CMFRI SPECIAL PUBLICATION
Number 56

MARICULTURE RESEARCH UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE

PART 5

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE,
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
DR. SALIM ALI ROAD, POST BOX NO. 1603, TATAPURAM - P. O.,
ERNAKULAM, 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 :

— provide adequate facilities to carry out research of excellence in mariculture/coastal aquaculture;

— 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.

Under the programmes of the PGPM at CMFRI, postgraduate courses leading to M. Sc. (Mariculture) and Ph. D. are offered in collaboration with Cochin University of Science and Technology since 1980.

Cover Photos by Shri K. Nagappan Nayar and Dr. C. S. Gopinadha Pillai.

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Published by : **Dr. P. S. B. R. James**
Director,
Central Marine Fisheries Research Institute,
Cochin - 682 014.

Citation : SUBHASH CHANDRA SONI AND P. VEDAVYASA RAO 1993. Pathological investigations in penaeid prawns. *CMFRI Spl. Publ.*, 56 : 9-14.

PRINTED IN INDIA
AT MODERN GRAPHICS, KALOOR, ERNAKULAM, COCHIN 682 017

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 Dissertations on the basis of a short-term research projects by the Junior Research Fellows and Theses by the Senior Research Fellows.

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/Theses 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 results of the research projects carried out by the Research Fellows are very valuable and practical. Therefore it is felt the highlights/summary of these works should be made available and utilised for further expansion of aquaculture.

The results, highlights, significant findings, etc. of the 29 research projects carried out by the Senior Research Fellows of the PGPM for their Doctoral degree are included as small summaries in this Special Publication No. 56.

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

Cochin - 682 014,
April 1993.

P. S. B. R. James
Director,
Central Marine Fisheries
Research Institute

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STUDIES ON THE ECOLOGY AND PRODUCTIVITY OF SALINE LAGOON

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Introduction

Considerable attention has been paid in recent years to study the ecology and productivity of the estuarine and connected inshore waters around India in order to ascertain the productivity of these areas for the utility of aquaculture practises. Very little information is available on these aspects in the coastal lagoons which are characterised by high salinity. In order to understand the suitability of such derelict saline lagoons for aquaculture practises, it is necessary to investigate their environment, nature of their fluctuations and the rate of productivity. With this in view, the investigations on the ecology and productivity of the coastal saline lagoon at Mandapam Peninsula along the southeast coast of India was taken up.

Material and methods

Pillaimadam Lagoon, located between 09°17'N to 09°17'48"N and 79°05'E to 79°8'24"E and situated along the Palk Bay coast of Mandapam, was selected for the present investigation. The lagoon is connected with the Palk Bay on the northern side and has a fresh inflow on the southern side. The period of study extended from July 1982 to June 1984. Five stations were located inside the lagoon and one station in the adjacent inshore area of Palk Bay, in order to carry out periodic investigations on the fluctuation in the ecological and productivity parameters.

Meteorological data pertaining to monthly mean, minimum and maximum temperatures, wind velocity, rainfall and relative humidity at this region were collected from the

Meteorological Department, Pamban. Samples for estimating hydrological parameters and nutrients were collected weekly, whereas estimation of primary productivity and analysis of sediment were carried out fortnightly. Using 0-50°C high precision thermometer, the air and water temperatures were recorded. Dissolved oxygen, salinity, soluble reactive phosphate, nitrate, nitrite and hydrogen sulphide contents of water were estimated following FAO (1975, *FAO Fish. Tech. Paper*, **137** : 1-238) methods. After determining the pH of water, *in situ* pH was calculated using the formula of Gieskes (1969, *Limnol. Oceanogr.*, **14** : 679-685). Ammonia was estimated following the phenol-hypochlorite method and the primary productivity by Light and Dark Bottle technique. The distribution and abundance of zooplankton were estimated for one monsoon season only during 1983-84. Zooplankton samples were collected with a cylindrically cone shaped nylon net with a mouth diameter of 0.5 m and mesh size of 202 µm. The duration of each tow was 10 minutes and the speed of the boat was kept constant at 2.5 knots/h. Samples were taken at 1900 hr, 2400 hr and 0500 hr with fortnightly intervals.

Total phosphorus and nitrogen contents of sediments were determined following FAO (1975, *loc. cit.*) methods. Eh of the sediment was estimated using platinum electrode. The method of Walkey and Black (1934) was used to determine the organic carbon content of the sediments. The total sulphide content of the sediments was estimated following the Standard Methods. A grab of 225 cm^2 gap and 15 cm high was operated five times to analyse the benthic macrofauna. In the laboratory, the animals were sorted out by hand picking and identified. Grain size analysis was done during premonsoon, monsoon and postmonsoon periods for two years in the upper layer of the sediment following "the sieving and pipette analysis" method to know the sand, silt and clay proportion. Core samples analysis was done once in two months. Organic carbon, total phosphorus and Kjeldahl nitrogen analyses were done following the procedures for regular analyses.

Four stations were selected to study the diurnal variations in the dissolved oxygen, surface and bottom salinities, soluble reactive phosphate, nitrate, nitrite and ammonia. The study was undertaken depending on the lunar cycle - full moon/new moon and also to cover three seasons *viz.*, premonsoon, monsoon and postmonsoon. The sampling was done from 0900 hr of one day to 0600 hr of the successive day at every three hours. The flora occurring more abundantly were collected and identified. Data were subjected to statistical analyses and correlations between all the ecological and productivity parameters were computed. In order to enhance the organic production of the Pillaimadam Lagoon, commercial N : P : K fertilizer was used. To reduce the autotrophic production d-glucose was used in combination with N : P : K fertilizer. Urea, superphosphate, potash and organic manure were also experimented with different concentrations and combinations. The experiments were conducted in three sets, of which two sets were on the polythene field enclosures and one set in the laboratory tanks.

Results and discussion

Maximum temperature of this region was experienced from March (32°C) to September (35°C). Southwest monsoon and northeast monsoon winds were observed to exert profound influence on the climatology of this region. The former is felt in summer months with an average speed of 15 to 22 km/hr while the latter blew from October to February and brought in monsoonal rains. Poor rainfall was usually felt in this region, with a record of 922.5 mm during the monsoon of 1982 and 1346.7 mm in 1983. Relative humidity was high during the monsoon periods, recording values from 77 to 87%. Freshwater supply into the lagoon was mainly due to the inflow of water during the monsoonal rains and sea water incursion was recorded during the northeast monsoon period. During the rest of the period, the barmouth usually remained closed, thereby implying the low stability of this ecosystem. Fluctuation in the level of water in the lagoon is chiefly dependent on the pattern of coastal circulation. Relatively high water level in the lagoon, recorded during the northeast monsoon period, was due to the

rise in the level of Palk Bay. During summer months, evaporation and seepage to the sea decrease the level of water in the lagoon, eventhough occasional innundations occur during lunar phases. The temperature of water was mainly dependent on the temperature of atmosphere and the level of water. During summer period, temperature of water varied from 27.0°C to 34.5°C and during northeast monsoon period from 24.0°C to 26.0°C. The positive correlation obtained during the present study between air temperature and water temperature explains the high values of the latter recorded in the summar period, and this is in agreement with the observations made by earlier workers.

During summer, the dissolved oxygen content ranged between 1.0 and 2.5 ml/l while during the northeast monsoon period its range fluctuated between 4.0 and 6.0 ml/l. The salinity of the lagoonal environment was found to be influenced by the freshwater inflow, seawater incursion and circulation during northest monsoon period. Salinity value, as low as 3‰ was observed during October-December. During summer period (April-August), hypersaline condition prevailed in the lagoon and salinity value recorded was more than 150‰. The variation in salinity in such lagoons is mainly dependent on the interaction of sea water and freshwater circulation. Ammonia and pH content in water showed positive correlation with each other ($r = > 0.67$). Soluble reactive phosphate, nitrate, nitrite and ammonia concentrations apparently showed an increase during the period of monsoonal rains. Nitrite concentration usually showed positive correlation with the nitrate concentration ($r = > 0.64$). The hydrogen sulphide content of water was low (< 3.5 µg-at H_2S-S/l) when active water circulation was observed in the lagoon. At Pillaimadam Lagoon, concentration of all the nutrients was observed to be maximum during the period of freshwater influx, suggesting that the loading of these elements in the ecosystem is from the freshwater source. The temperature of sediment showed positive correlation with the temperature of water ($r = > 0.73$). During northeast monsoon period the pH of sediment was comparatively more alkaline

(> 7.50) and Eh, though always evinced negative potential, was at comparatively high levels (> -100 mV). Organic carbon, total phosphorus and Kjeldahl nitrogen contents of the sediment increased during the northeast monsoon period, thereby implying that the major source of these elements is from freshwater. The positive correlations obtained between these parameters further emphasize a common origin for them. The average value of organic carbon and the total phosphorus content of sediment from this area, except at station V, was lower than the typical values recorded from coastal sediment by Mackay *et al.* (1972, *Mar. Poll. Bull.*, 3 : 7-11). The total sulphide content of sediment showed low level (< 20.0 µg/g wet wt) during northeast monsoon period. Gross primary production and net primary production rates showed relatively increased values during northeast monsoon period, but the overall rate of primary production in the lagoon was relatively less (< 450 mg/m^3/day) when compared to other coastal ecosystem.

After the northeast monsoon period, the lagoon remains cut off from the sea due to the formation of sand bar and fresh water inflow also diminishes after the cessation of rain. This resulted in the anoxic condition and the same got intensified with the advancement of the summer period. Dissolved oxygen content evinced a decreasing trend (< 2 ml/l). The water attained hypersaline condition (> 150‰). The pH of water indicated less alkaline condition (< 8.0). Soluble reactive phosphate, nitrate, nitrite and ammonia concentrations were relatively low being < 0.30 µg-at PO_4-P/l, < 0.80 µg-at NO_3-N/l, < 0.05 µg-at NO_2-N/l and < 5.00 µg-at NH_3-N/l respectively. Hydrogen sulphide content of water increased as the anoxic condition got intensified (> 10.0 µg-at H_2S-S/l). During the period of of anoxic condition, the pH of the sediment was almost near the neutral range. The Eh of sediment was very low (< -250 mV). Organic carbon, total phosphorus and Kjeldahl nitrogen contents during anoxic condition were comparatively low. The total sulphide content was high (> 65 µg/g wet wt). Sulphate reduction, denitrification, methanogenesis and mineralization might be at maximum level during the period of anoxic

condition in the ecosystem. The gross primary production (< 200 mgC/m^3/day) and net primary production rates were relatively low during anoxic condition and the latter frequently recorded nil values. The zooplankton occurred in the samples in the following order of abundance : amphipods, copepod eggs, decapod larvae, fish eggs, lamellibranchs, gastropods, coelenterates, cladocerans, ostracods, cumaceans, isopods, mysids, *Lucifer* sp., chaetognaths, polychaetes, heteropods, pteropods, appendicularians, fish larvae, copepod nauplii, ophiopleuteus larvae and insect larvae. Species specificity of the zooplankters was a characteristic feature observed during marine and brackishwater regimes in the lagoon. Bivalve spats, *Perinereis* sp., *Chironomus* sp., *Pulliella armata* and amphipods were recorded in the benthic macrofaunal community. The sediment is sandy in nature except at eastern region of the lagoon where it is clayey. Studies on the vertical profile of the chemical nature of the sediment revealed that organic carbon, total phosphorus and Kjeldahl nitrogen contents of the sediment decreased in their concentrations vertically downwards. In course of time, increase of their contents in the upper layer of sediment was discernible, but in the lower layers the temporal change in their concentration was of less in magnitude. The sand proportion of the sediment increased vertically downwards. The concentrations of soluble reactive phosphate, nitrate, nitrite and ammonia of water were apparently low during the day time and they showed an increasing trend during the night hours. The dissolved oxygen content of water was relatively high during the sunlit hours of the day. Salinity at surface and bottom layers usually followed the pattern of water circulation inside the lagoon. *Salicornia* sp. was found to be abundant with wide range of distribution around the lagoon. The nutrient enrichment experiments showed that gross and net primary production rates could be enhanced to more than seven-fold by employing commercial N : P : K Complex fertilizer or organic manure. d-glucose treatment was found to result in significant lowering of net primary production suggesting concomitant increase in the heterotrophic production. Experiments conducted using chemical fertilizers resulted in relatively less rate of gross and

net primary production, when compared to those in which enrichments were carried out employing commercial fertilizer. It is probable that this is indicative of the nonavailability of the minor nutrients for the phytoplankton. Individual enrichment experiments conducted using commercial fertilizers such as Potash (K), superphosphate (PO_4) and urea-N resulted in significantly less rate of primary production when compared with N : P : K Complex and organic manure. However, urea enrichment resulted in significantly higher rate of primary production than that of the superphosphate suggesting the primary deficiency of nitrogen in the lagoonal system. The phytoplankton in the enriched water was represented mainly by *Oscillatoria salinarum, Nitzschia closterium, Cymbella marina, Synedra* sp., *Navicula* sp., *Amphora* sp. *Oscillatoria salinarum* occurred in blooms in the experimental tanks.

