maximum of 51.4 and a minimum of 7.4 µg at/l were observed. At site III the values ranged between 18 and 82.6 µg at/l.

Inorganic phosphorus : The concentration of inorganic phosphorus in the surface waters ranged between 8.39 µg at/l in early

August and 17.37 µg at/l in early July. While variation in bottom water was between 8.56 and 17.48 µg at/l. At site II in surface waters, a range of 7.43 to 20.33 µg at/l was recorded and in bottom waters, the range was between 9.98 and 21.46 µg at/l. The value at site III ranged between 6.69 and 25.16 µg at/l.

Nitrite : A range of 1.6 and 13.6 µg at/l in surface water and between 2.2 and 12.8 µg at/l with a mean values of 5 µg at/l were recorded at site I. At site II in surface waters a range between 2.2 µg at/l and 12.22 at/l was recorded while the values for bottom waters were between 1.6 and 13.6 µg at/l. Values ranged between 1.4 and 11.2 µg at/l at site III.

Nitrate : In surface water, the values ranged from 5.8 to 17.8 µg at/l, while the values for bottom water ranged between 5.4 and 20.4 µg at/l. At site II, the surface water showed a maximum of 15 and a minimum of 5.2 µg at/l in the bottom water the maximum was 6.0 µg at/l. Nitrate values ranging from 3.8 and 25.6 µg at/l were recorded.

Silicate : At site I, the surface water showed a range of 9.37 and 100.86 µg at/l. In the bottom water the concentration showed a range of 8.6 and 103.75 µg at/l. At site II, silicate concentration in the surface water ranged between 8.65 and 95 µg at/l while the bottom waters showed a variation of 10.09 to 95.1 µg at/l. At site III a range from 12.25 to 93.66 µg at/l was observed.

Chlorophyll : Variation in *chlorophyll a* content at site I ranged from 7.67 and 77.5 mg chl/m^3, while at site II the range was from 3.91 and 108.3 mg chl/m^3. A range of 1.19 to 34.91 mg chl/m^3 was observed at site III.

Productivity : The productivity values for site I ranged from 0.45 $gm^3 d^{-1}$ to 3.76 $gm^3 d^{-1}$, while at site 2 the variation was from 0.66 $gm^3 d^{-2}$ to 3.38 $gm^3 d^{-1}$ and the site III had the production range of 0.05 to 3.02 $gm^3 d^{-1}$.

Discussion

While comparing the productivity and related parameters in the three sites which have been studied for a period of three to four months, the following conclusions could be arrived at.

The site III which has a greater influence from the back-water system showed a better correlation between the various parameters that have got a significant bearing on productivity. All the three areas have got a uniformly high rate of gross and net production reaching over 3 gC/m^3d^{-1} which is equivalent to the highest productivity that is normally observed in estuarine and inshore environments.

Qasim and Sankaranarayan (1970, *Limnol. Oceanogr.*, **15** (4): 574-578) has further suggested that the bloom of phytoplankton and growth of zooplankton in an estuarine system are some what of phase and that the phytoplankton production far exceeded the rate of consumption by the zooplankton harbivores which occur in the system as surplus. This surplus production was minimum during Februay and reached its maximum during July to October.

As discussed earlier sites I & II are enclosed environments where the tidal influx and consequent replenishment are almost nil except perhaps through percolation through the bunds. The thermal condition is dependent mostly on the monsoon precipitation. It has already been established that in Cochin Backwater and adjacent areas temperature has little significance in the production of organic matter (Qasim *et al.* 1969, *Proc. Indian Acad. Sci.*, 69 : 51-94).

The dissolved oxygen content of the water in the prawn culture fields showed little variation. Although the dissolved oxygen content has no direct role in the production of organic matter in the estuary, it is an index of the metabolic activities of the entire community comprising producers as well as consumers (Gopinathan *et al.*, 1982, *Proc. Symp. Coastal Aquacult.*, 1 : 368-382). Pillai *et al.* (1975, *Bull. Dept. Mar. Sci. Univ. Cochin*, 7 (1) : 137-150) observed that high values of dissolved oxygen were found during monsoon and premonsoon periods, which can be due to combined effect of photosynthesis and water movement.

Another significant productivity parameter is the occurrence of free ammonia in the water.

A scrutiny of the nutrients indicates that inorganic phosphorus is significantly higher to that of nearshore environment or in normal brackishwater environment. The high content of inorganic phosphorus can be attributed to leaching from agricultural fields around the culture ponds. It is possible that a large part of the fertilizers leach into the environment which accounts for high phosphorus and nitrate content. The agricultural activity also tend to increase the silicate values which were found to range from 12 to almost 9 µg/ at/l.

Therefore the above studies have again confirmed the fact that in such estuarine systems where there is considerable authropogenic activity, the expected relationship between micro nutrients and production may not be applicable. One thing that holds good in the aquatic ecosystem is that it is highly productive leaving a surplus production in excess of respiration.

Hence in enclosed waters or in areas which have got influx from the adjacent open areas, there is a large amount of surplus food which can be fully exploited by stocking at a higher density than the accepted norms and with less input. Among the areas studied by above authors the seasonal ponds appear to be more productive than perennial ones. The present study falls under the highly productive group. However after one or two culture operations during the summer months, it may be desirable to leave the culture system to rejuvenate by itself.

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CHANGES IN HAEMOLYMPH CONSTITUENT IN THE RESTING (OR NON-ACTIVE) AND ACTIVITY STRESSED *PENAEUS INDICUS* H. MILNE EDWARDS*

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Introduction

Recent advances in aquaculture practices have paved way for various manipulations aimed to scientifically improve the culture systems to obtain a higher production. But due to these man made and natural manipulations the aquaculture systems are frequently exposed to the variations occurring in the biotic and abiotic factors associated with it. Stress is a major condition to which the fishes and prawns are subjected to under intensive culture systems. The major stresses in these systems to which the individual prawn has to adapt inorder to maintain homeostasis are the deterioration in water quality, unnaturally high stocking density, handling, transport and exposure to different salinities, etc. The most suspected cause for the 'soft prawn' disease in the Indian white prawn *Penaeus indicus*, a species of great importance in brackishwater aquaculture in India, may be stress due to changes in the environment. Hence, in this study an attempt is made to quantify the changes occurring in the haemolymph of *P. indicus* exposed to three different types of stresses.

Materials and methods

P. indicus prawns of both the sexes of 100-150 mm TL were acclimated for 72 hrs in circular plastic pools with 30 ppt saline water. They were fed *ad libitum* with fresh prawn meat once a day and one third of the water was replaced everyday. The prawns were subjected to three different types of stresses and the haemohymph constituents were analysed for protein, calcium, magnesium, sodium and potassium.

*Prepared by the Editorial Committee.

Different salinity stress conditions : Ten groups of 25 prawns each were acclimated and 2 groups each were exposed to same salinity changes enabling to get replicas for each salinity. The experimental animals were abruptly exposed to 5 ppt, 15 ppt, 35 ppt and 45 ppt saline water. Haemolymph samples were collected at 0, 3, 9, 18 and 30 hrs after the exposure. Only prawns in the intermoult stage were used in the experiment.

Under activity stressed conditions : A total of 150 prawns were segregated to 10 groups of which 5 groups were kept as control assumed to be the 'resting' or 'minimal stressed' condition. The remaining 5 groups were chased continuously in the pool, until they did not respond to the stimuli any more which was assumed as the 'most stressed' condition. Haemolymph samples were collected at 0, 4, 8, 20, 24, 28 and 32 hrs after the experiment.

Effect of transportation stress : A batch of 50 prawns were seperated into 5 groups and transported in conventional open containers with 32 ppt saline water over a distance of 70 km involving $1^1/_2$ hr journey by jeep. The haemolymph samples were collected at 0, 6, 19 and 43 hrs after the journey.

Analytical procedures : The haemolymph of 0.2 ml volume were collected directly from the heart of each animal using a 1 ml syringe and 25 G needle. The samples were diluted to 0.6 ml using distilled water and mixed with a cyclomixer. All samples were deep frozen immediately. The protein samples were estimated within 48 hrs using biuret method. The sodium and potassium concentrations were determined on "Elico" digital flame photometer. For magnesium and calcium ions, a 49 fold haemolymph sample in lanthanum chloride solution was asperated on atomic absorption spectrophotometer. Standard statistical methods were used for computing the data using a Casio fx personal computer.

Results and discussion

The prawns exposed to different salinities exhibited rapid fluctuations in their haemolymph constituents soon after the exposure. The protein concentration of 61.3 mg ml^{-1} at 30 ppt

salinity increased to 87.82 mg ml^{-1} at 5 ppt salinity. This may probably be due to higher energy demand for maintaining homeostasis which cause an increase in the metabolic activity therefore requiring emergency mobilisation of proteins to the catabolic sites. The calcium concentration increased from 0.47 mg ml^{-1} at 30 ppt salinity to 0.5 mg ml^{-1} at 35 ppt salinity due to the salinity stress. The magnesium level decreased from 1.62 mg ml^{-1} at 30 ppt to 0.63 mg ml^{-1} at 5 ppt salinity. The decrease in sodium concentration from 9.9 mg ml^{-1} at 30 ppt to 9.3 mg ml^{-1} at 45 ppt salinity may be attributed to the tendency to maintain a hypo-osmotic haemolymph in higher salinity by the penaeid prawns. The potassium ions tend to be regulated within fairly narrow limits under different salinity being low in high salinity and still lower in low salinity.

Under the activity stressed conditions, the haemolymph concentration of proteins were high during the stress, but reduced during post-stress period. Animals exposed to transportation stress also showed a similar trend as that of the mechanically chased individuals. The magnesium concentration showed steep increase during the stress, but drastically reduced to very low levels and did not recover even after 43 hrs indicating over exhaustion where lot of energy has been spent to overcome the stress as magnesium plays an important role in ATPase activity. No significant change was observed in sodium concentration of haemolymph whereas potassium concentration increased slightly in case of both mechanical stress and transportation stress. The drastically low level of magnesium concentration even after 43 hrs of post-stress indicates that prawns could not be used for physiological experiments without sufficient acclimation.

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SEA WATER ANALYSIS WITH PARTICULAR REFERENCE TO DISSOLVED ORGANIC MATTER DURING PREMONSOON AND MONSOON MONTHS

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Introduction

Prawns are induced to spawn in sea water by unilateral eye stalk ablation and the larvae reared upto postlarval stages at the Marine Prawn Hatchery Laboratory of CMFRI (MPHL). At MPHL, sea water collected during high tide and stored, is used for maintaining the brooder prawns for spawning and also for rearing the eggs and larvae. The physical and chemical environmental parameters of the sea water used for these were monitored regularly. However, there have been frequent failures especially with the onset of monsoon and it was thought that the dissolved organic matter may have a role in the successful spawning and development of eggs and larvae.

Objectives

To find out the effect of dissolved organic matter (DOM) in the sea water used for culture experiments during premonsoon and monsoon months and to evaluate the possible effect of DOM on the successful spawning and rearing of the eggs and larvae, the present investigations was carried out.

Materials and methods

The water samples collected from the intertidal zone off Krishi Vigyan Kendra of CMFRI, Narakkal was labelled as "fresh" and those from the cement concrete seawater sump as "stored" sea water. Sampling was done every ten days for a period of five months from May 12, 1986 to October 10, 1986. Temperature and pH were recorded immediately, salinity by

Mohr-Knudsen procedure and dissolved oxygen by the Winkler's method were analysed. The sea water was analysed for reactive phosphate (PO_4-P), nitrite (NO_2-N), nitrate (NO_3-N) and silicate (SiO_4-Si). The fixation in extracellular production which is the main source of DOM and the particulate organic matter (POM) were estimated by Watt's method (1965, *Proc. Roy.*, 50 B 164 : 52-55) using C^{14} and liquid scintillation technique with an LSS 20 (ECIL) Liquid scintillation counter, Chlorophyll 'a', 'b', 'c', carotenoids and phaeopigments were estimated by using standard methods. In addition to this, the daily rainfall data from April to October 1986 was also obtained from Naval Meteorological Observatory, Cochin.

Results and discussion

The DOM concentration was highest during the SW monsoon (July-August) in fresh sea water and lowest during the postmonsoon (September-October). In stored sea water maximum was in the premonsoon and postmonsoon and minimum was during the monsoon with values not exceeding 1 mg Cm^{-3} hr^{-1}. The DOM in fresh sea water was found to have significant correlation at 1% level with POM and in stored sea water it had definite correlation with silicate at 5% level, with POM at 1% level and chlorophyll 'a' at 5% level.

The POM concentrations in fresh sea water were maximum during the monsoon and in the stored water during the postmonsoon. The POM values showed significant correlation with DOM and Chlorophyll 'a' 1% level in fresh sea water whereas in stored sea water the values showed correlation with DOM at 1% level.

The percentage extracellular release (PER) in fresh sea water was highest during monsoon whereas in other months it was fluctuating with lower values just after monsoon.

The chlorophyll 'a' showed maximum in monsoon in fresh sea water and in stored water during pre- and postmonsoon. It also showed significant correlation with nitrate,

silicate and POM at 10, 5 and 1% levels respectively. The chlorophyll 'b' values were high in fresh sea water during monsoon and postmonsoon and they were high in stored sea water during monsoon. The chlorophyll 'c' values, on the other hand, were high during postmonsoon in both fresh and stored sea water.

The carotenoid pigment values showed maximum in October and minimum in June in fresh sea water whereas these were maximum in September and minimum in May-August in stored sea water. The phaeopigments were maximum in fresh sea water in September and minimum in June. These were minimum in May, August and October in stored sea water and mostly uniformly high values were presented in other months.

The nitrite nitrogen (NO_2-N) was mostly uniform in both fresh and stored sea water with peak values in July and August respectively. The values were generally higher in stored sea water compared to fresh sea water. The nitrate-nitrogen (NO_3-N) was observed to be generally low in the stored sea water and mostly uniform whereas in fresh sea water it showed maximum during the monsoon with more or less uniformly lower values in other seasons. The reactive phosphate (PO_4-P) showed maximum in July and September and minimum during May in fresh sea water whereas it was maximum in August and September with minimum values in June in stored sea water. Silicate (SiO_4-Si) maximum in July in both fresh sea water and stored sea water.

The temperature and pH were mostly uniform in both fresh and stored sea water throughout, whereas except during the monsoon months the dissolved oxygen values were uniform in both fresh and stored sea water. During the monsoon months the dissolved oxygen concentrations were higher in fresh sea water. The salinity values were higher in fresh sea water during pre- and postmonsoon months with lower values during the monsoon period. The salinity was mostly higher and uniform throughout in the stored sea water.

The daily rainfall data from April to October at Cochin showed that the monsoon rainfall was erratic during this year, with the maximum rainfall in July. The reflection of this feature is seen on all parameters, in general, with more or less higher or lower values being presented intermittently. Thus, it cannot be clearly stated that the so termed pre-, postmonsoon, and monsoon features presented represent the true picture had the monsoon been in full swing and intensity. The irregularities in the values of the various parameters may be due to this feature.

Conclusion

It is well known that the phytoplankters release a part of their photosynthetic products as extracellular organic matter which is utilised in the higher food chain by other organisms. The physiological stress in phytoplankters is considered as one of the factors responsible for higher percentage of liberation of extracellular organic matter. Thus the phaeopigment concentration is much higher in stored sea water going upto 254 mg m^{-3} from a minimum of less than 1 mg m^{-3}, while the range is smaller upto 45 mg m^{-3} in fresh sea water. The maxima for both coincide in September suggesting that the chlorophylls that are synthesised during monsoon production, disintegrated to form the large mass of phaeopigments. Though the exact role of phaeopigments in the nutrition of culture organisms in not known fully, it can be inferred that the abundance of the phaeopigments is an index of a larger volume of detrital organic matter in the ambient water.

The DOM in sea water is used by the bacterioplankton and thereby returning to the particulate phase of the food web. This is of great ecological significance, as the stored sea water which is used for culture experiments would be able to support the growth of living organisms, in spite of the absence of active photosynthesis and primary production, especially during the initial stages. In natural environments, the DOM and its uptake and transformation by bacterioplankton forms an important pathway in the carbon cycle.

The results of this study indicate that POM is of a higher order of magnitude in fresh sea water than in stored sea water. The seasonal variability exhibited in the magnitude of particulate organic fraction is combined effect of photosynthetic activity which is generally higher in the coastal regions during the monsoon period. This is also the period when higher rate of precipitation carries larger amount of detritus and nutrients into the coastal regions due to discharge from rivers. A higher rate of particulate matter production along with land run off has resulted in the occurrence of higher magnitude of particulate fraction. In view of the increased availability of the utilization of the macronutrients, the instantaneous concentration of nitrite, nitrate and phosphate had been reduced in the ambient water, which perhaps explains the lower coefficient of correlation as compared to the levels of significance to which it should actually rise. The silicate fraction of the macronutrients indicate better relationships. This may be because of the fact that sampling was nearshore, where, due to the wind and wave action the supply of this nutrient exceeds the removal. Otherwise, all the factors governing the primary productivity in fresh sea water shows a positive correlation.

The higher PER observed in stored sea water may be attributed to the passive release of DOM by the disintegration of algal cells. In the recent studies, conducted by other workers, using diaflo membranes and fractional filtration techniques, it was shown that the smaller molecular weight fractions are more than the higher molecular weight fractions. This suggests that DOM with lower molecular weights are used by the heterotrophic bacteria, while the larger compounds will be removed at much smaller rates. So it can be concluded that in freshly collected sea water, the POM which is abundantly found, will be utilized by the hatchery produced larvae for their food which will be followed by the dissolved organic fraction converted by the heterotrophic bacteria. Further since the dissolved oxygen concentration is generally higher in fresh sea water compared to stored sea water, it is suggested that freshly collected sea water will be more beneficial for survival of newly hatched

larvae, whereas for the postlarvae and further stages stored sea water will not make much significant difference due to the heterotrophic uptake and transformation of the DOM available which made in the food chain, restricting the need for any additional input in the form of artificial or live feeds.

In conclusion, as DOM and POM were found to be more in fresh sea water than in stored sea water, it is inferred that freshly collected sea water is more beneficial for survival and growth of the newly hatched larvae, whereas for the postlarvae and subsequent stages stored sea water will not make much significant difference, rendering the required energy through heterotrophic uptake.

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STUDIES ON N-P-K RATIOS IN SOIL AND OVERLYING WATER IN SOME CULTURE PONDS IN RELATION TO PLANKTON BIOMASS*

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Introduction

The biological wealth of the culture ponds depends mostly upon the dissolved nutrients. The sediments are the major source of various organic and inorganic compounds entering the water. The nature and composition of the bottom sediment is one of the factors influencing the productivity of the culture ponds. The sediments store nutrients and control mineralisation of organic deposits at the bottom. Thus the nutrient status of both soil and the overlying water play the most important role in the production of the planktonic organisms in the fish ponds. The overall productivity of the ponds depends on the fertility of the bottom soil. The production of the plankton is mainly based on three major inorganic nutrient sources *viz.*, Nitrogen, Phosphorus and Potassium (N-P-K) of which Phosphorus is the major element regulating the phytoplanktonic growth in culture ponds. Nitrogen probably may be the limiting factor in brackishwater ponds.

The progresses made in the earlier studies have given ideas regarding the distribution of mud phosphate in the Cochin Backwater varying from 258 to 1320 g/gm of mud with a peak in the monsoon. The qualitative study of the phytoplankton, phosphate and nitrate showed that the rain washing of the southwest monsoon influences the phytoplankton production. The qualitative and quantitative studies on the phytoplankton of the Cochin Backwater described about 120 species of phytoplanktonic community occurring in the estuary. Time

*Prepared by the Editorial Committee.

Protein estimates were carried out by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard. (ii) Spectrophotometric method (Neilands, 1985. In : P. S. Colowick and O. N. Kaplan (Ed.) *Methods in Enzymology.* pp. 449-454).

The activity of LDH (DPNH produced) has estimated in the eye and skeletal muscle tissues of fingerlings of *L. parsia* and *M. cephalus* by measuring the rate of appearance of DPNH at 340 mμ.

Results

a. *Acrylamide concentration* : The lactate dehydrogenase system was expressed with all 5 isoenzymes at acrylamide (A) and bisacrylamide (BA) concentrations of 7% and 4% respectively. At this level of A and BA the homopolymers A_4 and B_4 were of equal intensity and thickness. All the 3 heteropolymers, A_3B_1, A_2B_2, A_1B_3 were also of equal intensity and thickness. At the other combinations of A and BA the intensity, thickness and electrophoretic mobility (Rf value) of the 5 bands were affected.

b. *Concentrations of substrate and co-factor* : When Lithium lactate (LL) and NAD concentrations were held constant at 5 mg and 1 mg/ml of staining solution, the LDH system was fully expressed as described above. At other combinations of LL and NAD the number, intensity and thickness of bands were greatly affected.

c. *Duration of storage* : Band characteristics such as intensity, number, separation and distinction have been found to be greatly affected with increasing hours of storage and frequency of thawings. The result of storage study indicate that eye and muscle tissue samples stored upto 72 hours of storage study indicate that eye and muscle tissue samples stored upto 72 hrs and 48 hrs respectively, can be used for genetic variation studies without causing any error in interpreting the band.

d. *Genetic variation* : No significant genetic variation was observed within the 34 specimens tested for LDH locus. Minor

variation in Rf value observed were within the experimental error.

e. *Measurement of enzyme activity* : (i) Serial dilution method: In the case of eye tissue of *L. parsia,* the last visible band was observed in the 9th tube and for muscle in the 6th tube. For eye tissue of *M. cephalus* the last visible band was observed in the 12th tube.

Specific activity of LDH in the skeletal muscle tissue of *L. parsia* was less, when compared to that in the eye tissue of *L. parsia.* The specific activity of LDH in the eye tissue of *L. parsia* was greater than that in the eye of tissue of *M. cephalus.* (ii) *Spectrophotometric method* : Eye LDH of *L. parsia* and *M. cephalus* exhibited almost identical rate of reaction. The activity of skeletal muscle LDH of both species was not comparable. In both species, the activity of skeletal muscle LDH was higher than that of the eye LDH.

Discussion

In the present study, the differences observed in the resolution of LDH varying combinations of acrylamide and bisacrylamide, offers scope for detecting more number of alleles. It has also been observed that the homo and heteropolymers of locus A and B react differently to changing concentration of NAD and Lithium lactate. Also the response to increased NAD concentration was found to depend on the level of substrate. The response of individual isozymes to varying concentrations of substrate and co-factor would enable one to study the isoenzyme of interest, without involving separation procedures.

The occurrence of extra bands due to storage, will affect the interpretation of genetic data. Extra bands of LDH have been observed in the sera of sock eye salmon stored for long duration. But in the present study no new extra bands were observed. Only the disappearance of bands, the conversion of A_4 homopolymer to A_3B_1 heteropolymer was seen. A_4 homopolymer of eye LDH was observed to be more stable than B_4.

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STUDIES ON LACTATE DEHYDROGENASE ISOZYMES IN *MUGIL CEPHALUS* AND *LIZA PARSIA*

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Introduction

Biochemical genetic studies have made a substantial contribution towards efforts at preserving genetic resources of fish. They allow identification and description of genetic variations in different populations of a species. They thus provide the basis for development of appropriate sampling and preservation procedures for particular species. Among the polymorphic enzyme systems in fishes, Lactate dehydrogenase (LDH) has been extensively used as a genetic marker for the investigations of several biological problems. The LDH pattern has been used to confirm or redefine taxonomical groupings. Hybrids between species have been identified with the use of LDH. Since LDH is one of the highly polymorphic enzyme in fishes, this enzyme has been extensively used for identifying discrete stocks and separating mixtures into component stocks.

Objectives

As the work on LDH of Indian mullets is very limited, the present work was carried out to (i) determine the effect of different levels of gel cross linkage on the LDH system; (ii) determine the effect of different concentrations of substrate (Lithium lactate) and co-factor (NAD) on the resolution of LDH system; (iii) find out the effect of storage at -4°C on the stability of LDH bands; (iv) detect genetic variation at LDH loci in adult *Liza parsia* and (v) estimate and compare the activity of LDH in the tissues of *M. cephalus* and *L. parsia* by two different methods.

Materials and methods

Juvenile (65-90 mm) and adult (100-160 mm) specimens of *Liza parsia* and *Mugil cephalus* were collected from the Chinese

dipnet at Cochin Barmouth. Fingerlings (30-55 mm) of both the species were collected from Kerala Agricultural University Fishery Station at Puduvypeen.

Polyacrylamide disc gel electrophoresis method as described by Davis (1964, *Annals, New York. Academy of Sciences.* pp. 404-427) with the modification suggested by Parag (1993, *CMFRI Spl. Publ.*, 53 : 48-51) was adopted.

Standardization

a. *Gel crosslinking* : The lactate dehydrogenase system in the eye tissue of juvenile *Liza parsia* has been resolved in 16 varying combinations of acrylamide (5, 7, 9 and 10%) and bisacrylamide (2, 3, 4 and 5%).

b. *Concentrations of substrate and co-factor* : The effect of 12 different combinations of Lithium lactate (5 and 10 mg/ml) and NAD (0.05, 0.5, 1.0, 2, 3 and 5 mg/ml) on the expression of eye LDH of *Liza parsia* has been tested.

c. *Duration of storage at -4°C* : Eye and skeletal muscle samples of *Liza parsia* both as tissues and as extracts were stored at -4°C. The samples were periodically subjected to repeated thawings (upto 8 times - 840 hrs storage). The stored samples were then analysed electrophoretically for LDH isoenzymes and compared with the expression of fresh and once thawed tissue (24 hr storage).

d. *Genetic variation* : Adult specimens of *Liza parsia* were examined to detect the presence of alleles at both the LDH loci.

e. *Measurement of enzyme activity* : (i) Serial dilution method (Klebe 1975, *Biochem. Genet.*, 13 (11/12) : 805-812) involves two fold serial dilution of the enzyme sample to a visible end point, which is determined electrophoretically. The activity of LDH in the eye and skeletal muscle tissues of *L. parsia* and eye tissue of *M. cephalus* stored for 48 hrs in deep freezer has been tested by this method.