The nutrient concentration of the water reveals that this lagoon falls under the category of "ultra-oligotrophic type" (O' Brien and deNoyeller 1974, *Hydrobiologia*, **444** : 105-125). The major deterrents in conducting mariculture practise in the lagoon can be categorised under two major heads : (i) Low rate of primary production due mainly to the "ultra-oligotrophic nature of the lagoon water and (ii) Periodical recurrence of low water level in the lagoon when it gets isolated from the sea. This results in poor plankton production, hypersaline condition and vulnerability of the cultured organisms to predation. In order to overcome these maladies, the lagoon can be fertilized by 17 : 17 : 17 : : N : P : K fertilizer to enhance the productivity at the primary level. The amount to fertilizer to be added for enrichment should be estimated based on the volume of water present in the area under consideration for mariculture practises. In short-term culture and quantity of water in the site should be taken into consideration. In the present investigations it is observed that the suitable level of water for aquaculture practises usually exists between the second half of October to first half of April. In addition to *Chanos chanos* being cultured at present, other euryhaline fishes such as *Aphanius dispar* (Ruppell) which can tolerate high salinity upto 70‰ (Jones *et al.*, 1978,

Estu. Coast. Mar. Sci., 6 : 253-262) may also be experimented in the lagoon, after investigating the biolgical impact of this species on the ecosystem. Perennial culture does not seem to be possible with the existing nature of the ecosystem. It is observed during the present study that the water currents of the Palk Bay and the Gulf of Mannar are monsoonal in nature. During the northeast monsoon, the surface currents are directed southwards with the resultant flow of water from the Palk Bay to the Gulf of Mannar. During the southwest monsoon, the condition is reversed. Due to the prolonged southwest winds, the speed of surface currents in this area may rise upto 2 knots/hr. Keeping this in view, it is suggested that a perennial water connection of about 2 km long could be maintained through an excavated channel connecting the Gulf of Mannar to the Pillaimadam Lagoon. Though the initial investment may appear to be high, the relief it would bring about would be permanent and atleast 200 hectares of area could be successfully made use of for intensive mariculture, as the level of water inside the lagoon will be maintained at the suitable level alternatively by the incursion from the Palk Bay and the supply from the Gulf of Mannar through the proposed canal.

CMFRI Spl. Publ., 1993; 56 : 9 - 14

PATHOLOGICAL INVESTIGATIONS IN PENAEID PRAWNS

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Introduction

During the past two decades there has been considerable advancement in the developed countries in the knowledge of diseases affecting the principal commercial species of penaeid prawns, their diagnosis and control. However, information on the diseases affecting the penaeid prawns of India is limited to a few descriptions and lists of parasites, and their biological consideration.

Objective

Since a knowledge of diseases, their causative agents and etiology is basic to control, and since this aspect is emerging as an important field of investigation, the present study was taken up initially to provide an insight into the common diseases encountered in natural and cultured populations of penaeid prawns and subsequently to make a detailed study of microsporidian-infected prawns, commonly known as "cotton" or "milk" shrimp.

Material and methods

The investigation was carried out from October 1981 to April 1985. The material was collected from the fishing operations off Cochin on the southwest coast and off Mandapam and Tuticorin on the southeast coast of India. Besides, the diseased prawns from the Narakkal Prawn Culture Laboratory (NPCL) and the seasonal and perennial culture fields at Vypeen Island near Cochin were also collected and examined.

Initially, a survey was conducted to obtain information and to understand the common diseases and anomalies

occurring in the penaeid prawns such as *Penaeus indicus, P. monodon, P. semisulcatus, Metapenaeus dobsoni* and *M. affinis,* in nature and those farmed in the study area. The pattern of infection/infestation, symptoms and pathogenicity of each of the cases encounttered were studied macro- and microscopically and by employing histopathological techniques. The diseased/ abnormal prawns were either preserved in ice, fixed in suitable fixative or transported live to the laboratory depending upon the condition of their availability. Environmental parameters relating temperature and pH of water were taken and water samples were collected to estimate salinity and dissolved oxygen. In the laboratory, prawns were first analysed for size, sex and maturity stage and then subjected to detailed light microscopic studies at CMFRI, Cochin and Mandapam Camp. Studies were also made of microsporidian infected prawns by using Electron Microscope at IIHR, Bangalore.

Laboratory experiments on transmission of microsporidian parasite to normal and healthy *P. semisulcatus* were conducted at the Regional Centre or CMFRI, Mandapam Camp. The transmission was attempted by contaminating the rearing medium with the pathogen by injecting the pathogen into the body of the prawns and by feeding the normal prawns with infected muscle tissue as well as their own faeces.

For proximate composition of abdominal muscle, ovary and hepatopancreas of normal and microsporidian infected *P. semisulcatus,* samples were collected from Mandapam fish landing centre. The tissues were analysed for moisture, ash content, total protein following the method of Lowry *et al.* (1951, *J. Biol. Chem.*, **193** : 265-275), total lipid as per the method of Folch *et al.* (1957, *J. Biol. Chem.*, **226** : 497-508) and for total carbohydrate by Anthrone method (Roe 1955, *J. Biol. Chem.*, **20** : 335-343). The data were statistically analysed and compared.

Observations

As a result of survey, ten cases of diseases and anomalies were reported. These include tumour-like growth, "soft" prawn, tail necrosis, brown-spot disease, red rostrum, ciliate infestation,

microsporidiosis, helminth parasitisation, metacercarial infestation and bopyrid infestation. Of these, the anomaly relating to the tumour-like growth on the carapace of a female *P. indicus* (152 mm TL) collected from a culture pond in Vypeen Island (Cochin) forms the first record of such an instance in penaeid prawns from India.

Among the different diseases and anomalies encountered during the survey, the "soft" prawn syndrome in the culture system of Vypeen Island, Cochin and microsporidiosis in the prawn catches landed at Mandapam, Tuticorin and surrounding localities were found to be the major disease problem. As the former disease syndrome was being studied elsewhere, the latter disease was taken up for intensive studies. The disease caused by microsporidian parasites, commonly known as "cotton" or "milk" shrimp disease, was found to infect the juvenile and adult populations of *P. semisulcatus* and *M. affinis*.

The nature, structure and characteristics of the different developmental stages and spores, as studied by light and electron microscopy and histological techniques, of the microsporidian parasites collected from *P. semisulcatus* and *M. affinis* revealed that they belong to three species, two of them assignable to the Family Thelohaniidae (Order : Microsporida; Sub-order : Pansporoblastina) and the other one to the Family Pereziidae (Sub-order : Apansporoblastina). Further detailed studies and comparisons with the described and known microsporidian species revealed that they were new to Science.

The sporont of one of the species belonging to the Family Thelohaniidae was found to undego a series of three successive binary divisions producing eight sporoblasts in a thin, subpersistent pansporoblastic membrane. These sporoblasts metamorphosed into free, mature spores which were ovoid, uninucleate, 5.0 to 5.5 x 2.5 µm in size and possessed isofilar polar tube measuring 14 to 22 µm in length. This species was described as *Thelohania semisulcata* sp. nov. and was found to infect mainly the body muscle, hepatopancreas, gonad and midgut of

P. semisulcatus. The other organs affected to lesser extent were the heart, eyes and the gills.

The other microsporidian assigned to the Family Thelohaniidae, showed a combination of characters of the Genera *Thelohania* and *Agmasoma*. This microsporidian was characterised by three successfive binary divisions of the sporont resulting in the formation of eight sporoblasts covered in a fragile pansporoblastic membrane. The mature and free spores were pyriform, uninucleate and measured 3.0 to 4.2 x 1.5 to 2.0 µm in size. The polar tube was anisofilar and formed about 9 to 10 undulations anterio-posteriorly inside the spore. In view of these characters, this microsporidian was assigned to a new genus *viz*. *Sulcovaria* and the species described as *Sulcovaria mannarensis*. This species, unlike *T. semisulcata*, was site specific and infected only the ovary of *P. semisulcatus*.

The third microsporidian attributed to the Family Pereziidae was apansporoblastic and disporous in nature and possessed ovoid, uninucleate spores which measured 2.2 to 2.5 x 1.0 to 1.5 µm in size. Their polar tube was isofilar type and measured about 25 µm in length. This was described as *Perezia affinis* sp. nov. and found to infect the body muscle, gonad and digestive tract of *M. affinis* and *P. semisulcatus*.

Symptoms of microsporidiosis caused by *T. semisulcata* were studied by qualitatively categorising the infection as light, moderate and heavy. The histopathological investigation revealed that *T. semisulcata* was an intracellular parasite and highly pathogenic in nature infecting important organs such as gonad, hepatopancreas, body muscle, midgut, heart, otpic nerves, retina and gills. The infection, though mild in the initial stage, spread gradually from gut sub-mucosa to all the vital organs of the prawn interfering with the normal funcations of these organs in the advanced state of the disease. The host response to the infection appeared to be least developed or effective as the pathogen did not apparently elicited any significant inflammatory response in the host. Most affected organs were found to be gonad, hepatopancreas and body

muscles bringing greatest damage to the individual as well as the population.

The laboratory experiments indicated that the pathogen followed oral route of transmission and did not require any intermediate host. However, it was observed that the spores of *T. semisulcata* had to undergo a process of conditioning by passing through the gut of the prawn before being capable of infecting the host. The initial site of infection was found to be the midgut. Infection was detected in the abdominal muscle around the midgut of the postlarvae of *P. semisulcatus*. The postlarvae were found to be more susceptible to infection by *T. semisulcata* than the juveniles or adults. Successful experimental transmission of the microsporidia in the postlarvae, but not in juveniles or adult prawns indicated that the prawns in their very early stage of life are more vulnarable to infection. On the other hand, microsporidian infected prawns with visible symptoms found in nature were either juveniles or adults. Such difference in the laboratory and field observations suggests that in the very young prawns although the infection exists, the disease may not be discernible for a long duration and by the time the symptoms are apparent, the prawn grows to larger size.

Study on the gross biochemical changes in the proximate composition of abdominal muscle, hepatopancreas and ovary of normal and those of moderate and heavily infected *P. semisulcatus* by *T. semisulcata* showed that the infection brings forth appreciable changes in the moisture content in the abdominal muscle, hepatopancreas and ovary. The variation in the ash content was generally observed in the hepatopancreas and abdominal muscle. Significant changes in total protein, lipid and carbohydrate content were observed in the hepatopancreas and ovary. The difference in the total protein content between the normal and infected ovary (45% and 58%) had been to the tune of about 11%, in lipid (27% normal and 21% infected) about 6% and that of carbohydrate (14% normal and 10% infected) about 4%. Similarly, the protein content of hepatopancreas showed an increase of about 11% (26% normal and 36% infected) while the lipid (40% normal and 34% infected) and carbohydrate (16%

normal and 14% infected) decreased by 6% and 2% respectively. This significant drop in lipid and rise in protein levels in the infected ovary and hepatopancreas indicates that the parasite largely depends on the easily accessible lipid from the host and multiplied faster in these organs. The increased level of total protein appears to be due to higher concentration of spores in ovary and hepatopancreas. The results of biochemical study thus showed that the most preferred site of infection by *T. semisulcata* is the ovary and hepatopancreas followed by muscle.

The studies carried out on the taxonomy, pathobiology and histopathology of the microsporidian parasites infecting the penaeid prawns of India, form the first detailed study on the subject from the country. It not only provides information *per se*, but also their relationship with the hosts. Various aspects studied and the information presented on the diseases and anomalies encountered in the wild and farmed population considerably add to the present knowledge on the pathology of penaeid prawns of India. The study would be helpful in averting the production loss and in the management of the exploited penaeid stocks both in the nature and in the aquaculture systems.

CMFRI Spl. Publ., 1993; **56** : 15 - 23

NUTRITIONAL STUDIES IN JUVENILE *PENAEUS INDICUS* WITH REFERENCE TO PROTEIN AND VITAMIN REQUIREMENTS

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Introduction

To develop cost-effective, nutritionally adequate practical feed formulations, prior knowledge of the essential nutrient requirements of the cultured species is a requisite. A great deal of contributions exist concerning the nutritional requirements of a number of species of prawns, primarily from Japan, the United States of America and France. In India, despite the extensive reports available on aspects such as the biology, fishery and culture of the penaeid pranws, very few studies have been carried out on the nutrition of prawns. The present study was carried out in the juveniles of the Indian white prawn *Penaeus indicus* H. Milne Edwards to define the protein and vitamin requirements.

Objectives

This investigation is to (i) study the effect of selected levels of protein in purified diets on growth, feed conversion and body composition and to determine the optimal protein requirement of juveniles, (ii) evaluate the nutritive value of a few protein rich ingredient sources (plant and animal origin) in compounded diets, (iii) study the deficiency symptoms associated with the deletion of water soluble vitamins (ascorbic acid, choline, thiamine, pyridoxine, pantothenic acid, niacin, riboflavin and inositol) from the diet, and (iv) determine the optimum requirements of important water soluble vitamins (ascorbic acid, choline, thiamine, pyridoxine, pantothenic acid and niacin) in purified diets.

Material and methods

Statistically designed experiments were conducted in triplicate aquaria for a stipulated number of days. Postlarvae of *P. indicus* of length 12-15 mm size, obtained from a single brood-stock from the Narakkal Prawn Hatchery of the Central Marine Fisheries Research Institute, Cochin were transported to the laboratory and acclimated by rearing in large prespex thanks for 10-15 days before commencement of the experiments. Fresh, filtered, UV-irradiated, dilute sea water of 20 ppt salinity was used for rearing the animals in 50 l capacity plastic tubs. The duration of the experiments was 30 and 45 days for protein and vitamin requirements studies respectively.