Protein estimates were carried out by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard. (ii) Spectrophotometric method (Neilands, 1985. In : P. S. Colowick and O. N. Kaplan (Ed.) *Methods in Enzymology*. pp. 449-454).

The activity of LDH (DPNH produced) has estimated in the eye and skeletal muscle tissues of fingerlings of *L. parsia* and *M. cephalus* by measuring the rate of appearance of DPNH at 340 mμ.

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In the present study, the differences observed in the resolution of LDH varying combinations of acrylamide and bisacrylamide, offers scope for detecting more number of alleles. It has also been observed that the homo and heteropolymers of locus A and B react differently to changing concentration of NAD and Lithium lactate. Also the response to increased NAD concentration was found to depend on the level of substrate. The response of individual isozymes to varying concentrations of substrate and co-factor would enable one to study the isoenzyme of interest, without involving separation procedures.

The occurrence of extra bands due to storage, will affect the interpretation of genetic data. Extra bands of LDH have been observed in the sera of sock eye salmon stored for long duration. But in the present study no new extra bands were observed. Only the disappearance of bands, the conversion of A_4 homopolymer to A_3B_1 heteropolymer was seen. A_4 homopolymer of eye LDH was observed to be more stable than B_4.

In *L. parsia* fingerlings of Puduvypeen area, the locus B in eye had been found to be polymorphic with two codominant alleles. But in the present study no polymorphism has been observed in both loci of the eye tissue of adult *L. parsia* collected from Vypeen Barmouth. This may be attributed to differences in the population size class studied.

Enzyme activity studies revealed that *M. cephalus* exhibits higher rate of activity for both eye and muscle. But that of muscle tissue is more pronounced. Though in the eye, two loci are present in both species, the muscle LDH activity is higher than that of eye LDH indicating that total enzyme activity is influenced by physiological role of tissues. A comparison of the two methods employed in the present study indicated that by serial dilution method it is not possible to detect significant variation between individuals of the same species as shown by the spectrophotometric method.

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OSMOTIC PRESSURE OF HAEMOLYMPH IN PREADULT AND ADULT *METAPENAEUS DOBSONI* ACCLIMATED TO DIFFERENT SALINITIES

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Introduction

Metapenaeus dobsoni (Miers) the most common penaeid shrimp occurring along the southwest coast of India and cultured extensively in Kerala, undergoes the spawning migration to the sea (by the adults) and come back to backwater (by the juveniles) and tolerate varied salinity conditions.

Adaptation of these shrimps to this changing environmental conditions is by means of a well established process the osmoregulation. This process is yet to be fully understood. An easy and reliable method of studying this process is by the estimation of the osmotic pressure. Though this outlines the process, a detailed study is usually done by the study of proteins which have been found to play a very important role.

Objectives

The present study was undertaken to understand the mechanism adopted by these animals to tolerate the environmental changes and also to estimate the isosmotic point of the animal and hence to determine the salinity most suitable for the prawn's existence so that it does not have to waste any energy on its adaptive mechanisms.

Materials and methods

The prawns were divided into three groups *viz.* preadults, adult females and adult males. The classification of the shrimps into preadults and adults was done based on the size at first maturity. The adult males and females were distinguished based on their external genitalia. These were then acclimated

under laboratory conditions to a salinity range of 0 to 45‰ for two periods *viz.* (i) one day and (ii) two days.

After each of this period the haemolymph was extracted for estimation of the osmotic pressure and protein content. The moisture content of the animal was found out by drying the animal overnight in an oven at 60°C. Hepatopancreas and muscle were utilised for estimation of the protein content and the ammonia excreted by the animal was estimated by utilising the water in which the animal was acclimated.

Standard procedures were utilised in all estimations. Statistical significance due to salinity and the effect of days of acclimation was confirmed by adopting the ANOVA two way classification.

Results

The variation in the osmotic pressure, moisture content, haemolymph, muscle and hepatopancreas protein and rate of ammonia excreted in the three groups of animals are given in Table 1.

TABLE 1. *Variation in different parameters of preadults, adult males and females between one day and two days.*

	Preadults		Adult females		Adult males	
	1 day	2 day	1 day	2 day	1 day	2 day
Osmotic pressure (Osm 1 Kg Water)	0.463-1.16	0.46-1.03	0.53-0.59	0.55-0.83	0.54-0.76	0.51-0.69
Moisture content (% body Wt)	71.73-77.40	70.95-76.03	72.46-77.03	70.66-77.32	75.04-77.35	74.65-75.75
Haemolymph protein (mg %)	71.38-160.00	68.54-140.15	100.15-316.32	155.20-238.10	148.66-236.33	131.00-218.66
Muscle protein % wet wt)	11.05-22.16	19.01-26.69	16.95-29.24	13.70-30.40	17.85-25.73	17.49-39.62
Heapto-pancreas protein (% wet wt)	7.08-18.53	7.85-14.96	5.80-29.84	10.75-15.46	14.47-19.51	12.91-22.51
Ammonia (Ug/L)	1.00-7.02	1.06-7.66	17.50-31.46	6.80-26.10	3.03-29.26	4.04-25.23

Conclusion

The results obtained indicated the presence of a well developed osmoregulatory capacity in this animal, by showing hyperosmoticity and hyposmoticity at salinities below and above the isosmotic point respectively. The results have also indicated a decrease in moisture content, ammonia and haemolymph protein content with rise in salinity whereas a direct relationship with salinity changes was observed in the case of hepatopancreas protein and muscle protein. This could indicate the mobilisation of the proteins from the tissues to the body fluids at lower salinities (salinities below isosmotic point) resulting in a corresponding increase in the blood serum peptide and protein material, the rapid deamination of which could lead to increased ammonia excretion. On the other hand at salinities above the isosmotic point, mobilisation seems to occur in the reverse direction *i.e.* from body fluids to the tissues thereby leading to a marked decrease in ammonia excretion and haemolymph protein level.

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INVESTIGATIONS ON FISH AND FISHERIES OF COCHIN BACKWATER

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Introduction

The Cochin Backwater supports a general sustenance fishery to a large number of fishermen. The backwater is popular for the stake net and Chinese dipnet fishing for commercially important prawns and finfishes. Castnets, gillnets, driftnets and dragnets are also widely used. The extended arms of Cochin Backwater form excellent sites for culture of prawns and fishes and the traditional paddy-cum-prawn fields are well known for centuries. The backwater forms a nursery ground for many of the commercial species of fishes and prawns suitable for culture. It also serves as a natural seed bank from where their postlarvae and juveniles are collected for stocking.

Large scale fishing operations here, inadvertently cause extensive destruction of the fish and prawns when they are undersized and uneconomical. The exploitation of seeds for stocking the culture ponds incidently results in heavy damage not only to the selected species, but also to others that co-exist.

Pollution also contributes to the destruction of fishery in the area. Heavy run-off of freshwater washing down insecticides and pesticides used in the vast stretches of agricultural fields, crowding by aquatic weeds like *Salvinia molesta* and *Eicchornia crassipes*, heavy siltation and periodic dredging interfere with the ecosystem.

Objectives

A knowledge on the fish is a pre-requisite for its management either in capture or in culture and it is scanty for

Cochin Backwater system. Therefore investigations on biology of important species were taken up and the results obtained are presented in this paper.

Materials and methods

Weekly observations on the fish landings at Pachalam, Vypeen, Fort Cochin, Thoppumpady and Thevara were made from February to October 1987. Experimental trawling was carried out with M. V. *Cadalmin* in the shipping channel.

Random samples of fish from these centres were taken for laboratory biological investigations. Daily landings were statistically estimated and raised for the month as follows:

The daily total (Yd) in respect of each type of unit was estimated as

$$Y_d = \frac{U}{n} \sum_{i=1}^{n} Y_i$$

where Y_i = catch of the ith unit,

U = Total number of units operated and

n = number of units observed.

The monthly total Y_m was estimated as

$$Y_m = \frac{D}{d} \sum_{i=1}^{d} Y_d$$

where D = total number of fishing days in a month and

d = number of observation days

The length-weight relationship was calculated using the formula, $\log W = a + b \log L$. Relationships in males and females were separately found and compared using F-test. When there was no significant variation a combined value for 'b' also was found out. The percentage distribution of the fishes in each length group is graphically represented.

Results and discussion

At Pachalam the gears were mainly dragnets (Koru vala) and driftnets (Ozhukku vala), at Thevara and Thoppumpady stakenets (Oonnivala) and at Fort Cochin and Vypeen dipnets (Cheenavala). Castnets have no fixed place of operation. The operation shift from place to place according to the availability of prawns and fishes and the catch are landed at the nearest centre.

During the period of observation (Fig. 1), the finfish formed 50% of the total catch in stakenets at Thevara, 46% in the stakenets at Thoppumpady, 45% in dragnets and 73% in driftnets at Pachalam, 82% and 83% in the dipnets at Vypeen and Fort Cochin respectively. An estimate of the fish landings in the backwaters *in toto* is difficult as a good part of the catch does not come to the landing centres. Monthly estimates at each place of observation only, were therefore attempted independently and results given in Fig. 2.

Stakenets are the important gears used in the backwaters. They are being used throughout the year for fishing and they contribute largely to the catches from the backwaters. During the present study the landings by stakenets were almost steady from month to month (Fig. 2). Similarly the dipnets are also being operated all through the year. Compared to stakenets and drag nets their yield is less. Fish dominated in these catches (Fig. 2). Monthly catches by dragnets showed wide fluctuations. Currently the catches were good during February-May and low during June-August. The driftnet operations are highly seasonal and their catches are comparatively poor (Fig. 2).

The fishes which constituted a major fishery were *Ambassis* spp., *Stolephorus commersonii, Leiognathus* spp., *Secutor insidiator,* Sciaenids, *Etroplus* spp., Catfish, *Trypauchen vagina, Cynoglossus* sp., Carangids, *Liza* spp., *Gerres* spp., *Sillago sihama, Hemiramphus* sp., *Thryssa* spp. and *Platycephalus crocodilus.* The species of fishes constituted a minor portion includes *Escualosa, Ehirava, Anadontostoma, Eleutheronema, Eutherapon, Lactarius, Sorotherodon, Pampus, Trichiurus, Anguilla* and *Austrobatrachus.*

Stray occurrence of *Elops saurus, Megalops cyprinoides, Scatophagus argus, Triacanthus* sp., young ones of *Rastrelliger kanagurta, Strongylura* and *Synaptura* were observed in the study. The

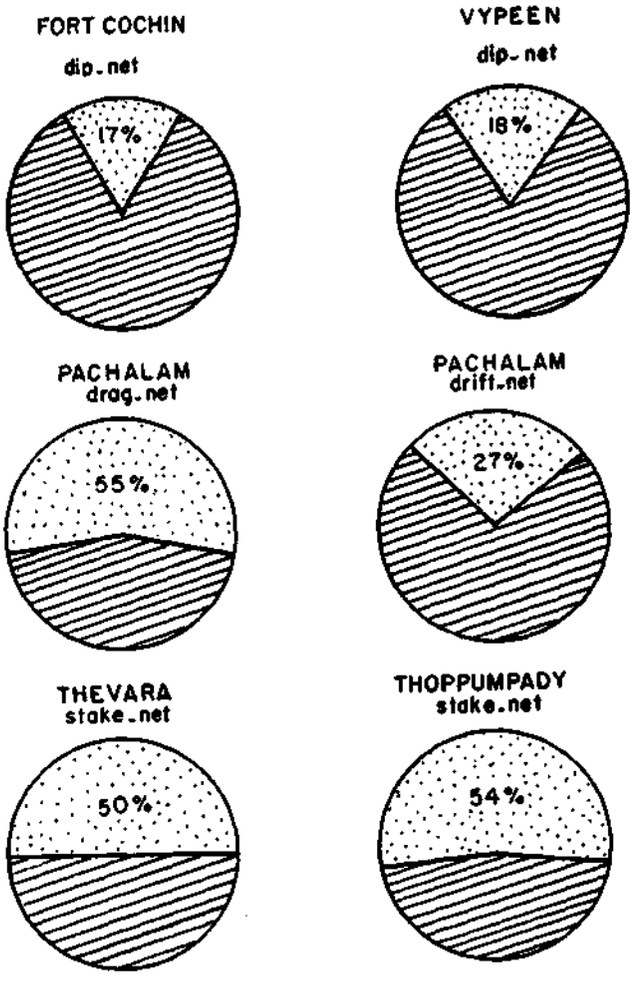

Fig. 1

fishes found in scant numbers in dipnets, with their months of occurrence in brackets are *Ctenochaetus strigosus* (July), *Sardinella*

longiceps (September), *Chanos chanos* (June), *Lates calcarifer* (June) and *Valamugil cunnesius* (July). Similarly the fish that occurred sparingly in stakenets, with months of occurrence in brackets are *Epinephelus* sp. (September), *Holocentrus rubrum* (October), *Siganus oramin* (March), *Cybium guttatum* (May), *Sphyraena* sp. (May) and *Tetradon* sp. (September).

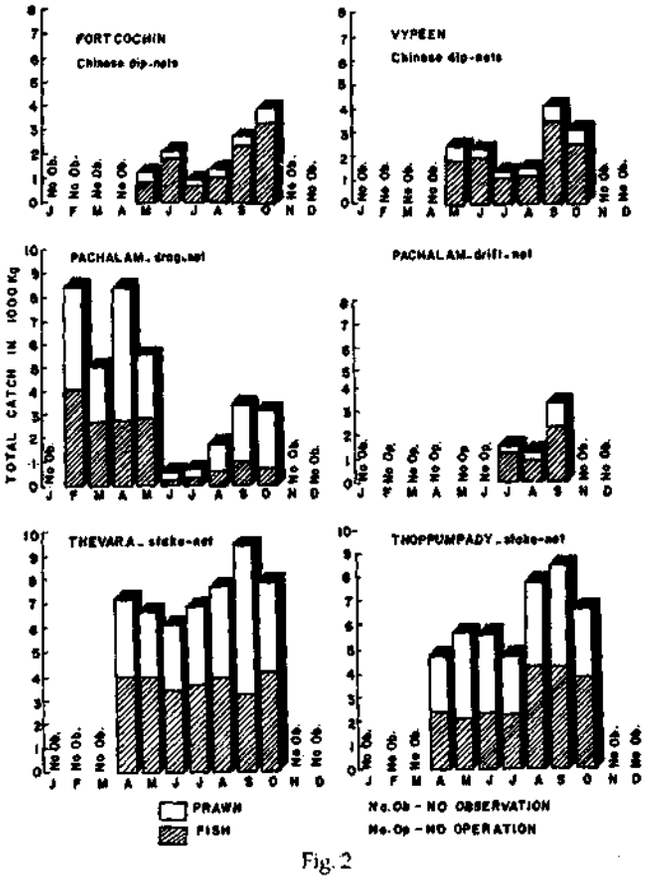

Fig. 2

Length distribution

In *Anadontostoma chacunda*, the mode at 70 mm in May shifted to 80 mm in July showing 10 mm increase in 2 months

time. During April-September (except July) *Stolephorus commersonii* ranging in sizes from 55 to 100 mm occurred. In July an insignificant percentage in 105-120 mm were also found. This species had faster growth as its mode at 65 mm in May moved to 80 mm in July. Widely separated size groups were peculiar to the catfish *Tachysurus* sp. in the fishery. *Liza parsia* also showed wide ranges in length during May-August with 110 to 210 mm of 10 mm size groups. Modal progression from 130 mm in June to 150 mm in July and 170 mm in August indicates 20 mm growth per month in this fish. *Ambassis gymnocephalus* with size range 40 to 80 mm occurred during March-September. There seems to be a regular 5 mm increase per month in its length with the mode at 45 mm in March reaching 70 mm in August. In May *Leiognathus bindus* ranged in size from 35 mm to 70 mm with mode at 50 mm. The species appears to be a fast growing one as the mode at 50 mm in June seems to have advanced to 80 mm group in September. The length distribution as collected in this study indicates continuous recruitment of *L. brevirostris* into the fishery.

Occurrence of *Trypauchen vagina* was continuous from April to September. The shifting of modal sizes indicates the fish to grow 30 mm, from 65 mm in May to 95 mm in August showing 10 mm increase per month.

Length-weight relationship

Among 14 species available in sufficient numbers to attempt length-weight reltionship, *Anguilla* sp. and *Leiognathus bindus* were available in smaller numbers and hence the length-weight relationship of their male and female could not be studied separately. The species thus studied for their length-weight relationships are given below:

Esculosa thoracata	log W=	-3.6002 + 2.2913 log L
Anadontostoma chacunda	-do-	=	-5.9555 + 3.5576 log L
Stolephorus commersonii	-do-	=	-5.6658 + 3.3296 log L
Anguilla sp.	-do	=	-5.9848 + 2.7849 log L
Liza parsia	-do-	=	-5.1603 + 3.0773 log L

Ambassis gymnocephalus	-do-	=	-4.5572 + 2.7563 log L
Gerres filamentosus	-do-	=	-3.5104 + 2.7316 log L
Secutor insidiator	-do-	=	-4.9883 + 3.0793 log L
Leiognathus bindus	-do-	=	-3.7922 + 2.4685 log L
L. brevirostris	-do-	=	-4.5710 + 2.8525 log L
Johnius belongeri	-do-	=	-4.1027 + 2.6409 log L
J. carouna	-do-	=	-5.7229 + 3.0133 log L
Trypauchen vagina	-do-	=	-4.5512 + 2.6261 log L
Cynoglossus sp.	-do-	=	-4.8747 + 3.0583 log L

Sex ratio

In most of the species the sexes were almost equalin proportion and shown below:

Species	Male (%)	Female (%)
Escualosa thoracata	47.6	52.4
Anadontostoma chacunda	50.8	49.2
Stolephorus commersonii	41.1	55.9
Liza parsia	33.3	66.7
Ambassis gymnocephalus	56.0	44.0
Gerres filamentosus	47.1	52.9
Secutor insidiator	40.8	59.2
Leiognathus brevirostris	47.8	52.2
Johnius belangeri	45.6	54.4
Johnius carouna	64.6	35.4
Trypauchen vagina	48.3	51.7
Cynoglossus sp.	33.3	66.7

But in *J. carouna* the male : female ratio was about 2:1, whereas in *L. parsia* and *Cynoglossus* sp. *vice versa.*

Gut content

The guts of fishes which occurred abundantly during the study were analysed for contents. *Johnius* spp., *Cynoglossus* sp., *Leiognathus brevirostris* and *Trypauchen vagina* feed mainly on benthic organisms, whereas *Escullosa thoracata, Anadontostoma chacunda, Stolephorus commersonii, Ambassis gymnocephalus* and *Sillago sihama* on planktonic organisms. In *Liza parsia* copepods were dominant. In catfish, crustaceans, mainly squilla, prawn, amphipods and Alima larvae were the major constituents. Remains of crustaceans were also found in good quality. Polychaetes and molluscs occurred in lesser number. Algal matter and sand particles were incidentally present along with the food.

Conclusions

As evident from the present investigations, the Cochin Backwater has a rich finfish and shellfish resource. At present the undersized ones caught in the commercial gears are discarded, without being put to any use. A step is to be taken in this context for the proper management measures regarding the mesh sizes of the different gears. Though the stakenets are licensed, they catch even very small fishes and prawns causing waste in the resource and fishery. This leads to the situation where the fishes are caught before attaining commercial size and are not given sufficient period to an optimum size, when they are ready to be caught.

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STUDIES ON THE MOVEMENT OF CULTIVABLE PENAEID PRAWNS IN RELATION TO DEPTH AND TIDE AT THE COCHIN BARMOUTH REGION

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Introduction

Juveniles of most of the cultivable penaeid prawns of the southwest coast of India are caught in large quantities from shallow brackishwater area, before they migrate to the sea for maturation and breeding. Some studies have been carried out on the fluctuations in the catches of juvenile prawns from the Cochin Backwater with reference to rainfall, tidal flow and lunar periodicity, and on their migration. The understanding of these aspects will definitely help in planning and formulating the strategies to collect prawn seeds for culture practices.

Objectives

As little is known about the biology of the juvenile penaeid prawns and their emigration from the Cochin Backwater, a detailed study was undertaken, on emigration of juveniles of cultivable penaeid prawns in relation to depth, tide and some biological factors such as species composition, abundance, size, sex, etc.

Material and methods

The study was conducted from February to September 1987 near Cochin Barmouth, the major outlet of Cochin Backwater. Samples were collected from the tidal pass, fixing a distance of about 1.3 km just outside the barmouth (from buoy No. 6 to 8) for trawling. Trawling was done adjacent to the shipping channel, at a depth of 6 to 10 m by M. V. '*Cadalmin*' (42.7 footer) of the Central Marine Fisheries Research Institute. A bottom trawl net and a surface trawl net of the same

dimensions with a cod end mesh size of 8 mm and a vertical mouth opening of 4 m were used for collecting prawn samples. The latter was fabricated by modifying the high opening midwater trawl net.

Trawling was done against the current during day and night low tides and day time high tide (2 to 3 hours prior to the peak hour of each tide) on full moon and new moon days, and first and last quarters. Samples collected were analysed for species composition, abundance by weight and number, size frequency distribution of male and female prawns, sex-ratio and maturity stages.

Results

The average catch per haul of prawns from bottom during low tide (day) was 1.299, 1.515, 0.182 and 0.377 kg during June, July, August and September 1987 respectively. Catch from the surface waters was much less when compared to that of bottom, the average catch coming to only 0.018 kg per haul. Average catch per haul of prawns (0.417 kg) from bottom during high tide was comparatively lesser than that obtained during low tide. The trend of decrease was more pronounced in the catches obtained from surface, during high tide.

Penaeus indicus, Metapenaeus dobsoni, M. monoceros and *Parapenaeopsis stylifera* contributed to the penaeid prawn fishery of this region. Stray occurrence of *P. monodon, P. semisulcatus* and *M. affinis* also has been recorded. A study of the catches obtained from the bottom during low tide shows that *M. dobsoni* which predominates the stake net catches from Cochin Backwater contributes to a lesser percentage when compared to *P. indicus*. However, the trend is just the reverse in the surface trawl catch. A comparison of these two species by number seems to be even more interesting. Whereas 19.2% of the total catch of *M. dobsoni* came in the surface haul and 80.8% in the bottom haul, only 5.3% of the total catch of *P. indicus* came in the surface haul, 94.7% coming in the bottom haul.

A significant observation made during the study was that the catch of *P. indicus* was much more during high tide than that during low tide. Catch per haul of the species almost doubled from 0.91 kg during low tide to 0.335 kg during high tide. Percentage composition by weight also increased from 32.8% of the total catch during low tide to 91.6% during high tide. Equally interesting was the decrease in the quantity of *M. dobsoni*.

Not much difference was noticed in the abundance or species composition of the catches during day and night. However the surface trawl catches were slightly better during night. Average catch per haul during low tide on the full moon and new moon days were 0.581 kg and 0.183 kg respectively. Catches were found to be comparatively better (1.123 kg/haul) during the first and last quarters. *P. semisulcatus* which occurred in very small numbers during March-June, disappeared totally in the following months presumably due to the advent of the southwest monsoon and the resultant drop in salinity. *P. stylifera* was well represented in the catches especially during low tide. The mean sizes of males and females of the species were found to be 73.4 mm and 75.1 mm respectively.

Mean sizes of *P. indicus* and *M. dobsoni* obtained in the bottom and surface hauls during low and high tides indicated that the mean lengths of prawns caught were more or less similar between water depths. However, the mean sizes of male and female *P. indicus* obtained during high tide were higher when compared to the sizes obtained during low tide. Sexes were found to be more or less equally distributed with a slight predominance of females. The catches were almost entirely constituted by immature prawns.

Discussion

The catch per haul of prawns during June-July was found to be higher than that obtained during the other months. It is interesting to note that this period coincides with the commencement of the southwest monsoon and the associated physico-chemical changes in the environment. Earlier researchers also have reported similar instances of rise in the catch of prawns during this period.

The present study shows that prawns which are usually bottom dwelling forms, are moving at least in the tidal pass through the column of water. However, the number caught from the upper column of water was much less, the catch per unit of effort increasing significantly from the surface to the bottom. A similar observation has been reported in the case of *P. setiferus* also.

The higher percentage of *M. dobsoni* in the surface trawl catch when compared to that of *P. indicus* may indicate more movement of the former through the column of water. The predominance of *M. dobsoni* in the stake net catches from the backwater of this area is worth mentioning here. Unlike the trawl net which sweeps the bottom, stake net when set in position against the current, filters the water column with the lower border of the mouth just touching the bottom or remaining only close to it.

The catch per haul obtained during high tide was lesser than that obtained during low tide. It is likely that prawns which emigrate to the sea try to avoid the tidal flow in the reverse direction. Similar instances of juvenile prawns resisting displacement during flood tides by staying on the bottom or along the sides of the canal and exhibiting short hopping movements have been reported in other penaeid species also. The significant increase in the quantity of *P. indicus* obtained during high tide when compared to that of low tide is not noticed in any of the other species. A similar observation has been made in the Godavari Estuarine system where "immigrants" (catch during high tide) are found to be poorer than emigrants with the exception of *P. indicus*. This may indicate the influence of the incoming tide on the population of *P. indicus* which incidentally, is the only species known so far to perform long distance migration in the Indian waters.

Size-frequency distribution of *P. indicus* and *M. dobsoni* shows that the catches are predominated by late juveniles or subadults. Sizes are found to be more or less similar between water depths. Sexes are almost equally distributed with slight

predominance of females, ruling out thus the possibility of sexual seggregation during movement. The higher mean size obtained and the stray occurrence of maturing females of *P. indicus* in the high tide catches may indicate the possibility of at least some of the prawns being carried back or swimming back along with the flood tide. The good catches of *P. stylifera* which is a marine species and is reported to move to the 41-60m depth zone during the southwest monsoon period may indicate the presence of a residual fishery of the species in the shallow inshore waters.

Catches obtained during the first and last quarters of the lunar phase were comparatively better. As the information already available on the influence of lunar phases on prawn catches from this region is based on catches obtained using stake nets, the operation of which itself is based on the tidal periodicity, it is desirable to conduct more experimental studies on this aspect.