Purified casein either lipid-free or vitamin-free, was used as protien source in the diets for most of the experimental studies. The other ingredients included were gelatin, egg-albumin, glucose, sucrose, starch, cod-liver oil (rich in w3 fatty acids), corn oil (rich in w6 series fatty acids), cholesterol, mineral mixture, vitamin mixture, additives and cellulose. However, in the case of protein sources evaluation experiments, ingredients like ground nut oil-cake, soyabean meal, gingely oil-cake, coconut oil-cake, fish-meal, crab-meal, shrimp-meal, clam-meal, mixture of plant protein sources or animal protein sources, or mixture of both plant and animal protein sources were also used to identify commercially viable protein sources. Similary, in vitamin requirement studies graded levels of the test vitamin were used in the diets. The ingredients were finely powdered, weighed and mixed in a Warring blender, and steamed before made into a dough having a consistency of 30% moisture content. The diets were adjusted to a specific pH before storing in polyethylene bags under low temperatures. The feeds were thawed before feeding the prawns to room temperature and made into small balls manually. The feeds were regularly made fresh every 15 days to maintain quality. The prawns were fed 10% of the body weight, twice a day. Residual feed was collected on the following day by siphoning from the tub.

Data on survival, growth, feed conversion, protein efficiency ratio, biochemical composition and ammonia

excretion rates (for few experiments) of the prawns, collected from each experiment were statistically analysed and expressed. Certain general observations on moulting, activity, presence of spots on the body and development of any lesions in the animals, etc. assoicated with different dosages of the test diets were also reported.

For biochemical analyses of the prawn and feeds, as well as for estimation of ammonia in water, standard analytical procedures were adopted. The data were statistically analysed using ANOVA and test for treatment mean differences.

Results and discussion

Protein requirement : Two sets of experiments were conducted to study the effect of selected levels (0 to 60% and 32.5 to 47.5%) of protein using casein as the major protein source.

Based on the survival and growth data from the first experiment the optimal dietary protein requirement of juvenile *P. indicus* was found to be within the range 30-40%. Besides, diets containing protein contents above 40% did not promote growth, but resulted in decreased growth. The subsequent experiment (32.5- 47.5% protein level) suggested the survival and growth was better at 37.5% protein level than any other protein level. The requirement has futher been substantiated by the data obtained on feed conversion, protein efficiency ratio and biochemical composition of the prawns fed at this level of protein in the diet. Ammonia excretion rates increased with increasing protein level in the diets showing the highest value at the maximum protein level. Thus, the study highlights significantly that the dietary protein level in juvenile prawns could be near 35.0 - 37.5% for fast growth, high survival and normal metabolic functions.

Evaluation of the nutritive value of protein sources : With a view to identifying suitable plant and animal protein sources for formulation of practical diets for juvenile prawns, the nutritive value of a few naturally occurring protein rich ingredients were evaluated. These included ground nut oil-cake, soyabean meal, gingely oil-cake, coconut oil-cake, fish meal, crab meal, shrimp

meal and clam meal. Besides these, nutritive value of a combination of plant protein sources, animal protein sources, and, both plant and animal protein sources were also studied.

The experimental study indicated that a combination of animal protein sources in the diet was superior to all other diets. Among the plant protein sources in the diet was superior to all other diets. Among the plant protein sources soyabean meal produced significantly higher growth than all other individual plant protein sources, which was comparable to the growth observed in juvenile prawns fed on diets containing certain individual animal protein sources. The growth of prawns fed with purified diet was also observed to be significantly higher than that recorded in many other feeds.

Effect of deletion of water-soluble vitamins from the diets : Using vitamin-free casein as the prime protein source, isonitrogenous and isocaloric diets were prepared to study the nutritional deficiency symptoms associated with the deletion of water soluble vitamins from the diets. The deleted vitamins were ascrobic acid, choline, thiamine, pyridoxine, pantothenic acid, niacin, riboflavin and insoitol.

From the results, it was evident that deletion of the above water soluble vitamins from the diets, significantly affect the survival of prawns, thus, indicating their essentiality in the diets. It was also observed that when choline and lecithin were deleted from the diets. It was also observed that when choline and lecithin were deleted from the same diet, survival was greatly reduced compared to the survival of prawns fed on diet with lecithin, but without choline. This indicates the significant influence of lecithin on choline and that lecithin may partially spare the dietary requirement of choline in the juvenile prawns. Data on growth, feed conversion, protein efficiency ratio and body composition of prawns showed significant differences between treatments. Significant variations were also observed in moulting frequency, activity and feed intake of prawns in different treatments especially in the case of ascorbic acid, thiamine, pantothenic acid and niacin deleted diets.

Effect of selected concentrations of water soluble vitamins in diets : A series of laboratory experiments were conducted using graded levels of ascrobic acid, choline, thiamine, pyridoxine, pantothenic acid and niacin in vitamin-free casein test diets and the optimum requirement for juvenile prawns have been worked out. The different ranges of concentrations of test vitamins used in the diets were as follows :

Ascorbic acid	0 to 2.4 g/100 g dry diet with an interval of 0.4 g.
Choline (choline chloride)	0 to 2.0 g/100 g dry diet with an interval of 0.25 g.
Thiamine (thiamine hydrochloride)	0 to 0.1 g/100 g dry diet with an interval of 0.01 g.
Pyridoxine (pyridoxine hydrochloride)	0 to 0.15 g/100 g dry diet with an interval of 0.01 g.
Pantothenic acid (calcium pantothenate)	0 to 0.25 g/100 g dry diet with an interval of 0.025 g.
Niacin (nicotinic acid)	0 to 0.25 g/100 g dry diet with an interval of 0.025 g.

From the experimental studies, it was observed that thiamine, pantothenic acid, pyridoxine and niacin are required relatively in lower concentrations in the diets of juvenile prawns than ascrobic acid and choline which are required in higher concentrations in the diet. However, these vitamins are indispensable for prawns in their diets for normal growth and survival. Based on ammonia excretion rates in prawns it was evident that some of these dietary vitamins significantly influence the metabolic activity. Higher or lower concentration of these vitamins resulted in poor feed conversion, protein efficiency ratio and significant alterations in the biochemical composition of the carcass. Deficiency of ascorbic acid and niacin resulted in the formation of 'black lesions' in the body and gills, whereas deficiency of pantothenic acid induced 'partial moulting' in prawns.

Some of the salient findings from these experimental studies are that (1) Deficiency of sub-optimal levels of protein in the diet significantly affects the growth, survival, ingestion and utilisation of food, synthesis of protein and general maintenance of body functions in prawns. Prolonged period of protein deficiency induced cannibalistic tendencies in prawns and resulted in near complete wiping of prawn population. (2) The optimal protein requirements of these juvenile prawn was found to be within the range of 35-40%. Beyond this range, the protein concentration had adverse effect on growth, survival, body composition and utilization of food and proteins. Excess of proteins in diets resulted in enhanced rate of protein catabolism resulting in increased ammonia excretion rates. (3) Amongst the natural protein sources, animal protein sources significantly imporved survival and growth in prawns. Protein sources of crustacean origin (prawn-meal and crab meal) have proven to be the best among individual protein sources. (4) Amongst plant protein sources soyabean meal and ground nut oil-cake were found to be best protein sources and proved to be the best supplements for animal protein sources. (5) Purified diet has shown relatively good growth and survival indicating that purified diets can be successfully used for nutritional studies in prawns. (6) The deficiency of the water-soluble vitamins, *viz.* ascorbic acid, thiamine, niacin, pantothenic acid, inositol, riboflavin and choline in the diets severely affected the survival and growth of prawns. All these vitamins have been found to be indispensable for prawns. (7) Prolonged deficiency of ascorbic acid in the diet resulted in general decline in the metabolic activity leading to poor food intake, aversion towards food and eventually death. The carcass composition clearly showed disturbances in the nutrient deposition. Histological examination of cellular layers of muscle and hepatopancreas showed lysis affected regions. The prominent externally visible changes observed in the prawns were blackening of gills and lesions in the abdominal region. Inclusion of ascorbic acid at concentrations of 0.4 g and 0.8 g/100 g dry diet significantly imporved growth, survival, food consumption, food conversion and protein efficiency as well as nutrient deposition. Histologi-

cal structures showed normal cellular layers in the muscle and hepatopancreas. Supra-optimal levels of ascorbic acid in the diet significantly influenced growth, survival and other parameters suggesting the deleterious effect of hypervitaminosis. (8) Deletion of both choline and lecithin from the same diet, resulted in significant decrease in growth, survival and affected the food consumption and protein conversion. The prawns showed aversion towards food after two weeks of feeding which resulted in passive activity of the prawns and utlimately resulted in death. Histological examination of the muscle, nerve and hepatopancreas of these prawns showed no changes in the cellular structures. Supra-optimal levels of choline in diets resulted in slower growth and survival suggesting that these prawns require optimal levels of choline in the diet for maximum growth and survival. (9) Deficiency symptoms associated with thiamine deletion in diets were instability, increased sensitivity to shock and aversion towards food. The optimal thiamine requirement in prawns was observed to be around 0.01 g/100 g dry diet, as high survival, growth and better food conversion and protein efficiency ratios were recorded at this concentration of thiamine. Supra-optimal levels of thiamine did not produce much variation in survival, yet resulted in poor growth, suggesting hypervitaminosis effect of thiamine on prawn's metabolism. (10) Pyridoxine deficiency did not significantly affect the survival, but the growth, efficiency of conversion of food and protein and the body chemical composition were significantly affected. The pyridoxine requirement in prawns was in the range of 0.01 g to 0.02 g/100 g dry diet for maximum survival and growth. Supra-optimal concentration of pyridoxine in the diet produced significantly lower growth rate and survival and caused significant variations in other parameters studied. (11) Niacin deficiency in the diet resulted in extremely high mortality rates, suggesting that the vitamin is indispensable for the prawns. The prominent dietary deficiency syndromes observed were early setting of anorexia and aversion towards food. Prolongation of niacin deficiency caused blackening of gills in some prawns which resulted in ultimate death of the prawns. Niacin when added in the diet

at 0.025 g/100 g dry diet produced high survival and growth. These prawns showed better food conversion and protein efficiency ratios and nutrient deposition. Supra-optimal dietary levels of nicotinic acid resulted in retarded growth and lower survival rate. (12) Deletion of pantothenic acid from the diet significantly affected the growth and survival of prawns. These prawns showed deficiency syndromes such as anorexia and aversion towards food. The most significant syndrome observed was partial moulting in the abdominal region in these prawns. The optimal pantothenic acid requirement was found to be around 0.075 g/100 g dry diet. The high survival rate and growth, the food conversion ratio, protein efficiency ratio, biochemical composition and other observations support these findings. The sub or supra-optimal dosages of pantothenic acid in the diet resulted in poor growth and survival in the prawns.

Conclusion

The present investigation in juvenile *Penaeus indicus* is one of the first attempts to be made on the nutrition of penaeid prawns in India. Besides, vitamin nutrition of prawns in one of the most neglected areas of research as compared to the energy-yielding nutrients in prawns. Thus, no prior substantial knowledge is available for a number of nutritionally linked deficiency syndromes occurring in prawns. The recommended dosages, though higher than that reported for finfish, seems to agree with some of the few studies that have been done in crustaceans. These recommended dosages seem to be optimal for the species if all the parameters like leaching of nutrients from feeds and moulting in prawns are given due consideration.

The present studies thus pave the way for many of the future nutritional requirement studies by highlighting some of the possible causes and then solutions for some of the nutrition related deficiencies and diseases. The protein requirement study suggests the near optimal requirement for juvenile prawns which would enable in practical feed formulations and future nutritional requirement studies. Studies on the evaluation of protein sources provide information on the relative efficacy

some of the plant and animal protein sources, which would help in the formulation of cost effective feeds on commercial scale.

CMFRI Spl. Publ., 1993; **56** : 24 - 28

STUDIES ON ECOPHYSIOLOGY OF *PENAEUS INDICUS* H. MILNE EDWARDS IN THE GROW-OUT SYSTEM

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Introduction

The present study was prompted as availability of maturing and mature *Penaeus indicus* in the estuarine area is meagre as compared to inshore marine area especially during monsoon and postmonsoon period when freshwater runoff and local precipitation, presumably, causes drastic alternation of physiochemical dynamics of the estuarine area. Therefore, in the present investigation, seasonal variation of physico-chemical parameters and some of the metals in water and sediment in the marine and the estuarine areas were studied. But the emphasis was to find out if season and ecosystem-specific variations of calcium, magnesium, phosphorus, copper and zinc in the water and the sediment has any role to play in altering the concentration of these elements in different tissues of *P. indicus* as evidenced by the seasonal studies in the grow-out (representing estuarine area) and the marine ecosystems.

Material and methods

The grow-out ecosystem, studied is an estuarine impoundment of 76 hectares, 1.5 m deep in Vypeen Island and connected to the Vembanad Estuary through a net-work of a few canals. The marine ecosystem lies at about 30 m depth zone on the continental shelf of the Arabian Sea off Cochin. Three sampling stations were fixed in each ecosystem. Samples of water, sediment and male and female *P. indicus* (intermoult) were collected fortnightly. Water samples were collected about 30 cm above bottom in both the ecosystem as penaeids are known to be benthic.