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EFFECT OF OCCURRENCE OF CLAMS ON THE GROWTH OF PRAWNS IN CULTURE FIELDS

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Introduction

Traditional prawn culture is being practised in three different types of ecosystems namely seasonal fields, perennial fields and coconut groves, of which the first two are the most important in Vypeen Island in Kerala. The adjacent Vembanad Lake supports a rich fishery of clams mainly constituted by the black clam *Villorita cyprinoides* var. *cochinensis*. Recently, there has been a report by the farmers on the occurrence of clams in the perennial fields of this area. Further, it has been reported that the occurrence of these clams result in a reduced production of prawns since the prawn culture is an important occupation and plays a major role in the economy of the island, this problem has attracted public interest and found a place in the news paper columns.

Objectives

Inspite of the voluminous literature on various aspects of prawns and clams, there is no published data available on this aspect. Therefore, the present work was undertaken to find out the reality, if any, behind this reported reduced production of prawns due to the presence of clams in culture fields. The experiment was designed to find out in what way the clams are affecting the growth of prawns, so as to lead to further research works in this line to overcome the problem and to enhance the income of the farmers.

Material and methods

Prawns were cultured in two pens made of Velon screen, in the perennial fields at Edavanakkad in Vypeen Island. In one

of the pens, prawns *Penaeus indicus* were cultured alone and in the other they were grown along with clams for a period of three months, without supplementary feeding. Fortnightly sampling, both in the surface and bottom waters, was made to study the various hydrographical and ecological parameters. In addition to this, the food and feeding habits of the prawns and the substrate selectivity of clams and its relation with other benthic animals were studied.

Table 1. *Average fortnightly values of different parameters in the culture ponds for a period of three months*

Parameter	PS	CS	PB	CB
Dissolved oxygen (ml/1)	3.29	3.21^{NS}	2.97	2.72**
pH	8.16	8.15^{NS}	8.26	8.15*
Ammonia-nitrogen (µg-at/1)	3.14	3.37*	4.32	7.05**
Nitrite-nitrogen (µg-at/1)	0.72	0.80^{NS}	0.78	1.09*
Nitrate-nitrogen (µg-at/1)	3.50	3.65^{NS}	3.18	4.59**
Reactive Phosphorus (µg-at/1)	7.15	7.15^{NS}	7.65	7.54^{NS}
Calcium (ppm)	135.71	135.14^{NS}	139.90	124.30**
Primary production † ($gC/m^3/day$)	1.72	1.62	0.98	0.45

* Significant at 5% level.

** Significant at 1% level.

NS Non-significant

PS Surface waters of the pen with prawns alone

CS Surface waters of the pen with prawns and clams.

PB Bottom waters of the pen with prawns alone

CB Bottom waters of the pen with prawns and clams.

† Statistical analysis was not carried out due to the less number of observations (monthly observations).

Results and discussion

The culture experiment showed that the growth of prawns was adversely affected by the presence of clams. The prawns with an average initial size of 34.65 mm attained average lengths of 102.0 mm and 93.6 mm when cultured alone and along with

clams respectively, within a period of three months. Among the parameters studied the phosphorus levels were found to be unaffected, whereas there was an increase in the levels of ammonia, nitrite and nitrate-nitrogen, while there was a decrease in the levels of dissolved oxygen, pH, clacium and primary production, due to the presence of clams (Table 1). Temperature and salinity exhibited the usual seasonal pattern during the study period.

There was a mixing-up of water between the pens which can be concluded from the highly significant difference of various parameters in the bottom waters compared to that in the surface waters. Though these differences were statistically significant, confirming that it is due to the clams, the actual differences are not to any considerable extent (Table 1). Therefore, these parameters cannot be considered as the major factors which affected the growth of prawns.

Studies on the food and feeding habits showed that the prawns did not consume the clams to any considerable extent (Index of preponderance 0.39), inspite of their occurrence in abundance. Substratum studies revealed an inverse relation between clams and other benthic animals which form the food of prawns and also the preference of clams towards a sandy bottom. These results indicate that the observed reduction in growth of prawns during the present study is possibly due to the lack of natural food available to them, since they were solely dependent on natural food as there was no supplementary feeding.

Polyculture and better management possibilities with supplementary feeding of prawns and introduction of genetically improved varieties of clams are suggested to enhance the income of the farmers.

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SIZE DISTRIBUTION AND ABUNDANCE OF CULTIVABLE PENAEID PRAWNS IN COCHIN BACKWATER DURING SOUTHWEST MONSOON

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Introduction

The estuaries and backwaters of Kerala are good nursery grounds for several species of prawns and play an important role in large scale prawn culture. Cochin Backwater system is well known for its traditional prawn farming and recently the traditional prawn cultivators are adopting scientific farming in the area. Hence an understanding on the availability, abundance, size distribution and seasonal fluctuation of seed and juveniles penaeid prawns in the region, is essential to collect seed and juveniles for culture practices.

Objectives

With the above idea in mind, the present study was undertaken to obtain first hand information on the availability, pattern of recruitment and distribution of cultivable penaeid prawn resources in the Ernakulam Channel of the Cochin Backwater system with special reference to their size, sex and relative abundance during the southwest monsoon (June to September) of 1987 in relation to physical and chemical characteristics of the environment.

Material and methods

The waterbody adjoining Cochin city between Vaduthala in the north and Thevara in the south was divided into three zones *viz.* north zone (Pachalam-Vaduthala), middle zone (Ernakulam foreshore) and south zone (Thevara).

The findings and conclusions are based on regular collections of weekly catch data on prawn landings from the

local landing centres of the respective zones, fortnightly data on hydrography and prawn seed and juveniles from the three zones, and monthly diurnal data on hydrography, postlarvae and seeds of cultivable penaeid prawns from the north and south zones. Zooplankton ring net with 50 cm mouth dia and 0.33 m mesh size was used for collection of postlarvae. Standard velon screen (VN) of 2 x 1 m and an experimental drag net (DN) of 50 x 40 cm mouth opening with a nylon bag net of 1.5 m long with 1 mm mesh were used for the collection of seed resources to cover each time an area of 10 m^2 and 2 m^2 respectively in the shallow areas. To keep uniformity in the operation of VN and DN, same persons were engaged throughout the course of field work. For the monthly diurnal studies, as far as possible, days with almost same tidal pattern such as the highest tide occurring in the afternoon were selected to facilitate comparison of data.

Seed and fishery data were treated separately. Seed was estimated and expressed in number per unit area of 100 m^2. Relative abundance of penaeid species was studied with reference to their number and weight. Specimens below 40, 35 and 35 mm in length for *Penaeus indicus, Metapenaeus dobsoni* and *Metapenaeus monoceros* were treated as seed and of size less than 55, 40, 40 and 45 mm respectively for *P. indicus, M. dobsoni, M. monoceros* and *Metapenaeus affinis* were treated as 'indeterminates' in the present study on sex distribution. Attempts were made to relate the abundance and distribution of seed and juveniles with local rainfall, depth, water temperature, salinity, dissolved oxygen, time and tide.

Results and discussion

Among prawns, penaeids contributed more than 90% and *M. dobsoni* was the dominant species in the entire ecosystem during this season, followed by *M. monoceros* in the north zone and *P. indicus* in the south zone. The penaeid seed resource potential was estimated as 1088, 644 and 304 Nos/100 m^2 for the north, south and middle zones in the order of abundance and on an average it was 680 seeds/100 m^2 with specieswise distribution of 616, 48 and 16 Nos/100 m^2 for *M. dobsoni,*

P. indicus and *M. monoceros* respectively for the entire ecosystem. *Penaeus monodon, Penaeus semisculcatus* and *Parapenaeopsis stylifera* together contributed less than 0.5% only. *M. affinis* seeds were exclusively absent during the southwest monsoon season.

An estimated penaeid prawn juvenile catch of 5,334 and 12,004 kgs from the landing centres of north and south zones for the season indicated the magnitude of fishery potential and the species composition being *M. dobsoni* (70.8 : 43.0), *M. monoceros* (15.9 : 27.9), *P. indicus* (10.3 : 25.0) and *M. affinis* (3.0 : 4.1) in percentage ratio by number and weight respectively in the Ernakulam channel. The ratio between number and weight may be used as an index to determine the size of juveniles in the population in space and time.

The absence of *M. affinis* seed and poor representation of juveniles during August-September indicate that the spawning season of *M. affinis* does not fall during the southwest monsoon season in the sea around Cochin, while in the case of *P. indicus* and *M. monoceros* the recruitment is meagre. The absence of larger sized juveniles in the shallow areas of less than one metre depth and their occurrence in the relatively deeper areas (>1 m depth) as evidenced from the prawn landings of the backwater confirm the movement of growing juveniles into deeper areas of the estuarine ecosystem; and the low percentage of larger size groups (Preadults) in the samples indicates their emigration to the sea.

The uneven size distribution and sudden occurrence/ addition of intermediate size groups in a particular fortnight or month in the fishery during this season, as observed in the case of *P. indicus, M. monoceros* and *M. affinis* may be attributed to their movement from upstream areas due to environmental stress by the monsoon rainfall. It is also evident from the progressive increase in the prawn landings from June to September, as observed from the increase in prawn catch data at north and south zones (3,397 and 8,185 kg respectively) during the later half of the monsoon season (August-September) which contributed 63.7 and 68.2% of the total monsoon catch (June-

September) in the respective zones. The representation of all size groups of *M. dobsoni* in the samples throughout the season may be related to its recruitment in higher magnitude and seggregation in the surveyed ecosystem.

The present study confirms the observations made by earlier workers in the area (Kuttyamma and Antony 1975, *Bull. Dept. Mar. Sci. Univ. Cochin*, 7 (3) : 503-510) on the dominance of females of *P. indicus*, *M. dobsoni* and *M. monoceros* and *M. affinis* in the ecosystem. It is also evident from the size distribution study of males and females of these four species that both the sexes are distributed in almost same size groups in the ecosystem.

Based on the progression of peak modes, growth rate of *P. indicus* was estimated as 20 mm/month between 63 and 93 mm and that of *M. dobsoni* as 13 mm/month between 38 and 58 mm. This finding is very similar to the observations made by Mohamed and Rao (1971, *J. mar. biol. Ass. India*, 13 (2) : 149-169) and Kunju (1978, *CMFRI Spl. Publ.*, 3 : 34-39). Progression of modes was not clear in the case of *M. monoceros* and *M. affinis*.

From the data with the shortest time available for this study and the efforts made, it is clear that seeds of *M. dobsoni* prefer low saline areas and of *P. indicus* in relatively higher saline areas and prawn seeds are generally abundant in shallow waters, in the morning hours, at low temperature and also during high tide.

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LOW AMBIENT OXYGEN TOLERANCE IN FRY AND FINGERLINGS OF *CHANOS CHANOS* (FORSSKAL) AND *MUGIL CEPHALUS* LINNAEUS

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Introduction

Knowledge of factors affecting metabolic rate; growth and survival of fishes can be applied to the practices of fishery management and can also be used as a means of assessing the responses of the fishes to environmental conditions. The multiplicity of involvement between environment and metabolic rate illustrates that within the bounds of knowledge the factors of dissolved oxygen, salinity, temperature and activity exert the greatest effect on survival of the fishes.

In the present study the experiments were performed at ambient oxygen concentrations near air saturation down to the asphyxial level of oxygen (concentration at which the fish begin losing its equilibrium) of *Chanos chanos* (Forsskal) and *Mugil cephalus* Linnaeus which are always subjected to transportation from the collection area or hatchery to culture sites. Total metabolic rates (oxygen consumption; carbon dioxide production and ammonia excretion) of the fishes concerned subjected to ambient oxygen concentrations below air saturation in 15 and 30 ppt salinity at 30 and 35°C with concurrent measurement of breathing rate (opercular movement) were investigated.

Materials and methods

Fry and fingerlings of *Chanos chanos* and *Mugil cephalus* were collected from the nursery ponds of Kerala Agricultural University, Poduveypu, a fish farm about 5 km north of Cochin Barmouth. Sixtyone milkfish ranging in total length from 5.4 cm to 9.0 cm and in weight from 1.13 g to 6.64 g and 64 mullet ranging in total length from 3.9 cm to 9.5 cm and in weight from 0.56 g to 10.89 g were used in the present study.

Fishes procured for the study were maintained in 'Perspex' stock tanks of 200 l capacity containing sea water adjusted to 15‰ and 30‰ salinity. Continuous areation by compressed air through air diffusers and recirculation of water through biological filters were provided. The fishes were fed *ad lib* once daily with formulated feed made of beaten egg, potato starch and cod liver oil mixed proportionately by boiling into a custared.

Acclimation of fishes was effected in the acclimation tank containing filtered sea water adjusted to the required salinity and temperature (using thermostatic relay) for a minimum period of 10 days. Continuous aeration through air diffusers by aerators and a biological filtering unit as in the stock tank were provided in the acclimation tank also.

Owing to the limited amount of experimental medium, a recirculation system was designed to use the same water throughout the experiment. For recirculation, water from the ground level tank was lifted to the overhead tank by air-lift, sometimes mechanically.

Experimental fish were starved for 36 hr before experiment in the acclimation tank, then weighed, measured and left in the round bottomed closed respirometer overnight with recirculation of the experimental medium.

The experimental set-up consists of a recirculation system and respirometer assembly. Initial water samples (60 ml each) for analysis of dissolved oxygen, carbon dioxide and ammonia were collected and the circulation of water through the respirometer was cut off. The ambient oxygen gradually fell down to the asphyxial level due to the respiration of the fish itself. Once the fish begin to lose equilibrium which is the indication of asphyxiation, the final water samples were collected as previously. During the closure period, the breathing rate was counted visually by using a digital mechanical counter at an interval of 15 minutes subsequent to the loss of equilibrium of the fish. It was revived by flushing the respirometer with air-

saturated water from the overhead tank. Each experiment lasted for 3-4 hours.

The modified Winkler method was followed for analysis of dissolved oxygen in the water sample. The standard titrimetric method was followed for the estimation of free CO_2. To calculate the total CO_2, the conversion factor based on alkalinity was applied. Ammonia was measured spectrophotometrically using the phenol-hypochlorite method with a slight modification - Ethanol was substituted by methanol to avoid high blank optical density.

Results

The milkfish was found to have the maximum tolerance to low ambinet oxygen at the highest temperature and the lowest salinity tested *i.e.* at 35°C and 15‰. The same was the case in the grey mullet too in addition to a maximum tolerance in lower temperature (30°C) and higher salinity (30‰).

In *C. chanos*, the lowest ambient O_2 was observed when the condition of the fish was relatively lower, irrespective of temperature and salinity. But in *M. cephalus*, the asphyxial O_2 level was found to be dependent on temperature and salinity.

The fry and fingerlings of *C. chanos* were found to be more tolerable to the lowest ambient O_2 in 15‰ and 30‰ respectively, irrespective of temperature. In the case of *M. cephalus*, fingerlings were more tolerant to low oxygen than fry in all salinities and temperatures tested.

Between the two species tested, *C. chanos* alone showed decrease in breathing rate on exposure to low oxygen. This dichotomy in response (behaviour) on hypoxic exposure may have a major role in the survival of the species.

The changes in the rates of O_2 consumption, CO_2 output and NH_3 excretion at different temperatures and salinities are reflected in RQ s and AQs.

The RQ values are significantly above unity in both the species tested. The AQ increased along with RQ under low oxygen.

Discussion

The major observation in the present study is the finding of the asphyxial level which is thus - the low lethal level of oxygen below which the test animals cannot live.

Another major observation in the present study is that the decrease in ambient oxygen from air saturation down to the asphyxial oxygen concentration, can cause different behavioural responses, as indicated by the trend of activity - breathing rate in fishes.

In the present experimental conditions, it can be taken that the magnitude of the RQ s over unity under hypoxic conditions is indicative of the intensity of anaerobic metabolism *i. e.* the higher the RQ over unity, the higher the extent of anaerobic metabolism. It is assumed in the present study that the magnitude of AQ is indicative of relative protein utilisation by fish.

To study the effect of temperature and salinity on asphyxial O_2, breathing rate, metabolic rates and quotients under hypoxic condition, analysis of variance was carried out. Comparison of means of all these parameters were made using the LSD (Least Significat Difference) test. Within the framework, the discussion was made for both the species for each parameter.

Mean comparison analyses showed that the difference in asphyxial O_2 level between 15‰ and 30‰ at 30° and 35°C are highly significant in *C. chanos*, but in the case of *M. cephalus*, the significance at 5% level was found between 15‰ and 30‰ at 35°C only.

The difference in the tolerance limit to low ambient O_2 between the two species tested is species specific, as observed in earlier studies.

The trend in breathing rate at 15‰ and 30‰ salinities and 30°C and 35°C showed marked differences in *C. chanos* and *M. cephalus*. Between the two species tested, *C. chanos* alone showed decrease in breathing rate on exposure to low oxygen, whereas *M. cephalus* indicated the reverse trend.

The changes in the rates of O_2 consumption, CO_2 output and NH_3 excretion at different temperatures and salinities are reflected in RQ s and AQs. The RQ values are significantly above unity in both the species tested and clearly suggest that during the hypoxic phase, considerable amount of anaerobic metabolism has taken place, resulting in the release of extra CO_2. The AQ increased along with RQ under low oxygen, suggests a coupling of the increased NH_3 excretion and increased CO_2 output.

The results of the present study have thrown some light on low ambient oxygen tolerance in fry and fingerlings of milkfish and grey mullet, especially in view of its importance as cultivable warm-water fishes in culture practices.

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CHROMOSOME STUDIES ON *MERETRIX CASTA*

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Introduction

Clams form an important group of edible bivalves. Species of *Meretrix* are widely distributed in India. Though not cultivated at present in India on a commercial basis, they have a very high potential for culture. The shells of these species are used as raw material in lime and cement based indutries.

With the realisation of higher yields due to the application of scientific principles to practical aquaculture, there has been a growing awareness of the potential role of genetics in improvement and control of both growth and inbreeding.

Objectives

The scope of the present study was to select a number of methods for chromosome studies based on investigations on fishes, molluscs, etc. and try them on *Meretrix casta* in order to evolve a suitable method. Different modifications were also tried. The chromosomes of *M. casta* were studied for their diploid number and also to examine variations in the diploid number if any.

Materials and methods

The method of Bantock and Cockayne (1975, *Heredity*, 34 (2) : 231-245) was used with modifications. The animals were allowed to reside for 3-4 hours in a 0.04% colchicine solution containing algal suspension. The excised gill was treated hypotonically in 25% sea water (28 ppt) for 30 minutes. In another modification the excised gills were exposed to colchicine and cell suspensions prepared with different concentrations of acetic acid.

Gradual fixation was carried out in methanol : acetic acid (3:1). In some cases the gill was directly put into the fixative. The tissue was taken through four changes of fixative at 15 minute intervals and stored in fixative under refrigeration after a few hours at room temperature.

For slide preparation, small bits of the tissues were transferred to 25% acetic acid, allowed to stand for about 5 minutes and shaken vigorously till a cell suspension was formed. One or two drops of methanol were added to the cell suspension to retard further action of acetic acid. The suspension was dropped on slides heated to about 40°C. Each drop was sucked back into the pipette and the ring of cells deposited, heat dried. The slides were dried, stained in 2% giemsa, rinsed in tap water and dried.

Chromosome spreads were examined from 21 animals. The modal diploid number was found to be 38. It was observed in the maximum number of cases *i.e.*, in 36 metaphases. Another mode also was observed at 34 (2n) and was counted from 23 metaphases. The remaining diploid numbers observed seem to fall into the pattern of normal distribution. The lower diploid counts were due to aneuploidy *i. e.* loss of chromosomes. Differences observed in the morphology of chromosomes are due to the effect of colchicine exposure on the particular cell. A cell entering metaphase immediately after commencement of colchicine treatment would be subjected to a longer duration of colchicine exposure while a cell entering metaphase towards the later part of colchicine treatment would be less affected by colchicine treatment and would show less shrinkage of chromosomes. Morphology of chromosomes is revealed clearly when shrinkage is minimal.

Results and discussion

The constancy of diploid numbers at the family level or generic level has been a matter of continuous discussion among cytologists. Findings by various workers have thrown up evidence for both the view points. Even intraspecific and intra-

individual chromosome variations have been documented by earlier workers.

Earlier workers have referred to the constancy of chromosome numbers in the different families of bivalves. However this view does not find acceptance in the light of findings of Ieyama (1977, *Jap. J. Malacologia*, 36 : 25-28). Hence it is difficult to maintain that the modal number for the family Mytilidae is 2n = 38. It is necessary to obtain more evidence from other genera in the family (Ieyama 1977, *loc. cit.*).

In the present study, 38 was found to be the diploid number in the maximum number of cells. This is in agreement with the diploid numbers reported for other clams. However, a second mode was observed at 34 (2n) suggesting the existence of intraspecific variation in *M. casta*. Perhaps with a larger screening the mode at 34 may get obliterated. The present results however do suggest the possibility of variation within the species.

In conclusion, it may be said that indication of chromosome polymorphism in *M. casta* is of cytological interest and points to the possibility of intraspecific variations. The population cytology of *M. casta* requires further investigations in the light of the present evidence of autosomal polymorphism.

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BIOCHEMICAL ASPECTS OF OVARIAN MATURATION IN *LIZA PARSIA* (HAMILTON-BUCHANAN)

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Introduction

Liza parsia, one of the common grey mullets in the Cochin Estuary, constitutes a thriving fishery in estuaries and back-waters of India. This species by virtue of its high nutritive value, extreme tolerance limits to salinity and temperature and the ability to transform nutrients into fish flesh at minimum expense of energy, is considered as a full fledged source of culture.

For successful hypophysation for seed production to meet the requirements of the fish farmers, a proper understanding of the biological strategies, physiological requisitions and biochemical composition of parent fish is essential. Studies of biochemical composition of eggs can help us to assess the quality of the eggs and hence the quality of the emerging young ones. There is no published account on this aspect or very little is available. This work is therefore taken up, with a view to understand the pattern of mobilisation of energy sources during the process of maturation of gonad in *Liza parsia*.

Material and methods

The fish samples were collected bimonthly from February to September 1987 from the Cochin Barmouth area. The females, caught in the Chinese dip net operated during the hours of high tide every day, were studied. They had a length range of 115-236 mm. The fish after washing thoroughly were measured for their total length, standard length and weight. Maturity stages were determined based on macro and microscopic examination.

Tissues of muscle, liver and gonad were removed from the samples. Muscle tissue was taken from the base of the first

dorsal fin, care being taken not to include any skeletal parts. Weights of the whole liver and gonad were also noted to the nearest milligram. All the values of biochemical analyses are given in wet weight basis.

Biochemical analysis : Moisture content of the samples was determined by drying the flesh at 105.0°C to constant weight. For estimation of total protein, the method of Lowry *et al.* (1951, *J. Biol. Chem.*, 177 : 751-766) was followed, where trichloroacetic acid was used as deproteinizing agent. The supernatant was removed and used for glycogen analysis, the latter following the method suggested by Viles *et al.* (1949, *Analyt. Chem.*, **21** : 950) using anthrone reagent. Lipids were estimated by chloroform-methanol mixture in the ratio 2:1 v/v (Bligh and Dyer, 1959, *Can. J. Biochem. Physiol.*, 37 : 911). Cholesterol was estimated following Hestrins' method (1949, *J. Biol. Chem.*, **180**) and modified by Henley (1957, *Analyst.*, 82 : 286). To estimate carotenoid content, Olson's method (1979, *Nutrition Report International*, pp. 807-813) was followed where the chloroform stabilized with 0.75% absolute ethanol was used to extract the carotenoids. Ash content was determined by incineration of dry tissue to constant weight.

All the biochemical analyses except ash content analysis, were done 5 times and the values were pooled up to work out the standard deviation. Analysis of variance was also carried out to ascertain the possible difference in the contents of various parameters in the muscle, liver and gonad at 5% level of significance.

Liver index was calculated in specimens of different maturity stages using the formula :

$$\text{Liver index} = \frac{\text{weight of liver} \times 100}{\text{weight of fish}}$$

The average of the liver index values at each maturity stages was calculated.

Results

Five maturity stages were identified based on the size, shape and colour of the ovary (Graham, 1924, *Fish Invest. London,* 2 : 1-77) and the microscopic structure of the ova as suggested by Qasim (1973, *J. Biochem.*, 121 : 847-852) and adopted by Kurup and Samuel (1983, *Mahasagar,* **16** (3).

Stage I (Immature) : ovary pinkish, translucent and jelly like, the ova with no yolk deposits ranged from 26μ to 196.3μ. *Stage II* (Maturing virgin and recovering spent) : ovary slightly yellowish. Eggs with traces of yolk and had a diameter range of 26μ to 288μ. *Stage III* (Ripening Mature) ova becoming yellowish and opaque with deposition of yolk eggs with diameter range of 26μ to 560 μ. *Stage IV* (Ripe) : ovary deeply yellowish and has conspicuous blood vessels. Ova diameter ranged from 26μ to 694μ. *Stage V* (Spent) : ovary dark pinkish, flaccid, translucent and shrunken. Ova diameter ranges from 26μ to 650μ.

Proximate compsition

Muscle : Moisture content was in the range of 76.30 to 80.23%. The value decreased from stage I to III and thereafter showing an increase in stage V. Protein content was the highest in stage III being 18.0%. The concentration showed a declining trend in the succeeding stages reaching a lowest level of 15.72% in stage V. In stage I and II, the values were 17.30% and 17.15% respectively. Lipid content was the minimum in stage II (0.87%) and the maximum in stage III (2.54%) and it decreased thereafter to 1.92% in stage V. Glycogen content was comparatively less in the muscle with a range of 0.12% (stage III) to 0.20% only (stage II). From stage III onwards, an ascending trend was obvious. Cholesterol showed a pattern similar to that of glycogen with the values increasing from stage III (0.14%) to stage V (0.18%). Ash content generally showed an alternate increasing and decreasing pattern with the values ranging from 1.03% to 1.94% in stages V and IV respectively. Carotenoid content was at its minimum in stage V being 1.80μg/g with the

maximum values noticed at stage II (7.72 µg/g). The levels generally showed a steady decrease from stage II to stage V.