Water was analysed for temperature, dissolved oxygen, pH, Eh, salinity, net primary productivity, nitrate, nitrite, ammonia, calcium, magnesium, total phosphorus, copper and zinc. Sediment samples were analysed for temperature, pH Eh, calcium, magnesium, total phosphorus, copper and zinc. Different tissues *viz.* exoskeleton, muscle, hepatopancreas, ovary/testis and the haemolymph of male and female *P. indicus* were analysed for calcium, magnesium, phosphorus, copper and zinc content.

Results and discussion

The results of seasonal variation study from November '82 to October '84, showed that the physico-chemical conditions, in general, remained same in both the ecosystems during non-monsoon period, but major noticeable variations occurred when considerable rainfall poured during monsoon and postmonsoon period. The physico-chemical parameters generally recovered to their premonsoon level by the end of postmonsoon period.

In the grow-out ecosystem, freshwater runoff from the catchement area and the local precipitation during monsoon and postmonsoon had enormous dilution effect on the premonsoonal marine waters of Cochin Backwater resulting in decreased salinity of water, and calcium and magnesium of the water and the sediment. The decreased temperatures and Eh of the water and the sediment were noticed during monsoon and postmonsoon period. The considerably increased nutrients, copper and zinc, observed during monsoon and postmonsoon period resulted in increased primary production as a result of which ecosystem became more alkaline as shown by high values of pH during same period. The erratic fluctuations of nitrite were recorded. Total phosphorus showed increased values during transition period and early monsoon. The dissolved oxygen level showed more or less uniform values, but for a minor decrease during monsoon. The increased primary production caused increase in consequent 'death and decay' and 'sinking'. This resulted in increased level of total phosphorus, copper and zinc in the sediment during monsoon and postmon-

soon as recorded in the present study. It is explained that the freshwater discharge brings alongwith suspended silt rich in nutrients and organic matter, to which copper and zinc are presumably absorbed. A part of this silt settles down on estuarine floor. Besides, the presence of a major Port, Udyogamandal Industrial Complex, Shipyard and shipping activity, Rare-earth mining complex and other Industrial and geo-chemical activities alongwith domestic pollution, and flushing of these area also contribute to increase the metal and nutrient levels during monsoon and postmonsoon.

In the marine ecosystem, the seasonal variations of salinity, calcium and magnesium of water, and calcium and magnesium of sediment are found to be little. The considerable decrease of water temperature, dissolved oxygen and Eh was noticed during monsoon and postmonsoon period. Relatively low pH was recorded during monsoon and postmonsoon period. The considerably increased nutrients, net primary production, copper and zinc recorded in the marine ecosystem during monsoon and postmonsoon period might be partly due to the 'sedimentation' of organic matter rich in silt of riverine origin and 'sinking' of organic debris. It is observed that the incursion of offshore water are of primary significance and the effect of freshwater discharge and local precipitation is not quite apparent in the marine ecosystem. The marine ecosystem, in comparison to the grow-out ecosystem, provides a relatively stable seasonal picture.

While comparing the seasonal variation in both the ecosystem, the following aspects become clear. Both the ecosystems showed decrease in temperature of water and sediment during monsoon and postmonsoon, but the quantum of decrease was considerably higher in the marine than in the grow-out ecosystem. The pH of water and sediment showed stable values in the marine ecosystem with a little decrease during monsoon period, whereas considerable increase of pH was noticed during monsoon and postmonsoon period in the grow-out ecosystem. The Eh in both the ecosystems behave in

a similar fashion all round the year. The level of dissolved oxygen was considerably higher in the grow-out than in the marine ecosystem and the quantum of seasonal decrease of dissolved oxygen was relatively mild in the grow-out ecosystem. The salinity, calcium and magnesium of the water and the sediment showed considerable decrease to almost freshwater values during monsoon and postmonsoon in the grow-out ecosystem, in contrast to the stable seasonal values recorded in the marine ecosystem. The behaviour of net primary production, nutrients; total phosphorus, copper and zinc of water and sediment was similar in both the ecosystem.

The results of present study showed that the seasonal variations of bio-elements, in different tissues of male and female *P. indicus*, were little during non-monsoon period and the variations, whatsoever recorded, occurred mainly during monsoon and/or postmonsoon period only.

The behaviour of calcium and magnesium in the tissues was similar to that of the same in the water and the sediments of both the ecosystems. During non-monsoon period, calcium and magnesium variation in water, sediment and various tissues was little in both the ecosystems. Considerable decrease of each of these metals was noticed in the water, sediment, haemolymph, muscle, hepatopancreas and gonad of male and female *P. indicus* during monsoon and postmonsoon period in the grow-out ecosystem only. The exoskeletal calcium showed little decrease during monsoon and postmonsoon period. The marine ecosystem showed relatively stable seasonal magnesium values in water, sediment and various tissues of *P. indicus*. There were no sex-specific differences in either of the ecosystems. Calcium was found to be always higher in the haemolymph than in the ambient medium, but the reverse was true for magnesium.

The haemolymph phosphorus showed erratic seasonal variation in both the ecosystems. The relatively lower values of exoskeletal phosphorus were noted during monsoon and postmonsoon, in both the ecosystems. Muscle phosphorus was variable in both the ecosystems, though slightly higher values

were noted in the monsoon and postmonsoon especially in the grow-out ecosystem. Phosphorus values of hepatopancreas and testis/ovary were low during monsoon and postmonsoon period in the grow-out ecosystem whereas, in the marine ecosystem there was no much change in the values. There was little effect of seasonal variation of total phosphorus in water and sediment on the variations of the same in animal tissues in either of the ecosystems.

The seasonal behaviour of copper and zinc in various tissues of male and female *P. indicus* was similar in both the ecosystems. Increased levels of these metals in haemolymph and muscle were noted during monsoon and postmonsoon in both the ecosystems. Exoskeletal copper and zinc levels were recorded to be more or less seasonally uniform in both the ecosystems. The hepatopancreatic copper and zinc showed minor decrease during monsoon and postmonsoon period in both the ecosystems. Copper and zinc levels in the ovarian tissue remained seasonally uniform in the marine ecosystem. In the grow-out ecosystem, relatively lower levels of copper and zinc were noted during monsoon and postmonsoon period. The testicular copper and zinc too behaved like ovarian tissue. The seasonal variation of copper and zinc in the haemolymph and the muscle are significantly correlated to variations of the same in water and sediment in both the ecosystems.

In general, the uniform seasonal tissue levels of calcium and magnesium noted in the marine ecosystem suggest that the animals were bathed in a stable unaltered milieu involving least stress on osmo-regulatory activity. On the other hand, the drastic alteration of the grow-out ecosystem was stressful for *P. indicus*. The animal has to keep on attaining the dynamic chemical equilibrium with the ambient medium.

The findings of present study has its own inborn limitations. However, it may serve as the baseline *in vivo* information which can be utilized as a spring-board to launch more useful *in vitro* studies to understand the operating physiological principles and to arrive at definitie conclusion.

CMFRI Spl. Publ., 1993; 56 : 29 - 34

STUIDES ON LARVAL NUTRITION IN THE PEARL OYSTER *PINCTADA FUCATA* (GOULD)

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Introduction

Over the past two decades, India has made rapid strides in mariculture in concerted efforts to augment production from the sea. Apart from shrimp and finfishes, molluscs offer a great scope for aquaculture production and technology for the culture of several molluscan species has already been established. Availability of seed, however, has been one of the major constraints in promoting commercial production of molluscs primarily because of unpredictable supply and heterogeneity of stocks. The problem of "seed" is particularly grave in the case of pearl oysters, as the natural pearl oyster resources in the Gulf of Mannar and Gulf of Kutch have not been productive for over three decades. The development of hatcheries, therefore, are essential for the establishment of pearly oyster production systems and efforts on development of technology for hatchery production of pearl oyster at the Central Marine Fisheries Research Institute has been successful with pearl oyster larvae being reared upto spat stage.

One of the vital components in the hatchery production of seed has been recognised to be larval nutrition. Nutritional requirements, if not fulfilled, can often result in disease and eventual mortality of all larvae. A critical study on larval nutrition of pearl oyster was considered necessary to improve and standardise larval food and feeding techniques to achieve maximum efficiency in larval rearing and spat production. "Studies on larval nutrition in the pearl oyster *Pinctada fucata* (Gould)" is the result of the above practical necessity.

Material and methods

Larval rearing procedures : Pearl oyster broodstock were obtained from the pearl banks of the Gulf of Mannar or from the CMFRI pearl oyster farm at Tuticorin Harbour. Larvae for the experiments were obtained either through natural spawning or by induced spawning through pH stimulation. Seawater for larval rearing was collected from the bay, passed through a biological filter and a UV chamber prior to use. Larval rearing was done under static conditions and water was changed on alternate days. Feeding was done once a day. Larvae were maintained until spat setting was complete.

Criteria for nutritional evaluation : A comprehensive set of parameters was chosen for evaluation of results. The parameters were (a) growth of the larvae as characterised by mean size, size frequency, growth rate, growth regression and growth curve; (b) survival rate at the stages of umbo, eyed-umbo and spat; (c) spat setting and production as evaluated by day of first setting, duration and amount of spat fall, and (d) algal cell consumption at the end of 24 hr after feeding. Appropriate statistical tests were employed for evaluation and comparison of results.

Results and discussion

Standardisation of larval density : As the first step of the study it was necessary to standardise the larval density and algal cell concentration through experimentation so that all other experiments could be carried out on that basis to make the results comparable for deriving conclusions. Pearl oyster larvae were reared in the density range of 1 to 10 larvae/ml at the uniform algal cell concentration of 25 cells/µl of *Isochrysis galbana*. Results indicated that the density of larvae in the culture has an influence on growth and spat setting. Relatively poor growth and setting were observed at very low densities (1/ml and 3/ml) or high densities (8/ml and 10/ml). Lower growth in heavily crowded cultures, poor growth has been attributed to reduced availability of food, frequent collisions among larvae and increased levels of metabolic products. At high densities, available food in the rearing medium gets reduced leading to

wastage of food cells. For pearl oyster larvae, density of 5/ml was seen to be optimum for larval rearing.

Standardisation of algal cell concentrations : Maintaining larval density at 5/ml, two species of algae namely, *I. galbana* and *Pavlova lutheri* were independently fed to pearl oyster larvae at four increasing cell concentrations from 10 to 100 cells/µl.

The concentration of algal cells in the rearing medium is of obvious importance as it strongly influences filtration rate and consequently algal consumption. At high cell concentrations, there is a decrease in sweeping rates accompanied by an increase in pseudofaeces formation. Concentration of algal cell in the rearing medium can also affect the retention time of cells within the gut thereby affecting assimilation. For both algal species, maximum growth of pearl oyster larvae was observed at the algal cell density of 25 cells/µl. Maximum spat setting was observed at 25 cells/µl with *I. galbana* and at 10 cells/µl with *P. lutheri*. Algal cell concentration of 25 cells/µl was used as standard in all larval rearings.

Evaluation of nutritional value of algae : The two chrysophycean flagellates *Isochrysis galbana* and *Pavlova lutheri* which are almost universally used in bivalva larval rearing, another chrysophycean *Chromulina freiburgensis*, cyanophycean *Synechocystis salina* and prasinophycean *Tetraselmis gracilis* were the five species studied for their nutritional value to *Pinctada fucata* larvae. Larval growth, setting and survival were seen to be greatly influenced by the algal species used as diet. Larval metamorphosis occurred only with *I. galbana, P. lutheri* and *C. freiburgensis*. Good larval growth was observed with *I. galbana* and *P. lutheri*, but very poor growth with *S. salina* and *T. gracilis*.

Evaluation of algal combinations : The following combinations of microalgae were studied for their comparative nutritional value at the total cell concentration of 25 cells/µl in each combination. *I. galbana + P. lutheri; I. galbana + C. freiburgensis; I. galbana + S. salina; I. galbana + T. gracilis; S. salina + T. gracilis,* and *I. galbana + S. salina + T. gracilis*. The diet combination of *I. galbana + P. lutheri* yielded the best results by way of larval growth and

spat setting. Setting was observed only for larvae fed with the diets of *I. galbana* + *P. lutheri*, *I. galbana* + *C. freiburgensis* and *I. galbana* + *S. salina*.

The nutritional value of the above algal species used singly and in combination has been discussed with reference to their physical characteristics.

Food value index : Based on larval growth regression values (log µm/day) obtained for different diets, a food value index was prepared to rank the tested diets for nutritional value.

Efficacy of stratified feeding : The algal cell concentration of *I. galbana* was increased from an initial cell concentration of 10, 15, 20 and 25 cells/µl by a step of 5 cells/µl at the three larval stages of straight-hinge, umbo and the eyed-umbo. Results indicated that there is an increase in the quantitative requirement of cells with larval development.

When *I. galbana*, *T. chui* and *Chlorella salina* were provided either singly or in combination at the different larval stages, growth and setting showed variable results.

Nutritional value of mixed phytoplankton : A culture of mixed phytoplankton from the open sea was evaluated for its food value to the pearl oyster larvae. It promoted growth and setting when fed to the eyed-umbo stage, but yielded very poor results when fed to the D-shape larvae.

Estimation of filtration rate : The rate of filtration of algal cells by pearl oyster larvae was measured at four increasing cell concentrations of *I. galbana* between 10 and 100 cells/µl. It was observed that filtration rate increased with larval growth and decreased with higher cell concentration.