Liver : In liver, moisture content ranged from 68.06% in stage III to 76.42% in stage II. The level showed an alternative increasing and decreasing pattern. Protein content indicated an increasing pattern from stage II to stage V, except for a decrease in stage IV (16.06%). Lipid levels showed an equable decrease from 5.77% in stage I to 4.76% in stage IV. In stage V, the level showed a recovery reaching a level of 5.50%. Glycogen marked its peak level in liver. From a level of 2.12% in stage I to 0.79% in stage II, and thereafter increased steadily upto stage IV. Cholesterol content showed a pattern of continuous decrease from stage I to IV. Later the Cholesterol content increased to slightly higher values in stage V (0.79%). Ash content declined from stage I to II, shooting up again in stage III and declining thereafter. Carotenoid in liver showed almost a steady value around 10µg/g.

Gonad : Moisture content was found highly fluctuating in the gonad. The value showed a decrease from stage II (80.68%) to stage IV (56.77%). In stage V, the value recovered to 79.16%. Protein concentration increased steadily from 15.26% in stage I to 21.81% in stage IV, declining thereafter in stage V to 15.51%. Lipid followed a pattern similar to that of protein. The value increased from stage I (5.37%) to stage III (18.03%). Glycogen showed an increase from 0.43% in stage I to 0.93% in stage II and decreasing thereafter to levels of 0.12 and 0.11% in stages III and IV respectively. Stage V showed an increase upto 0.80%. Cholesterol level indicated a constant increase from 0.26% in stage I to 1.13% in stage IV, with the value decreasing to 0.53% in stage V. Ash content was at its maximum in stage I (1.77%) and minimum in stage II (1.16%). The value increased upto 1.30% in stage IV with a further decrease to 1.21% in stage V. Carotenoid content of gonad was found increasing from 3.20 µg/g in stage I to 12.05 µg/g in stage IV with a lag to 2.18 µg/g in stage V.

Liver index

The liver index increased gradually from immature stage (0.682 in stage I) to mature stage (1.500 in stage IV), declining thereafter to 1.130 in stage V.

Statistical analysis

Analysis of covariance showed that moisture and lipid level were not significantly different in various tissues. Protein and ash content level were highly insignificant. On the other hand, glycogen, carotenoid and cholesterol were found to show significant difference between various organs statistically at 5% level.

Discussion

Major body components like protein, lipid, cholesterol, glycogen, minerals, etc. undergo variations during the process of reproduction with an overall purpose of making the developing eggs self sufficient.

In the present study, protein levels in stage I and II in the muscle remains almost constant. This may be attributed to lesser drainage of protein during early development of gonad when the fish feeds actively supplementing adequate protein for growth. Protein content of the liver in the present case also appears drained in the early stages of development, while an immediate reflection of a corresponding accumulation in the gonad is not discernible, which may perhaps be due to its utilisation as soon as it is drawn. In stage III, protein in all the tissues appears to be at a high level indicating peak period of synthesis and mobilisation as gonad build up advances. In stage IV, when gonad is fully ripe, muscle and liver showed a decline with a corresponding all time high in protein level of gonad suggesting a withdrawal of protein for gonad build up. This has been substantiated in species like flounders (Sorvachev and Shatunovskii, 1968, *Po. Ekol. Treski. Servernoi Atlantiki*, pp. 133 - 143) and *Clarias batrachus* (Yagana Bano 1977, *Proc. Indian Acad. Sci.*, 85 B : 147-155). In stage V, there is an obvious fall in the protein level in the muscle and also in the gonad, while it

increases in the liver. It may be surmised here that, while the decline in the muscle protein is due to enormous tissue break down, that in gonad can be attributed to strains imposed by extrusion of eggs.

Liver as the site of protein synthesis during maturation has already been proved histologically in Ayu Fish (Aida *et al.* 1973, *Bull. Jap. Soc. Sci. Fish.*, 39 (11) and in flounders (Sorvachev and Shatunovskii 1968, *loc. cit.*). In the present study, the high protein level noticed in liver during stages I, III and IV further substantiates the probable role of liver in protein synthesis.

A close scrutiny of the data on lipid shows that this component is transferred from the liver to the gonad in the early stages of maturation and from muscle also during advanced stages, thereby following a pattern similar to that of protein.

Further, studies on the ovary of *L. parsia* indicated a parallel relation between protein and lipid thus making the view of Marias and Erasmus (1977, *Aquaculture*, 10 (1) : 75-86) positive that protein and lipid content contribute together for the development of the fish, showing an inverse relationship with moisture.

Glycogen content with a narrow range of 0.1 to 0.2% in the muscle did not show any definite trend. However, the liver which has a comparatively high glycogen content (0.34 - 3.75%) is found depleting during stages II and V, with a corresponding increase in the ovary substantiating the view of Fontaine and Hatey (1953, *Physiologia Comp. Oceanol.*, 3 : 37-52) that in female fish, the glycogen of the liver, the main carbohydrate store house is preferentially depleted.

A gradual accumulation of cholesterol in the ovary is evident from stage I through IV, with a corresponding descent in the liver. In the muscle, on the other hand, with an insignificant level of 0.1-0.2%, cholesterol does not appear to play and decisive role in germ building. Depletion of liver cholesterol to meet the physiological demand of gonad

development has already been established by Chaturvedi *et al.* (1976, *Matsya*, **2** : 16-18).

Carotenoid levels in muscle increased from stage I to II, thereafter declining upto stage V. Correspondingly, the gonad shows an increase in its carotenoid content from stage I to IV. This indicates that in muscle, the amount of carotenoid drastically decreased with advancement of maturation, thus substantiating the view of Kithara (1983, *Comp. Biochem. Physiol.*, B 76 : 97-101). Further, the gradation of pigmentation of eggs in different stages of maturity is so profound that it can be used as a reliable index for determining the maturity stages of fishes.

Ash content in the muscle increased from stage I to II with the corresponding increase in the gonad. This may be due to the fact that, in the early growing stages minerals like calcium and phosphorus get accumulated in the body for overall development. In stage III, the mineral content in the muscle shows a decline with a more pronounced increase in the liver than in the gonad. It may therefore be stated that in the gonad the accumulation is not reflected, probably due to the utilisation by the developing eggs, while in the liver, the high level obvious in stage III and a subsequent decline in stage IV and V indicate transfer of essential inorganic ions through the liver to gonad.

Moisture levels show a slight decrease in stage II and III in muscle thereafter increasing in the forthcoming stages. In liver, the moisture content is increasing in stage II with a decrease in stage III again to increase and remain constant in stage IV and V. Gonad water level shows a wider range of variation with an increase from stage I to II decreasing drastically in stage III and IV again to increase in stage V.

An inverse relationship of moisture with lipid, though obvious in all the three tissues is more pronounced in the muscle and gonad. A similar relationship is also discernible between moisture and protein in all the tissues and more evidently in liver and gonad. As protein is removed from the muscle, the water content rises steadily and hence can be used as a useful index of the state of depletion of fish (Love 1960 *Nature*, **185** :

692; 1962, *J. Cons. perm. int. Explor. Mer.*, 27 : 34-42). Another inverse relationship obvious in the gonad is between moisture and cholesterol and such a relationship is but natural as cholesterol forms one of the sterol component of the total lipid.

Liver index is an important indicator of the state of liver during maturation and liver weight is said to increase in the female until gonadal maturation has definitely started and then decline with progressive maturation upto the stage when ova are ripe (Krivobok 1964, *Von. Ikhtiol.*, 4 : 483-494). In the present study, liver index in various stages was found increasing upto stage IV and then decreasing in stage V thereby substantiating the above statement.

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COMPARATIVE STUDIES ON THE ECOLOGY OF BOTTOM MACROFAUNA IN SEASONAL AND PERENNIAL FISH PONDS AND IN ADJACENT BACKWATERS

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Introduction

The flora and fauna at or near the bottom or subbottom layer of aquatic ecosystem may be broadly considered as benthos. Though there are many studies on the benthic fauna and their interaction with the environment, spatial distribution, species abundance, seasonal fluctuation in coastal waters, estuaries and backwaters, only little informations are available on the comparative studies of macrobenthos in different ecosystems such as backwater and perennial and seasonal culture systems. The present study aims at the qualitative and quantitative abundance and ecology of bottom macrofauna in relation to different parameters. A comparative account on the faunistic composition and their seasonal distribution and abundance in perennial and seasonal culture systems, adjacent to backwater area is also attempted.

Material and methods

The present study was carried out at five different stations extending from Cochin Barmouth to Narakkal along the backwaters, station B I was situated at the confluence of the sea; station B II was in the marine zone of the backwater; station B III situated towards the southern side of Cochin; station B IV was near Bolgatty Island and station B V represented the region between Edavanakad and Narakkal. Samples were collected from two perennial fields and two seasonal fields. Out of the two perennial fields, one was experimental pond attached to prawn culture laboratory of CMFRI (Designated as NP) and the

other one was the traditional culture field at Edavanakad (EP). Two seasonal fields were located at Narakkal (NS) and Edavanakad (ES) respectively.

Water, sediment and benthos were collected fortnightly from March to September '87 except for the first fortnight of June. Macrofauna and sediment samples were collected using a vanveen grab. After separation by sieving the benthos, samples were preserved in 5% formalin with rose bengal (1 gm/l). Species diversity using 'Shannon diversity index' and biomass were determined for each station.

The sediment samples were analysed for organic carbon reactive phosphorus and grain size. Bottom water samples were analysed for salinity, dissolved oxygen and pH by using standard techniques.

Results

Temperature : In the backwater stations and culture fields temperature remained more or less same and the peak values were obtained during premonsoon and showed a declining trend during monsoon months. It ranged between 24.5 - 32.5°C. The biomass showed positive correlation with temperature in station B II and B III. In culture fields, the benthic fauna was not affected by seasonal changes in temperature. The data indicated that temperature around 28.2 - 32.3°C was found favourable for the growth and survival of benthos.

Salinity : Salinity also showed peak values during premonsoon months in all the stations. It ranged between 7.75-34.48‰ and 1.25-29.78‰ in backwater station and culture fields respectively. The correlation between biomass and salinity showed that 'r' values were statistically non-significant in backwater station as well as in 'Pokkali' fields, whereas in perennial fields this was highly significant.

Dissolved oxygen : All the stations showed low oxygen values during premonsoon and an increase during the onset of monsoon. Dissolved oxygen values were high during peak monsoon month (July) in all stations.

An inverse correlation between biomass and dissolved oxygen was obtained in perennial culture fields. Low dissolved oxygen content in the backwater station may be a major factor responsible for reduction in species diversity as well as their population density. But species like *Ancistrosyllus constricte* and *Diopatra neopolitana* were recorded throughout the period of study irrespective of the dissolved oxygen content indicating their adaptability to survive in very low oxygen content.

pH : It remained more or less same during premonsoon period, but with the onset of monsoon the values increased. The peak values raised from 8.20 to 8.35 in backwater stations and 8.8 to 9.2 in culture fields. The correlation between biomass and pH was statistically significant in stations B I, B II, B III, NP and EP. An inverse correlation between biomass and pH showed that alkaline medium was unfavourable for the growth of macro-benthic fauna, especially in perennial culture fields, where the values were high. During the regime of high pH (9.2) the benthic biomass disappeared completely from the perennial culture field (EP).

Sedimentology

Organic carbon : Relatively low values of organic carbon were recorded during premonsoon month, but with the onset of monsoon its percentage composition registered an increasing trend. The values were generally high *i. e.* 3.23 (B I), 3.81 (B II), 4.96 (B III), 3.40 (B IV) and 1.73% (B V) during the monsoon season. The highest value (4.96%) during July, with a total absence of benthic fauna, was noticed in station B III.

Culture fields also showed the same trend as that of the backwater. Higher values, 3.74 & 3.61% were recorded at NP and EP respectively in the months of August and July. The fluctuation was very high in seasonal ponds (1.8-9.5% in NS and 2.10-11.56% in ES). High organic carbon content in 'Pokkali' field is due to the decay of roots and stumps of paddy left to rot after harvest.

Biomass was found to be significantly correlated with organic carbon content at 1% level in EP, NP and B III, whereas in B I the relationship was significant only at 5% level. Polychaete population was significant only at 5% level. Polychaete population was significantly correlated with organic carbon only in station B III.

Reactive phosphorus : The values were more or less uniformly high during premonsoon months. But with the onset of monsoon, it registered a declining trend. Among the backwater stations, B III showed higher values (4.12-9.12
May. In perennial fields also the range was more or less same and EP showed the highest values (5.92
compared to NP (5.30
fields also showed the same trend with high values of 5.2 to 6.7

There was significant correlation between mud phosphate and biomass in station B I, B II and B III and in culture fields the relationship was non-significant. This indicated that macrofaunal population was affected by a fall in mud reactive phosphate probably through food chain.

Grain size : The station B I showed clayey sand sediment with 71.75% sand and supported only 42.56 gm/m^2 of biomass. Station B II with equal proportion of sand, silt and clay supported the biomass as high as 130 gm/m^2. The muddy (B IV) and thick clay (B III) substratum supported very poor faunal assemblage. In B IV & B III the molluscan population was only 2% and 0.6% respectively of the total population, which can be attributed to rich clay content of the substratum. The major component of sediment in NP was sand whereas in EP and in seasonal fields it was silty sand.

Inspite of the sandy substratum in B V and NP the polychaetes constituted about 42% and 32% respectively of the total population. The dominance of *Dendronereis aestuarina* in these stations indicated that it can thrive well in sandy substratum. This fact is further supported by the occurrence of same species in B I (clayey-sand substratum). Though the type

of substratum was similar in both 'Pokkali' fields (silty sand) there was a complete absence of polychaete and crustaceans in NS. This shows uneven distribution of macrobenthos in similar type of substratum.

The present study shows that more biomass was associated with fine sand with silt and clay, whereas soil with thick clay holds less biomass.

Bottom fauna

Polychaete : 17 species of polychaetes belonging to 12 genera were collected. The species composition in backwaters and culture fields showed marked differences. *Ancistrosyllis constricta, Diopatra neopolitana, Lumbriconereis* sp., *Dendronereis* sp., *Dendronereis aestuarina, Nereis cavifrons, Prionospio pinnata* and *Nereis chilkensis* were the dominant species in backwater stations. However with the onset of monsoon, their population density declined gradually. Other species of stray occurrence were *Nereis unifascinata, Perinereis cavifrons, Nereis* sp., *Heteromastidus bifidus, Notopygos* sp. and *Paraheteromastus tenuis.*

Polychaete species spectrum in the culture fields was narrow when compared to the backwater stations. In station NP *Dendronereis aestuarina* and *Notopygos* sp. formed the dominant species; whereas in EP *D. aestuarina, Perinereis cavifrons* and *Prionospio pinnata* were dominant. In station NS polychaete population was totally absent. But, *D. aestuarina, Heteromastidus bifidus* and *P. cavifrons* were the dominant species at station ES.

Crustaceans : This group also showed an increasing trend upto monsoon season and thereafter declined. Penaeid prawn seeds (*Penaeus indicus, Metapenaeus dobsoni, M. monoceros* and *M. affinis)* were available throughout the period of study in all stations. The dominant species recorded from the backwater stations were *Alpheus paludicola, Grandidierello gilesi, Apseudeus chilkensis, A. gymnophobia, Synidotea variegata, Cirolana fluviatilis.* Species like *Alpheus malabaricus, A. paludicola, Cirolana* sp. showed irregular distribution in some stations. *Milita* sp. were recorded from June onwards.

Very few species of crustaceans were recorded from perennial and seasonal culture fields compared to backwater stations. *Apseudeus chilkensis, A. gymnophobia* and *Milita* sp. were the common forms in stations NP, EP and ES, whereas no crustaceans were encountered in NS.

Molluscs : *Modiolus* was the dominant bivalve species in B I, B II and B III. Economically important species like *Meretrix casta* and *Paphia* sp. were also recorded in station B I. *Pandora* sp. occurred both in culture fields and backwaters. Settlement of bivalve was noticed with the onset of monsoon. Among the gastropods *Littorina* sp. occurred frequently in culture fields.

Others : Fishes like *Cynoglossus* sp. and *Anguilla* sp. were recorded in B I and gobids from ponds. Nematods occurred in large numbers from almost all stations except EP.

Biomass

Declining trend of biomass was noticed from barmouth station to upstream adjacent to culture fields. In almost all stations, except B III, high biomass was observed during premonsoon months. Highest values were recorded at station B II (130 gm/m^2). Comparatively low values of biomass were recorded in culture fields. It fluctuated from 2.25-34.2 gm/m^2 in perennial fields and 1.25-12.12 gm/m^2 in seasonal fields.

Species diversity

Polychaetes : The polychaete population showed a declining trend with the onset of monsoon. The maximum species diversity was noticed in station B I (H = 1.9360). There was varying species diversity in culture fields as the systems harbour only less numbers of species.

Crustaceans : Station B II showed the highest value (H = 1.9302) among the different backwater stations, whereas at NP and EP the values were 1.0549 and 1.0609 respectively.

The present study suggests that the species diversity and population density in culture fields were low when compared to backwater stations. The distribution and abundance of

macrofauna in perennial culture fields is primarily influenced by salinity followed by pH, dissolved oxygen and organic carbon. In seasonal culture fields biomass was mainly affected by organic carbon content. Salinity played only a secondary role. In backwater station near the barmouth, the faunal assemblage was affected by mud reactive phosphorus, pH and temperature, salinity indistinctly controlled the benthic population. Apart from physico-chemical conditions nature of substratum also played a vital role in the distribution and abundance of faunal assemblage.

Discussion

The behaviour, distribution and abundance of bottom fauna in space and time are influenced very much by the changing ecological parameters such as temperature, salinity, pH, dissolved oxygen, micro- and macro-nutrients and sedimentalogical characters. The hydrographical and sedimentalogical features of the presently studied backwater and culture systems, both seasonal and perennial, showed drastic changes with the onset of southwest monsoon. The low benthic biomass production and relatively low species diversity during south-west monsoon in the presently investigated areas indicated the possibilities of destruction of stenohaline species or their migration to adjacent sea, which is in agreement with the observations of earlier workers. This study revealed that the bottom water temperature and abundance of macro fauna had a positive correlation in backwater stations with seasonal temperature variations of 7 to 8
systems this relationship was nonsignificant statistically with seasonal temperature variation of 3.0 to 3.4
suggests that lesser the seasonal variations in temperature lower the correlation between temperature and biomass abundance.

The correlation between the biomass and salinity showed that the 'r' values were non-significant in backwater as well as seasonal fields, whereas in perennial fields this relationship was highly significant.

The dissolved oxygen content in the culture fields remained high throughout the period of study unlike in backwater stations; the values remained as high as 4.9 to 7.04 ml/l in the perennial fields, probably owing to effective mixing of surface and bottom waters. An inverse correlation between biomass and dissolved oxygen was noticed in the perennial fields. The decline in benthic biomass of the culture fields during monsoon, therefore, may not be due to dissolved oxygen content.

An inverse relation between biomass and pH showed that alkline medium was unfavourable for the growth of macro-benthos, especially in perennial culture fields. Srinivasan (1982, *M.Sc. Dissertation, CUSAT,* 96 pp) and Sugunan (1983, *M. Sc. Dissertation, CUSAT,* 76 pp) also noticed similar results from the culture systems.

The organic carbon content in bottom sediment showed seasonal variations. The values were high during July in Station B III, where polychaetes constituted 68% of the total population showing their affinity to substratum with high carbon content and low levels of dissolved oxygen. The correlation between biomass and organic carbon of the bottom sediment was significant in the perennial fields as well as in stations B III and B I. The study indicated that sediment with high clay content was found to hold maximum of organic carbon (station B III with 25.7% clay and 4.96% organic carbon). A similar relationship was recorded from the Vembanad Lake by Murthy and Veerayya (1972, *Ind. J. Mar. Sci.,* **1** (1) : 45-51). Sorokin (1978, *Mar. Ecol.,* **4** : 501-616) reported that when the organic matter ranged from 4-8% meiobenthic biomass was 50-200 gm/m^3, while the ecosystem with 1-4% of organic matter sustained higher biomass of 100-500 gm/m^3.

Higher values of mud reactive phosphate were recorded during premonsoon, but in monsoon its concentration decreased considerably. In the backwater stations the correlation with mud phosphate and biomass was significant; whereas in culture fields this relationship was non significant. Sugunan (1983, *loc. cit.*)

found positive correlation between meio-faunal population and mud reactive phosphate in culture systems.

The sediment type near the mouth (B I) was clayey sand with 71.75% sand supported only 42.56 gms/m^2 of biomass. Kurian (1969, *Int. natl. Symp. on Fertility of the Sea. Abst.*) recorded similar results from the same area. Whereas the station B II with 43.3% of sand and almost equal proportion of clay and silt supported the biomass of as high as 130 gm/m^2. The muddy (B IV) and thick clay (B III) substratum supported poor faunal assemblage.

Maximum biomass was associated with fine sand with equal admixture of clay and silt. Biomass as high as 130 gms/m^2 was noticed in backwater stations whereas in culture fields the maximum value was 34.2 gm/m^2. Kurian (1967, *Bull. Nat. Inst. Sci. India*, 38 : 649-659) and Ansari (1977, *Mahasagar*, 10 (3 & 4) : 160-171) recorded uneven distribution of macrofauna in similar types of substratum.

Species diversity declined with the onset of monsoon in backwater stations and culture fields. Species diversity of ploychaetes and crustaceans were more in the backwater than in the culture fields.

Besides physico-chemical environmental parameters, human intereference like dredging also was one of the factors responsible for changing benthic ecology as well as their species diversity.

MANUALS OF RESEARCH METHODS AND SPECIAL PUBLICATIONS ISSUED UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE, CENTRAL MARINE FISHERIES RESEARCH INSTITUTE, COCHIN.

*1. Manual of research methods for crustacean biochemistry and physiology. *CMFRI Spl. Publ.*, 7, 1981, 172 pp.

*2. Manual of research methods for fish and shellfish nutrition. *CMFRI Spl. Publ.*, 8, 1981, 125 pp.

3. Manual of research methods for marine invertebrate reproduction. *CMFRI Spl. Publ.*, 9, 1982, 214 pp.

*4. Approaches to finfish and shellfish pathology investigations. *CMFRI Spl. Publ.*, 11, 1983, 43 pp.

*5. Application of genetics in aquaculture, *CMFRI Spl. Publ.*, 13, 1983, 90 pp.

*6. Manual of research methods for invertebrate endocrinology. *CMFRI Spl. Publ.*, 14, 1983, 114 pp.

*7. Production and use of *Artemia* in aquaculture. *CMFRI Spl. Publ.*, 15, 1984, 74 pp.

*8. Manual on marine toxins in bivalve molluscs and general consideration of shellfish sanitation. *CMFRI Spl. Publ.*, 16, 1984, 100 pp.

*9. Handbook on diagnosis and control of bacterial diseases in finfish and shellfish culture. *CMFRI Spl. Publ.*, 17, 1984, 50 pp.

*10. Mariculture research under the Centre of Advanced Studies in Mariculture. *CMFRI Spl. Publ.*, 19, 1984, 109 pp.

*11. Water quality management in aquaculture. *CMFRI Spl. Publ.*, 22, 1985, 96 pp.

*12. A practical manual for studies of environmental physiology and biochemistry of culturable marine organisms. *CMFRI Spl. Publ.*, 25, 1986, 45 pp.

13. Theorems of environmental adaptation. *CMFRI Spl. Publ.*, 26, 1986, 50 pp.

14. A manual for hormone isolation and assay. *CMFRI Spl. Publ.*, 41, 1986, 46 pp.

15. Manual of techniques for estimating bacterial growth rates, productivity and numbers in aquaculture ponds. *CMFRI Spl. Publ.*, 42, 1986, 27 pp.

16. Nutritional quality of life food organisms and their enrichment. *CMFRI Spl. Publ.*, 43, 1987, 28 pp.

17. Mariculture research under the Postgraduate Programme in Mariculture, Part 2. *CMFRI Spl. Publ.*, 53, 176 pp.

18. Mariculture research under the Postgraduate Programme in Mariculture, Part 3. *CMFRI Spl. Publ.*, 54 (in press).

19. Mariculture research under the Postgraduate Programme in Mariculture, Part 4. *CMFRI Spl. Publ.*, 55 (in press).

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* Out of print.

PREFACE

The Centre of Advanced Studies in Mariculture commenced in 1979 at the Central Marine Fisheries Research Institute, Cochin under one of the sub-projects of the ICAR/UNDP Project on **'Postgraduate Agricultural Education and Research'**. It is now continued as a regular **'Postgraduate Programme in Mariculture'**. Under this programme, postgraduate courses leading to M.Sc. and Ph.D. degrees are offered in collaboration with the Cochin University of Science and Technology since 1980. The courses and syllabii are well designed to catalyse research and education in mariculture consisting of basic science, marine biology, coastal hydrography, physiology, endocrinology and cytogenetics of marine animals; a general fisheries programme introducing the students to the foundation of marine, brackishwater and freshwater fisheries, fisheries economics and administration, and fish and fishery biology; core programme on mariculture involving fish farm engineering technology and culture of finfishes, crustaceans, molluscs and seaweeds, management of mariculture and extension; and research methodology and preparation of dissertation on the basis of a short-term research projects.

There is ever increasing demand for Postgraduates in mariculture from this institute especially in the private sector aquaculture projects. The feed-back from the industry on their performance has been very encouraging. This is essentially due to their background knowledge in practical aspects of aquaculture which enables them to handle problems straightaway on the field. It is on record, the students occupy very high and key positions not only in leading aquafarms, but in all other Government organisations/agencies and research institutes as well. The research topics for their dissertations in partial fulfilment for the degree, are well identified in priority areas such as nutrition, physiology, pathology, genetics, reproductive biology and physiology, and ecophysiology of cultivable marine organisms, culture systems, etc.

The research results of the short-term projects carried out by the M. Sc. Mariculture students are very valuable and practical. Therefore it is felt the highlights of this work should be made available and utilised for further expansion of aquaculture.

The first part of the results was included in Special Publication No. 19, issued in December 1984. This Special Publication covers 38 topics investigated by the students of the third, fourth, fifth and sixth batches of the PGPM.

The students deserve all appreciation for their hard and sincere work to bring out useful results within the shortest time available. I thank my colleagues who have efficiently supervised and guided the students in their research work.

I place on record my sincere thanks to the Editorial Committee for their efficient screening, editing and printing of this Special Publication.

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April 1993.

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