Uptake and retention of algal cells : Radioactive C^{14} labelled algal cells of *I. galbana*, *P. lutheri* and *C. freiburgensis* were used to estimate the uptake and retention of algal cells by pearl oyster larvae at the two cell concentrations of 25 and 50 cell/µl. The uptake of *I. galbana* and *P. lutheri* was greater than that of *C. freiburgensis*. Doubling the cell concentration from 25 to

50 cells/µl did not result in substantial increase in the uptake of algal cells. About 67 to 87% of the cells taken up were retained by the larvae.

Evaluation of non-living diets and dissolved nutrients : Two types of non-living diets *viz.*, freeze-dried *I. galbana* and carrageenan bound microparticulate diet (CBMD) were evaluated for their nutritional potential for pearl oyster larvae. Freeze dried *I. galbana* was found to be a promising source of nutrition for the after stages of larval development and not for the earlier stages. The response to CBMD was very poor and did not result in larval metamorphosis.

The addition of dissolved nutrients in the form of amino acids and vitamins are found to be of no significance in enhancing the larval growth when provided alone or as dietary supplements.

Biochemical investigations of pearl oyster larvae and algal diets : The increase in total organic matter from the D-shape stage to the eyed umbo stage was largely in the form of protein and lipid and to a lesser extent in carbohydrate. With the onset of metamorphosis, levels of lipid, protein and carbohydrate were seen to decrease. It is suggested that a part of this energy is diverted for use during metamorphosis. Neutral lipid seems to be more important for larval growth. Gross biochemical analysis did not reveal any major difference in composition among the algal species used in this study.

Role of environmental parameters on larval growth and setting : In monofactorial experiments conducted to study the effect of parameters such as temperature, salinity and pH on pearl oyster larval growth, a direct relationship between larval growth and temperature was seen. Larvae reared at 21°C did not grow beyond the straight-hinge stage although they survived for 21 days. Consumption of algal cells was greater at higher temperatures (28° and 32°C). The influence of salinity in the range of 26 to 38.1‰ on larval growth and setting was not notable. Within the pH range of 7.5 to 9.0 maximum growth and setting was observed at 8.1 (ambient pH).

Some aspects of water quality management : The effects of aeration, antibiotic treatment and a continuous flow system on larval growth and setting were studied. Streptomycin sulphate enhanced larval growth marginally and total spat production remarkably when provided as "washes". Spat production increased when the antibiotic was added to the rearing medium. Aeration depressed growth rate when introduced during the D-shape stage, but enhanced growth and setting when introduced during the eyed-umbo stage. The continuous flow system was beneficial in rearing post-umbo stages resulting in increased spat production.

CMFRI Spl. Publ., 1993; 56 : 35 & 36

STUDIES ON SPORULATION AND PROPAGATION IN SELECTED AGAROPHYTES

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Summary

Studies on sporulation of 4 commercially important red algae (Agarophytes) namely *Gelidiella acerosa, Gracilaria corticata, G. edulis* and *Hypnea musciformis*, growing in the vicinity of Mandapam Coast, were carried out from October 1981 to September 1983. During this period fruiting behaviour in the natural population of these species was investigated. Laboratory experiments were carried out with the four algae to collect information on seasonal aspects of spore production and diurnal variation of spore shedding. Studies were also carried out to understand the effect of some selected environmental factors such as desiccation, salinity, temperature, light intensity and photoperiod on spore output in the above four species.

Population of all the four species occurred throughout the year along the coast of Mandapam. Tetrasporic plants of these species were observed in all the months of the year. The carposporophytes of *G. corticata* and *G. edulis* were seen throughout the year. In *H. musciformis*, however, they were found only in some months. Cystocarpic plants were not found in the population of *G. acerosa*.

Maximum output of tetraspores and carpospores were observed mostly on the first day of the experiment in the four red algae studied. The tetraspore output decreased from 2nd day onwards. Rhythmic liberation of carpospores, with peak shedding of spores at intervals on different days was also observed. In the laboratory conditions, the tetraspore output was observed for a period of 6 - 14 days in *G. acerosa*; 6-27 days in *G. corticata*; 3-30 days in *G. edulis* and 3-23 days in *H. musciformis* during

different months of the year. Carpospore liberation was found for 6-30 days in *G. corticata;* 10-30 days in *G. edulis* and 2-24 days in *H. musciformis*. Seasonal periodicity was not observed in the liberation of tetraspores and carpospores in the four algae studied. Diurnal periodicity in the liberation of tetraspores with a prominent peak between 1400 and 1800 hrs was observed in *G. acerosa*. A definite peak at a period of the day in the sheding of tetraspores and carpospores was not seen in *G. corticata, G. edulis* and *H. musciformis* and the maximum liberation of spores occurred in these three species from 1000 hrs to 1400 hrs. Peak shedding of spores was seen in 30‰ S in *G. edulis* and *H. musciformis,* at 40‰ S in *G. acerosa* and at 30-40‰ S in *G. corticata*. peak sporulation was observed at low light intensity of 500 lux in *G. acerosa, G. corticata* and *G. edulis* and of 1000 lux in *H. musciformis*. Maximum spore output in *G. acerosa* was observed at 25°C, in *H. musciformis* at 30°C, and in *G. corticata* and *G. edulis* between 25°C and 30°C.

CMFRI Spl. Publ., 1993; 56 : 37 - 41

STUDIES ON THE HISTOLOGICAL AND BIOCHEMICAL CHANGES DURING SPERMATOGENESIS IN *MUGIL CEPHALUS* LINNAEUS AND RELATED SPECIES

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Introduction

Mullets form a major group of economically important cultivable finfishes throughout the world. In India too, being distributed along both the coasts, they constitute an important group in the culture fisheries of the coastal waters. While information on the biology, bionomics and some culture aspects of this group is available, there is a dearth of information on the reproductive physiology and gametogenesis of these fishes, which is considered an essential prerequisite for the control of sex and effective management of the broodstock. In view of this, one aspect of reproductive physiology relating to spermatogenesis in two species of mullets is taken up in the present study. A comprehensive account of the process of spermatogenesis and the results of the preliminary experiments on the cryopreservation of the milt of the two species are presented here.

Material and methods

Mugil cephalus Linnaeus the largest and the most important of the cultivated species of mullets, was selected as the candidate species for the investigation and *Liza parsia* (Hamilton-Buchanan) one of the smaller verieties found abundantly in the Cochin Backwater, was chosen as the second species. The material for the study was collected from the Cochin Barmouth from October 1981 to April 1985. The gear used was the Chinese dipnet.

Results and discussion

Organisation and structure : Based on the anatomical and histological observations, it was found that the male reproductive system of *M. cephalus* and *L. parsia* consisted of a pair of elongated testes, vasa deferentia and a common sperm-duct. There were no seminal vesicles or other associated secretary glands. Initially the testes make their appearance as two thread-like strands of tissue attached to the dorsal side of the body wall by the mesorchium. As development proceeds they grow in size become flattened and reorient themselves in such a way that the mesorchium in the mature gonad appears to be connected from the inner lateral sides of the testes to the body wall.

Internally the body of the testis was found to be composed of seminiferous lobules. The lumen of the lobules was continuous with that of the vas deferens. The inter lobular tissue was composed of connective tissue strands, boundary cells and interstitial cells. Each testis was surrounded in the periphery by a thick coat of connective tissue (*tunica albuginea*). The spermatogenic cells at different stages of development formed cysts lining the inner margin of the seminiferous lobules all along their length. Hence the testes belonged to the unrestricted type. Each seminiferous cyst was surrounded by cytoplasmic extensions from the Sertoli cells present in the seminiferous lobule.

During the process of development the testes underwent changes in the morphological and histological characters depending on the degree of maturation. Making use of these criteria six stages of maturity were identified. These were - immature, early maturing, late maturing, mature, oozing and spent. Once maturity was attained the condition of the testes oscillated between the late maturing and the spent stages depending on the seasonal reproductive cycle.

Spermatogenesis : Light and transmission electron microscopic studies of the gonads revealed the presence of six types of spermatogenic cells in both *M. cephalus* and *L. parsia*. These are

the primordial germ cells, the spermatogonia, the primary and secondary spermatocytes, spermatids and spermatozoa. The primordial germ cells are characterised by their irregular outline, eccentric nucleus with distinct nucleoli, large number of cytoplasmic organells and low nuclear cytoplasmic ratio. The spermatogonia are the largest cells in the testis with a regular membrane, an electron dense cytoplasm and a large nucleus with granular chromatin and a smooth outline.

The primary and secondary spermatocytes are synchronously dividing cells which show a conspicuous decrease in cell size and increase in the nuclear cystoplasmic ratio. A number of cytoplasmic bridges are also seen between adjacent secondary spermatocytes.

The spermatids are characterised by a series of substages showing the gradual condensation of chromatin material, formation of the midpiece and the reorientation of the head with respect to the tail.

Spermatozoa are the fully developed male gametes lying freely in the lumen of the seminiferous lobule. They are distinguished by a kidney-shaped head made up of highly condensed chromatin matter, a mid-piece or neck composed of four mitochondria arranged in the form of a collar below the head and a long tail or flagellum showing the typical fibrillar organisation of 9 doublets in the periphery and two singlets in the centre.

The sequence of spermatogenesis and the structure of the various cell types are similar in the two species, but there are differences in the actual size of the cells and the nuclear cytoplasmic ratios.

Environmental factors : The environmental parameters such as temperature, photoperiod, salinity and dissolved oxygen, monitored throughout the period of the study were correlated with the condition factor and the gonadosomatic index (GSI) of the two species. The study revealed that under natural conditions of the study area, where these paramaters were found

to fluctuate within a small range, the reproductive cycle of the two species were governed by an endogenous rhythm. *M. cephalus* showed distinct peaks of GSI during November, May and June while *L. parsia* had a single protracted period of high GSI extending from October to May.

Biochemical changes : Biochemical analysis of protein, carbohydrate, lipid and cholesterol in the testis, muscle, liver and blood serum during the different maturity stages revealed that there was a distinct depletion of these body resources from the somatic tissues during maturation. The depletion was found to be partly due to the translocation of these substances to the testes for the synthetic activity during gametogensis and partly for meeting the energy requirements of the fish during the final stages of maturation, when the fish abstains from feeding.

Qualitative histochemical tests revealed the distribution of the different types of proteins, carbohydrates and lipids in the various cell types of the testicular tissue at each stage of maturity.

Spermatological studies : The studies conducted on the milt of *M. cephalus* and *L. parsia* revealed that the former had a mean sperm count of 7.62×10^{10}/ml of milt while the latter had about 2.69×10^{10}/ml of milt. The motility scores of the milt samples were estimated by using a six point scale. It was observed that within 15 minutes of dilution of milt with sea water at room temperature, the motility score of the milt samples of both the species was reduced to '2'. The undiluted milt of both the species however could be preserved at 5°C up to 48 hours without reduction in motility.

Cryopreservation : Cryopreservation of milt plays an important role in modern aquacultural practices as it reduces the need for maintaining a large number of males in the broodstock and also helps genetic manipulation. Preliminary experiments on cryopreservation were carried out with ten extender solutions. Mrine fish ringer solution formed the major component of seven of these extenders with glycerine and dimethylsulphoxide

(DMSO) acting as cryoprotectants. Chao's extender, Tris-egg yolk citrate and Mounib's extender were the other three extenders used.

Two fertilization experiments were conducted with the milt of *L. parsia* cryopreserved for 15 and 20 days. A fertilization rate of about 70 to 80% was obtained in both the cases. The study was the first of its kind on cryopreservation of milt of marine finfish in India.

CMFRI Spl. Publ., 1993; 56 : 42 - 44

STUDIES ON INDIAN CICHLIDS

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Introduction

The endemic Indian cichlids are represented by two species *Etroplus suratensis* and *Etroplus maculatus*. They occur along the coastal tracts of peninsular India and some of the land locked freshwater lakes of the country. *E. suratensis* the larger of the two, is a delicious table fish and an important species for culture in brackish and fresh waters. Considerable attention has been given in recent years to research and development programmes relating to the culture of this fish. *E. maculatus* due to its smaller size is of lesser economic importance as a cultivable fish. It is however caught in the wild and also reared as an aquarium fish.

In view of wide spread degradation of natural aquatic environments, often with decline or extinction of fish stocks, it has become important to evaluate the genetic diversity of fish resources. During the last decade considerable progress has been made towards understanding the genetic make-up and variability of the wild as well as farmed fish stocks for the management and improvement of their genetic resources. However, our knowledge of these aspects in the Indian cichlids is scarce.

Objectives

The study aims to clarify the taxonomical status of the species through study of morphometric and meristic variation and protein patterns varivation of fishes from different geographical areas of peninsular India.

Attempts have also been made to understand the genetic make-up of populations of both species in Cochin Backwater

through isoenzyme study and to see the variability in biochemical constituents of fish tissues during the life cycle.

Studies on chromosomes and induced breeding have also been made.

Material and methods

The material was collected from Cochin Backwater, estuarine areas of Mangalore, Karwar, Goa, Pondicherry, Muthukadu, Pulicat Lake and freshwater lake of Hyderabad, during December 1981 to December 1985.

Results and discussion

Variations in morphometric and meristic characters of populations of different localities have been described. Morphometric study revealed that the populations of *E. suratensis* in Cochin and Hyderabad, and Karwar and Pondicherry are homogenous, since they showed no significant variation in respect of morphometric and meristic characters considered among them. Populations of Mangalore and Muthukadu, Mangalore and Pulicat Lake, Muthukadu and Pulicat Lake and Karwar and Goa showed homogeneity in respect of only morphometric character; whereas populations of Muthukadu and Hyderabad showed homogeneity in respect of meristic characters only.

Populations of *E. maculatus* showed homogeneity in respect of the morphometric characters considered in Cochin, Muthukadu, Pulicat Lake and Hyderabad, whereas in respect of meristic characters the populations were observed to be heterogenous.

The biochemical parameters such as moisture, protein, carbohydrate, lipid and ash were estimated in the blood, muscle, liver and gonad at each maturity stage. A close relationship was observed between the fluctuations in bio-chemical composition of these tissues with the stage of maturation of gonads. As the maturation of the gonads advanced there was a distinct drain of the body resources to the gonads.

Proteins and isoenzymes were studied electrophoretically in both species. Protein pattern showed variation among tissues, between sexes, maturity stages and in different size groups. Muscle protein patterns varied in different localities also. Expression of isoenzymes lactate dehydrogenase and esterase in *E. suratensis* and for esterase and acid phosphatase in *E. maculatus*.

Various dosages of hormones and steroids were tried to induce spawning in *E. suratensis* in the laboratory conditions.

The present studies carried out on the morphological, biochemical and chemotaxonomical aspects on the two species of Indian cichlids *E. suratensis* and *E. maculatus* form original contributions and considerably add to the present knowledge on cichlids. The results of this investigation, it is hoped, would help in the management and improvement of the aquaculture schemes of Indian cichlids and related species.

CMFRI Spl. Publ., 1993; 56 : 45 - 47

LARVAL BIOLOGY OF SPINY LOBSTERS OF GENUS *PANULIRUS*

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Introduction

The spiny lobsters with their ever increasing demand as a delicacy all over the world have great importance in the commercial fishery. Through the export of marine products from this valuable crustacean, a considerable amount of foreign exchange is earned by our country. The export oriented industry of frozen lobster tail in India wholly depends on the commercial exploitation of the spiny lobsters occurring along Indian Coasts, the important species exploited being *Panulirus homarus, P. polyphagus, P. ornatus* and *P. versicolor.*

In recent years considerable decline in the total production of these lobsters have been noticed indicating necessity for measures of conservation of fishery. One way of augmenting production is through artificial culture of the spiny lobster. This has attracted attention of researchers all over the world and various attempts have been and are being made to culture the spiny lobsters through all the larval stages in the laboratory. However, so far attempts to rear commercially important spiny lobsters from egg through all the phyllosoma stages and subsequent commercial culture have not been successful. To a large extent, knowledge about the biology of the phyllosoma larvae remains to be revealed in order to make commercial culture possible. Development of culture systems largely depends on evolving an efficient technology for commercial rearing of the larvae.

Knowledge about the phyllosoma larvae of spiny lobsters of India is mostly limited to larval samples from nature through plankton collections. A few attempts have also been made by

some authors to rear the larvae in the very early stages. Therefore the Indian spiny lobster *Panulirus homarus* was selected to study the larval biology by hatching the eggs in the laboratory and subsequent rearing of the phyllosoma larvae through the stages and also by conducting experiments to determine optimum conditions of environment to minimise larval mortality and ensure successful growth and moulting of the larvae.

Material and methods

Fully alive berried specimens of the lobster *Panulirus homarus* for hatching and rearing experiments were collected from Kayalpattinam near Tuticorin during January 1984 to June 1986. The phyllosoma larval samples for analysis were obtained from the zooplankton samples collected during cruises of the Fishery and Oceanographic Research Vessel *Sagar Sampada* of the Department of Ocean Development, conducted off Indian Coasts in 1985.

Results and discussion

By keeping live berried specimens in brood tanks in the laboratory successful hatching of the eggs were obtained several times. Experiments conducted on rearing the larvae giving *Artemia* nauplii and jellyfish as food and the different stages obtained are described. In spite of several attempts to rear the larvae through successive moults only 4 stages were obtained in the laboratory. One important observation is that more than one moult occurs between two stages.

The presence of the naupliosoma in the life history of *P. homarus* was confirmed, although it was observed that there is no moulting from this to the Ist stage.

Another important result is the fact that chaetognaths and jellyfishes are acceptable to the phyllosoma larvae as food in addition to *Artemia* nauplii. It was also noticed that the major cause of the larval mortality was the lack of suitable food for different larval stages.

Experiments conducted to determine the influence of Salinity, pH, temperature and dissolved oxygen on the survival and moulting of the larvae in the laboratory yielded useful results. Statistical analysis of results obtained by rearing batches of phyllosoma Ist stage larvae in sea water of different grades of salinity, pH, temperature and dissolved oxygen in triplicate experiments showed that the optimum salinity, pH, temperature and dissolved oxygen to be in the range of 28 to 30 ppt, 8 to 8.6, 31°C and 4ml/1 respectively.

Taxonomic studies on the phyllosoma larvae collected from the plankton indicated the occurrence of different stages of larvae of *P. homarus, P. versicolor, P. penicillatus, P. longipes, P. polyphagus* and *P. ornatus* in the samples analysed. The following stages are described with full details and figures in the original Thesis.

P. homarus	- stages	3 to 9
P. versicolor	- stages	7, 8, 9 and 10
P. penicillatus	- stages	9, 10 and 11
P. polyphagus	- stages	6, 7 and 9
P. ornatus	- stages	4 and 10
P. longipes	- stage	9

The taxonomy of phyllosoma stages collected during one of the cruises of the Research Vessel *Skipjack* in 1983 off Andhra Pradesh Coast is also included in the Thesis.

The contribution adds considerably to the present knowledge of larval biology of spiny lobsters of India and it is hoped that these original contributions would significantly help in developing a technology for artificial culture of spiny lobsters.

CMFRI Spl. Publ., 1993; 56 : 48 - 56

BIOCHEMICAL GENETICS OF SELECTED COMMERCIALLY IMPORTANT PENAEID PRAWNS

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Introduction

In spite of the additional effort, the prawn production in recent years from the fishing grounds, is almost at a stagnant level and this is causing serious concern for the proper management of the fishery. In this context study of population and stock structure and other aspects of the species of prawns contributing to the fishery at various places gains importance. Although a good deal of research work has been carried out on various biological aspects of prawns, the role of biochemical genetics in delineating different stocks, determination of genetic resources, etc. has not received serious attention so far. Therefore a detailed work of allozymic variation to quantify the amount of racial divergence among geographically separated natural populations of *Penaeus indicus* and *Parapenaeopsis stylifera.*

Delineation of stocks, which is one of the most essential parameters necessary for effective management of a fishery is made easier. The results obtained in the present study are expected to help in a big way in solving some of the problems envisaged.

Material and methods

Collection of specimen : Live *Penaeus indicus* was collected from Cochin, Tuticorin, Madras and Waltair. In Cochin Backwater the white prawn was taken from cost net and chinese dip net

catches. Live specimens were also collected from prawn culture laboratory at Narakkal. In addition, collection of *Parapenaeopsis stylifera* and *Penaeus indicus* was made by operations of trawl nets from CMFRI Research Vessel Cadalmin. *P. stylifera* was also collected from Bombay.

Sample preparation : All enzymatic proteins were analysed in 3 different tissues. *viz.* eyes, hepatopancreas and muscles tissues. Prawns of intermoult stages with immature gonad conditions were collected. Tissues taken from freshly sacrificed prawns were dissected in ice cold condition. Tissue was washed with precooled distilled water and the water content was removed by wiping it with blotting paper. Definite quanitity of tissue was measured and homogenized with ice cold distilled water in machanical homogenizer at 80 rpm inside ice box. Then it was centrifuged for 10,000 rpm at 4°C for 20 mts. The supernatant was taken and it was freezed for further use in electrophoresis. The quantity of protein used as the sample loaded for electrophoresis was determined by Lowry's method (1951, *J. Bio. Chem.*, pp. 193-265).

Electrophoretic studies : Zone electrophoresis method using pol-acrylamide gel was employed for separating various enzymatic proteins. Acrylamide with different percentage was tried for best resolution and separation of proteins (Samuel 1987, *Ph. D. Thesis, Univ. Cochin*). 10% concentration of acrylamide which gave good resolution and separation was followed for further genetic analysis of different enzymatic proteins.

Eight different buffers were tried for different enzymes to find out the buffer which helps in good separation of enzymes. Staining of different enzymes was done by histochemical staining methods.

After the calculation of allele frequency, estimation of heterozygosity of observed and expected was found out. Genetic identity and distance of the two species were also calculated.

Results

Alternative enzyme forms derived from different loci are isozymes whereas multiple enzymes encloded by alternative alleles at a locus are allozymes.

Acid phosphatase

Penaeus indicus : Acph-3 locus was found to be polymorphic only in muscle tissue analysed in all the localities. A two banded phenotype of presumed heterozygotes is consistent with a monomer submit structure of the enzyme found with 2 alleles.

Parapenaeopsis stylifera : Acph-2 locus in muscle tissue exhibited two banded heterozygote expression indicating monomer structure of the enzymes. Genetic variation studies in the Acph-2 found in muscle expressed polymorphism in all the localities.

Alcohol dehydrogenase

P. stylifera : Genetic variation studies in eyes and hepatopancreas confirmed Adh-2 locus in hepatopancreas to be polymorphic. Different genotype pattern was observed.

Aldehyde oxidase

P. indicus : Ao-1 locus located in slowest migrating zone in hepatopancreas exhibited polymorphism in all the localities.

Aldolase

P. Indicus : Polymorphism was observed in all locations with 3 alleles.

Alkaline phosphatase

P. indicus : Alkaline phosphatase showed polymorphism in muscle tissue. Phenotypic expression showed a 2 banded heterozygote revealing it to be having monomeric submit structure.

P. stylifera : Muscle was tested for genetic variation. Heterozygote showed 2 bands indicating monomeric structure of the enzyme. Alph-2 locus analysed showed allelic variants.

Esterase

P. stylifera : Exhibited polymorphism in esterase enzyme collected from Cochin and Bombay. Genetic analysis was carried out in eye tissue.

Lactate dehydrogenase

P. indicus : Two zones of lactate dehydrogenase activity were observed. Eye and hepatopancreas expressed one band each having different mobility. Since consistent pattern couldnot be observed these were not utilized for further electrophoretic analysis work.

Malate dehydrogenase

P. indicus : Malate dehydrogenase expressed monomeric pattern by its 2 banded heterozygote nature in eye. Mdh-1 analysed in eye tissue showed allelic variants and polymorphism in all the localities sampled.

P. stylifera : Expressed two bands of enzyme activity like *P. indicus* in eyes and muscle tissue. Mdh-1 locus was found to be polymorphic in all the localities. Two banded heterozygotic nature revealed its monomeric form with 2 alleles.

Malic enzyme

P. indicus : Malic enzyme expressed one loci in eyes and another in muscle. Me-1 found in eyes expressed polymorphism with 2 alleles. Two banded heterozygote was seen.

P. stylifera : Electrophoretic analysis revealed one locus each in eye and muscle. Me-1 in eye was found to show polymorphism at Cochin.

Octonol dehydrogenase

P. indicus : The enzyme expressed zones of enzyme activity in different tissues. The loci observed were Odh-2 in eye, Odh-1 in hepatopancreas and Odh-3 in muscle. The Odh-2 locus showed polymorphism only at Waltair. Phenotypic expressions with one and 3 banded patterns in homozygous and heterozygous nature respectively, suggested a dimeric polypeptide structure for Octanol dehydrogenase enzyme.

6-Phosphogluconate dehydrogenase

P. indicus : There are two loci for 6-Phosphogluconate dehydrogenase giving rise to a fast 6-Pgdh-1 and slow 6-Pgdh-2 bands of activity. 6-Pgdh-2 expressed polymorphism with 2 alleles in all the localities.

Tetrazolium oxidase

P. stylifera : This enzyme exhibited 2 zones of activity. To-1 in hepatopancreas showed allelic variant with a monomer pattern. To-2 expressed low level of allele frequency so it is discarded from further calculation.

Heterozygosity analysis was carried out to find out the amount of genetic variation in the population. So, observed and expected frequency of heterozygotes in these populations was calculated. The difference arose between these two was tabulated for statistical significance as seen in Acph-3, Ald-1, Ao-1, Alpj-1, Mdh-1, Me-1, Odh-2 and 6-Pgdh in *P. indicus*, Mdh-1, Me-1 and To-1 in *P. stylifera* and insignificant in To-2, Ao-2, Odh-2 and Pgdh and Pydh for *P. stylifera*. Significant values obtained in polymorphic loci were due to the excess of homozygotes/deficiency of heterozygotes in these two prawn species.

Genetic variation within prawn populations

Overall estimate of average frequency of heterozygotes per locus H in a population was calculated by averaging observed frequency of heterozygotes (Ho) overall loci sampled. The average frequency of heterozygotes per locus (H) for *P. indicus* in Cochin was 0.0114 ± 0.02, in Tuticorin 0.0114 ± 0.02, in Madras 0.0096±0.02 and in Waltair 0.0142 ± 0.02. Total estimate of average frequency of heterozygotes per locus H is 0.0105 ± 0.02. The same estimate was calculated for *P. stylifera* collected from Cochin and Bombay. The average frequency of heterozygotes per locus H for *P. stylifera* collected in Cochin is 0.3 ± 0.03 and for Bombay it is 0.025 ± 0.03. Total average frequency of heterozygote per locus H was found out to be 0.026 ± 0.03 for *P. stylifera*.

Proportion of polymorphic loci P was calculated for *P. stylifera* in Cochin and it was found out to be 0.304 and for *P. stylifera* in Bombay it was 0.273. For *P. indicus*, the proportion of polymorphic loci was found out to be 0.261 in Cochin, Tuticorin and Madras and 0.33 in Waltair. Average proportion of polymorphic loci per population Pp is 0.2885 for *P. stylifera* and 0.279 for *P. indicus*.

Mean number of alleles per locus (A) seen in each population was found out. For *P. indicus* in Cochin, Tuticorin and Madras it was 1.304 and 1.375 in Waltair. For *P. stylifera*, mean number of alleles per locus was 1.545 in Cochin and 1.575 in Bombay. For these populations in total, the mean number of alleles per locus was found out to 1.558 for *P. stylifera* and 1.322 for *P. indicus*.

Genetic variation between prawn populations : Genetic divergence among *P. indicus* and *P. stylifera* population was quantified by Nei's measure of genetic distance (D) and Genetic identity (I) considered as the average probability per locus of selecting two electrophoretically identical alleles, one from each of 2 different populations. This is calculated directly from gene frequency data with Nei (1972, *Am. Nat.*, **106** : 283-292) definition. Genetic distance (D) is defined as the negative natural longarithmic transformation of I and with average number of amino acid substitutions per protein that have diverged from one another (Nei, 1973, *Proc. Natl. Acd. Sci. USA*, 70 : 3221-3223).

Population samples of *P. indicus* collected from 4 different areas are genetically similar. Genetic identity and genetic distance estimates of Nei & Roger's are given in the Thesis. Likewise, populations of *P. stylifera* which were sampled from Cochin and Bombay are also genetically identical. It shows genetic identity of 0.991 and genetic distance 0.009. According to Roger's analysis, the distance is 0.03 and similarly S is 0.97. In *P. indicus*, the genetic similarity and distance for different enzymes of geographic populations like Cochin-Tuticorin, Cochin-Madras, Cochin-Waltair, Tuticorin-Waltair, Madras-Waltair, Tuticorin-Madras and genetic variants calculated for

different enzymes between Bombay and Cochin population of *P. stylifera* are elaborately given Tables in the original Thesis.

Discussion

Electrophoretic investigations of different isozymes in *P. indicus* and *P. stylifera* have enabled to detect seven polymorphic loci in *P. indicus* and six in *P. stylifera*, out of 23 and 22 loci analysed in the respective species.

The observation of apparent polymorphism in the enzyme octanol dehydrogenase alone in the Waltair samples of *P. indicus* would suggest the probable existence of an isolated population of the species there.

In *P. stylifera* out of the various enzymes analysed acid phosphatase alone showed some difference in the phenotypic distribution and allele frequency between Cochin and Bombay samples.

The lack of goondess of fit as per Hardy-Weinberg equilibrium, in the distribution of different phenotypes in all the population of *P. indicus* and *P. stylifera* tested from samples from different places may be due to deficiency of heterozygotes and excess of homozygotes.

Genetic identity and genetic distance estimates following the analysis of Nei as well as Roger suggests that the population samples from four locations in the case of *P. indicus* and the population samples from two location in the case of *P. stylifera* are genetically similar as seen in American and European species of the lobster (Hedgecock *et al.* 1977, *Biol. Bull.*, 152 : 41-50) and *Limulus polyphemus, Phoronopsis viridis* and *Tridacna mixima.*

Most of the prawn species occupy an extensive geographic area like *P. indicus* found in Indo-Pacific, S. Africa to China, New Guinea and Australia and *P. stylifera* seen in Indo-West Pacific. Besides this they have mixed life cycle, to include a broad-niched species. Long larval period faced by these animals result in unpredictable environment with consequent selection of few alleles.

Berger's (1973, *Biol. Bull.*, **145** : 83-90) findings in genus *Littorina* supports the inverse relationship between the capacity of larval dispersal and the extent of population.

Mixing of prawn populations has been proved by tagging studies. New light on the migration of the *P. indicus* using tagging studies revealed that Tirunelveli Coast is replenished by prawns migrated from the backwaters of Cochin. Suggesting that lack of heterozygosity in *P. indicus* and *P. stylifera* as observed in the present study need not be due to subdivision and separation of these species with genetically different populations.

Mixed life cycle in decapods shows a change during the life cycle from a pelagic and planktonic larva to a free swimming adult. This life cycle heterogeneity might force the animal to perceive its environment as coarsegrained and select a few generalised rather than many specialised alleles. Because of this a low level of genetic variation have been found in decapods with a more heterogeneous life cycle. The tropic encironment for prawn is shown to be highly heterogeneous (Moriarty 1977, *Aust. J. Mar. Freshw. Res.*, **28** : 113-118). Marine invertebrate species such as thirteen penaeid prawn species subject to wide range of physical and tropic environments were characterised by low levels of heterozygosity (Mulley and Latter 1980, *Evolution*, **34** : 904-916).

According to the environment the specialist species, select several narrow-range alleles and is characterised by high heterozygosities. In the generalists the individuals bear "flexible" alleles which are few and wide ranged alleles characterised by low heterozygosities (Smith and Fugio 1982, *Mar. Biol.*, **69** : 7-20). This same observation was seen in *P. indicus* and *P. stylifera*. Penaeid prawns can be included in generalists which occupy wide range environment and feed and exhibit low levels of heterozygosity.

A hybrid environmental heterogeneity trophic diversity model has been proposed to explain genetic variability in decapod Crustacea (Nelson and Hedgecock 1980, *Am. Natl.*,

116 : 238-280). In 51 species of coastal, intertidal, temperate and tropical decapods, the specialist species which are characterised by small, less mobile animals occupying a number of sub-niches has high variability in Group I, Glucose-metabolising enzyme, Aldolase, Alpha Glycerophosphate dehydrogenase, Malate dehydrogenase, Malic enzyme and 6-Phosphogluconate dehydrogenase enzymes and low variability in Group II enzyme, Non-glucose metabolising enzymes Alcohol dehydrogenase, Acid phosphatase, Esterase, Tetrazoliumoxidase, Octanol dehydrogenase, Pyrroline dehydrogenase, Alkaline phosphatase and Aldehyde oxidase and thus act as trophic specialists with narrow range of substrates promoting low variability. In the same way the generalist decapods, species with a fine-grained are characterised by low genetic variation in Group I and high genetic variation, in Group II enzymes. These include large, mobile crustaceans like prawns. As trophic generalists they face a wide variety of food species and heterogeneous tropic environment (Moriarty 1977, *loc. cit.*) showing high variability in the external substrate (Gourp II) enzymes. This habitat specialist-generalist model would classify the decapods as generalists and observed low variability which is observed in the case of *P. indicus* and *P. stylifera* analysed in the present study.

There is little evidence to show that the prawns *P. indicus* and *P. stylifera* are subdivided into two or more genetic stocks. For management purpose all the production of these two species of prawns sampled from different locations, namely *P. indicus* from Cochin, Tuticorin, Madras and Waltair and *P. stylifera* from Cochin and Bombay appear to belong to a single unit biochemically.

Genetic characterisation of *P. indicus* and *P. stylifera* enable to determine the extent to which prawn stock differ genetically along their spatial range of distribution. Delineation of stock is one of the most essential parameter necessary for the effective management of the fishery. Genetics play an important role in breeding and hybridization work to increase productivity through aquaculture. The results obtained at present would definitely help in achieving some of these goals.

CMFRI Spl. Publ., 1993; **56** : 57 - 65

STUDIES ON CERTAIN NITROGEN CYCLE BACTERIA IN THE PRAWN CULTURE FIELDS OF KERALA

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Introduction

In the Southwest coast of India, certain paddy fields found in the vicinity of Cochin Backwater, traversed by tides, are used for a traditional prawn culture. In the perennial fields, prawns alone are cultured throughout the year and in the seasonal fields known as "Pokkali fields", prawns and paddy are cultivated on a rotation basis depending upon the prevailing ecological conditions.

The productivity of these brackishwater fields or ponds depends to a great extent on the physicochemical properties of the water and sediment, particularly the availability of the nutrients such as nitrogen and phosphorus and the production of prawn food organisms. The salinity of the water in these ponds does not remain constant throughout the year, but fluctuates during the different seasons of the year, attaining maximum values during the summer and minimum during the monsoon. Such changes in the water salinity during the different seasons are likely to bring about profound influence on the bacteria associated with the biogeochemical transformation of the nutrients. A series of chemical and biochemical reactions continuously takes place within the bottom soil resulting in the release of nutrients to the overlying water which are mediated by specific groups of micro-organisms including bacteria. The dynamics of these processes influence greatly the growth and population of micro-organisms in these ponds.

Objectives

Despite the extensive studies on the Cochin Backwater detailed investigations on the microbial ecology of these prawn

culture ponds are lacking. Recognising the importance of nitrogen in the productivity of aquatic ecosystems and the involvement of various groups of micro-organisms in the turnover of nitrogenous matter, the present study was carried out on the nitrogen cycle bacteria in selected perennial and seasonal prawn ponds.

Material and methods

The investigation was carried out during 1982 - 1985, at Narakkal (76°14'E - 10°03' N), a fishing hamlet in the Vypeen Island, about 15 km from Cochin. Four ponds were selected for the study : two of which are perennial prawn culture systems (ponds A and B) located within the premises of the prawn culture laboratory of the Central Marine Fisheries Research Institute and the other two are seasonal prawn culture fields (ponds C and D), where prawns and paddy are cultivated during the intermonsoon (October to May) and monsoon (June-September) periods respectively.

Water and sediment samples were collected fortnightly from four fixed sites from each pond for enumeration of the nitrogen cycle bacteria and for monitoring the environmental parameters. Samples collected from the four sites of the same pond were pooled, thoroughly mixed and representative samples were taken. Water samples for bacteriological studies were collected aseptically in sterilized BOD bottles of 300 ml capacity. Sediment samples were collected with a impact corer (100 cm x 4 cm diameter) made of perspex material. Sediment from the upper layer of 10 cm was aseptically removed with a sterile spatula and collected in sterilized glass bottles.

Enumeration of bacterial groups involved in the nitrogen cycle, such as aerobic total heterotrophs, proteolytic, ammonifying, nitrifying (ammonia - oxidizers), denitrifying and nitrogen-fixing, were carried out for two years (October 1982 to September 1984) using standard microbiological methods with a view to study the seasonal variations in their abundance.

Environmental parameters such as temperature, pH, Eh, salinity, nitrite, nitrate, ammonia, dissolved oxygen, organic carbon, total nitrogen and total phosphorus were regularly monitored from all the ponds to study their effect on the distribution and abundance of the bacterial groups in water and sediment. Studies were also carried out on the *in situ* bacterial nitrogen fixation rate fortnightly, using micro-kjeldahl method and the effect of environmental factors on nitrogen fixation was computed.

Linear multiple regression analysis of the data was carried out to determine the influence of the various environmental factors on the distribution of each of the bacterial groups, as well as on the aerobic bacterial nitrogen fixation rate in the water and sediment in the ponds.

Thirty nitrogen fixing *Azotobacter* strains isolated from water and sediment were identified based on their morphological, physiological and biochemical characteristics. Experimental studies were made to elucidate the effect of salinity on the growth and nitrogen fixing ability of thirty isolated strains of *Azotobacter* in the laboratory. Effect of incubation time on nitrogen fixing ability of the thirty strains of *Azotobacter* was also studied by sampling on the 15th, 30th and 45th days of incubation.

Experimental studies were also carried out on nine selected *Azotobacter* strains (*Azotobacter chroococcum* - 3 strains; *A. vinelandii* - 3 strains; *A. beijerinckii* - 3 strains) to evaluate the effect of pH, certain vitamins (cyanocobalamine, biotin, ascorbic acid and thiamine) and trace elements (cobalt - $CoCl_2.6H_2O$; copper-$CuSO_4.5H_2O$; zinc - $ZnSO_4.7H_2O$ and iron - $FeCl_3$) on the growth of the strains. Data obtained from the experiments were statistically analysed, following standard procedures, to ascertain if there were any significant influence of the test parameters on the growth of the bacteria. The optimum requirements for pH, selected vitamins and trace elements for maximum growth of the 9 strains have also been found out.

Results and discussion

Environmental data showed that the physico-chemical conditions, in general, remain more or less steady in both the perennial and seasonal culture systems during the premonsoon period, but major noticeable variations occurred during the monsoon and postmonsoon months as a result of heavy rainfall. The physicochemical parameters, generally, recovered to their premonsoon level by the end of postmonsoon period. Fresh-water run-off from catchment areas and local precipitation during monsoon and postmonsoon had enormous dilution effect resulting in decreased salinity of the water.

Water temperature and Eh of the water and the sediment showed decrease during the monsoon and postmonsoon periods. But the quantum of decrease in temperature was considerably higher for the seasonal ponds. The pH of water and sediment was quite stable in the perennial ponds, with a marginal decrease during the monsoon period; whereas considerable increase of pH occurred during monsoon and postmonsoon periods in the seasonal prawn culture systems. The Eh in both the systems behaved in a similar fashion throughout the period of study, except for more reduction in the seasonal prawn culture fields during the premonsoon season. Depletion of dissolved oxygen content was more pronounced during the premonsoon season in the seasonal ponds as compared to the perennial ponds. The considerable increase in nitrite, nitrate and ammonia levels in water observed during the monsoon and early premonsoon periods resulted in increased primary production, and consequently the culture systems became more alkaline as evident from high pH values during the same period.

Total phosphorus and organic carbon showed increased values during the postmonsoon and in the early monsoon, also in certain months of early premonsoon season. Nitrogen level was more in the premonsoon and monsoon than in the postmonsoon season. The dissolved oxygen level showed more or less uniform values, but for a slight decrease during monsoon.

During most part of the investigation, in all the ponds, sediment was found to harbour more number of bacteria than the water. This is attributed to various factors such as gradual deposition of bacteria from the overlying water, increased propagation of the bacteria indigenous to the sediment; settlement of the particulate substrates during the process of sedimentation. In general, seasonal ponds were found to be more bacterial productive than the perennial ponds due to the decomposition of more organic matter and disintegration of paddy-stumps after harvesting the paddy, and also due to the application of organic manure for the paddy crop.

Heterotrophic and proteolytic bacteria occurred in greater numbers during the permonsoon season than the monsoon and postmonsoon seasons, but the ammonifiers were more in the postmonsoon season than the monsoon and the premonsoon seasons in both the culture systems. Nitrifiers (ammonia-oxidizers) did not show any consistent trend during the period of investigation. Denitrifiers were abundant in premonsoon than the postmonsoon and monsoon seasons. In general, total heterotrophs, proteolytic and ammonifiers were more abundant than the nitrifiers, denitrifiers and nitrogen fixers in all the ponds.

The linear multiple regression analysis of the data revealed that water temperature, dissolved oxygen, salinity, nitrate, nitrite, ammonia, water pH, sediment pH and total phosphorus had a significant influence on the distribution of total heterotrophs, proteolytic, ammonifying, nitrifying, denitrifying and nitrogen fixing bacteria in both the sediment and the overlying water of all the ponds, except for minor changes occurred in some of the groups in certain ponds. Water Eh, sediment Eh, organic carbon and total nitrogen had not shown any significant influence on the different groups of nitrogen cycle bacteria, except for *Azotobacter*. Sediment and water Eh and organic carbon had significant influence on the distribution of *Azotobacter* besides other parameters.

The abundance of ammonifiers, nitrifiers and denitrifiers was negatively influenced by water and sediment pH and NH_3-N in both the water and the sediment. Water temperature had a negative influence on the distribution and abundance of denitrifiers in most of the ponds; ammonifiers in ponds B and C, and proteolytic in pond B sediment. An inverse relationship was found for ammonia and heterotrophic, proteolytic, ammonifying and nitrogen fixing bacteria in pond C. Salinity also had a negative influence on the distribution of total heterotrophs, proteolytic and nitrogen fixers only in pond B water and sediment.

Partial regression analysis indicated that the abundance of *Azotobacter* is mostly influenced by nitrogen. Phosphorus and NO_2-N compared to other parameters like dissolved oxygen, water and sediment Eh and water temperature.

Nitrogen fixation in the ponds was more during the premonsoon season than the postmonsoon and monsoon seasons during the first year; but in the second year, more nitrogen fixation occurred in the postmonsoon than the premonsoon and monsoon seasons. Besides, nitrogen fixation was found to be more in the seasonal ponds than the perennial ponds, and nitrogen fixation rates during the first year was superior to that of the second year. Multiple regression analysis showed that bacterial nitrogen fixation in water was significantly influenced by water temperature, water pH, sediment pH, dissolved oxygen, salinity and NO_2-N and in the sediment by factors like sediment pH, salinity, nitrate and total phosphorus in all the ponds.

Morphological, physiological and biochemical characterisation of 30 different free-living aerobic nitrogen fixing *Azotobacter* strains was carried out to identify them upto the species level. Of these : 13 strains belonged to *A. chroococcum* : 9 strains to *A. vinelandii* and 8 strains to *A. beijerinckii.*

Experimental studies were carried out on all the 30 strains to assess their nitrogen fixing capacity for a period of 45 days. Relative nitrogen fixation efficiency of *Azotobacter* strains

revealed that good nitrogen fixation occurred even upto the incubation period of 45 days in *A. beijerinckii* and *A vinelandii;* but only in one strain of *A. chroococcum* (Azc6) a decline was noticed in nitrogen fixation after 30 days of incubation. Nitrogen fixation was found to be the highest in two strains belonging to *A. beijerinckii* which fixed 11.74 and 11.42 mg NH_3-N/100 ml after the maximum incubation period of 45 days, followed by two strains belonging to *A. chroococcum* which fixed 10.6 mg NH_3-N/100 ml and 10.70 mg NH_3-N/100 ml of medium.

Salinity showed a significant influence on the growth and nitrogen fixation in all the strains of *Azotobacter*. Salinity leveled above 40 ppt and below 10 ppt in general, resulted in reduced growth of *A. vinelandii* and its nitrogen fixation was limited at salinity levels < 15 ppt and > 50 ppt. The estimated optimum salinity levels for growth ranged from 23.52 to 30.82 ppt and nitrogen fixation from 27.17 to 40.83 ppt for the 9 different strains of *A. vinelandii*. Salinity levels below 5 ppt and above 50 ppt were found to be non-conducive to the growth of *A. croococcum* and its nitrogen fixation was depressed at salinity levels < 10 ppt and > 45 ppt. The estimated optimum salinity levels for growth ranged from 23.98 to 27.85 ppt and for nitrogen fixation from 24.49 to 35.88 ppt for the 13 different strains of *A. chroococcum*. For *A. beijerinckii* salinity level below 5 ppt and above 40 ppt were detrimental and their nitrogen fixation also was very low at salinity levels < 20 ppt and > 50 ppt. The estimated optimum salinity levels for growth ranged from 24.01 to 29.96 ppt and for nitrogen fixation from 30.15 to 39.78 ppt for the 8 different strains of *A. beijerinckii*.

pH showed a significant influence on the growth and nitrogen fixation in all the strains of *Azotobacter*. The estimated optimum pH level for growth varied from 7.75 to 8.82 and for nitrogen fixation from 6.64 to 6.81 in *A. vinelandii* strains. In *A. chroococcum* strains optimum growth was at pH 7.59 to 8.28 and nitrogen fixation at pH 7.79 to 7.91. However, in *A. beijerinckii* strains optimum growth was at pH 7.10 to 7.67 and nitrogen fixation at pH 7.12 to 7.52. In general, pH below 6.5

and above 8.5 resulted in reduced growth and nitrogen fixation of the *Azotobacter*.

The selected trace elements *viz.* zinc, iron, cobalt and copper and vitamins had a significant influence on the growth of *Azotobacter* strains. The optimum levels of trace elements ranged from 219.3 to 391.0 µg/l for cobalt, from 132.32 µg/l to 463.43 µg/l for zinc, from 260.05 to 420.87 µg/l for iron, and from 251.81 to 378.26 µg/l for copper for the nine selected strains. The optimum levels of vitamins for growth ranged from 78.25 to 172.36 µg/l of ascorbic acid, 11.42 to 21.91 µg/l of biotin, 14.38 to 43.39 µg/l of thiamine, and 4.26 to 5.57 µg/l of cyanocobalamine.

Conclusions

The present investigation clearly revealed the considerable variation in the relative abundance of selected nitrogen cycle bacterial groups between ponds, between sediment and water and between the different seasons of the year. A number of environmental variables had significantly contributed to the observed variations indicating that the microbial productivity in these prawn culture systems depends largely upon the physical and chemical conditions of the water and the sediment.

Substantial differences were observed in the efficacy of the thirty *Azotobacter* strains to fix nitrogen. Among the 3 species, strains of *A. beijerinckii* are the most efficient with relatively greater nitrogen fixation rates. Most of the *A. vinelandii* strains preferred a salinity range of 15 to 35 ppt for their optimal growth, though they were obtained from ponds with salinities ranging from 1.28 to 36.48 ppt. This indicates that the genera existed in the brackishwater were comprised of strains originated from the estuarine and marine environments and perhaps include halo-tolerant freshwater forms. In certain strains, while the optimum growth occurred at one salinity level its maximum nitrogen fixation was at another salinity level. These results suggest that growth efficiency and nitrogen fixation efficiency of the *Azotobacter* need not have a direct relationship and that

the strains require specific salinity requirements for growth, which is independent of the need for nitrogen fixation.

From the present observations it is clear that the optimum pH for the growth and nitrogen fixation of *Azotobacter* was near or slightly above neutral.

Vitamin and trace element studies revealed that all the strains require optimal levels of cyanocobalamine, thiamine, biotin, vitamin C, cobalt, zinc, iron and copper for normal growth and propagation.

CMFRI Spl. Publ., 1993; 56 : 66 - 72

STUDIES ON THE PHYSIOLOGY OF MOULTING IN THE PENAEID PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Available informations suggest that among 15 species of shrimps and prawns occurring in Indian waters, which are deemed suitable for aquaculture, the Indian white prawn *P. indicus* is identified as one of the most important commercial species. Considering the increasing importance as an accepted species for prawn culture and its good quality for exports, *P. indicus* was selected for the present study. In the life history of prawns, moulting is an important event, which enables the animal to achieve growth. This dynamic physiological event continues through out the life span of the prawn, linking almost all biological activities with this process. Hence, a good knowledge pertaining to the physiology of moulting is imperative to understand the growth process. This knowledge will be of great use in the scientific prawn farming, so as to achieve high prawn production.

Pioneer workers in the past in 1960s initiated the classic studies on crustacean moulting physiology. In subsequent years a great deal of information has been added to this particular field by a number of workers *viz.* Passano, Aiken, Cooke and Sullivan, Stevenson, Skinner and Fingerman. In spite of these great contributions, only very limited attention was received to the moulting physiology of natantians especially of penaeids. In this context an effort is made, through the present work, to study some aspects of the moulting physiology of *P. indicus*.

Objectives

The main objectives of the present study were (i) to draw a scheme for the moult cycle classification and identification of sequential stages of the moulting process, and (ii) to understand the physiology of moulting in relation to following aspects :

a. The structure and function of endocrine systems in relation to moulting process.

b. Studies on the behaviour of important metabolites during the moulting cycle.

c. The structure of the cuticle and mapping of some of the important minerals *viz.* Calcium, Magnesium and Phosphorus and investigation on the mobilisation of these minerals during moulting process.

d. The effect of some environmental and other factors on moulting process.

Results and discussion

Characterisation and classification of complete moult cycle of *P. indicus* have been worked out on the basis of setal development and epidermal retraction in the uropods (setogenesis). Based on the setal morphology, the moult stages were identified as postmoult (stages A and B), intermoult (stage C) and premoult (stages DO, D1', D1", D1"' and D2-3).

The setogenic moult staging was verified on the basis of structural changes in integumentary tissue during the moult cycle. On the basis of cuticle histology moult stages A and B (postmoult), C (intermoult), and D1"' and D2-3 (late premoult) were identified. Moult staging used in the present study was found to be a rapid and simple technique for determining the different stages of the moult cycle. Since excision of appendage is not required, this technique is non-destructive and permits repetitive moult staging of an individual.

The average time duration of one moult cycle with relative duration of each stage was determined in the adult *P. indicus.*

Premoult occupied the major part of the moult cycle (71%) followed by intermoult (18.35%) and postmoult (10.45%).

A linear relationship was observed between the size or age of the prawn and the moult cycle duration. The duration of the moult cycle was found to be more with increase in size or age of the prawn.

Observation on the moulting behaviour showed that majority of the prawns moulted during the late hours of the night, especially between 0000 and 0400 hrs. Prawns were found very active prior to moult and the actual process of ecdysis was very short which lasted only for 30-50 seconds.

Structure of neuroendocrine centres such as X-organ sinus gland complex of eye, brain and thoracic ganglia and their structural changes during the moult cycle were studied using histology and histochemistry. Neurosecretory cells of different sizes and shapes were observed in the above centres. The neurosecretory cells have been classified into five different types on the basis of size, shape and staining characterstics as 1. Giant neuron (G-type cell) with a diameter of 75±12 μ, 2. Large oval cell (A-type cell) with a diameter of 40±17 μ, 3. Small oval cell (B-type cell) with a diameter of 23±4 μ, and 5. Small round cell (D-type cell) with a diameter of 9±4 μ. G type cell is the smallest. Type A and B cells are the most common and widely distributed, whereas the C type cell showed limited distribution.

Histochemical tests showed that the neurosecretory material is predominantly a protein with small amount of carbohydrates and lipids. Strong positive nature of the neurosecretory cells except D type cell, to PAF, CHP and MTP revealed the neurosecretory nature of these cells. Electron microscopial study revealed that the neurosecretory material is made up of hallowed-dense-core vesicle of spherical shape with 120 to 130 nm in diameter.

Except D cell, all the neurosecretory cells exhibited a secretory cycle, with an active neurosecretory phase and passive neurosecretory phase. Secretion and release of neurosecretory

materials were seen in the active phase, while the passive phase showed the non-neurosecretory phase or inactive phase. In the eyestalk, percentage of active neurosecretory cells was high during the postmoult and intermoult when compared to the premoult, whereas in brain and thoracic ganglia, high percentage of active neurosecretory cells was noted in the premoult and low in postmoult and intermoult.

Eyestalk neurosecretory system in *P. indicus* was found to be composed of X-organs and sinus gland. The central axis of the eyestalk was found to consist of the optic ganglia, which was further divided into three different medullae *viz.* medulla terminalis, medulla interna and medulla externa. Two medulla terminalis ganglionic X-organs (MTGXO I and MTGXO II) were detected in the medulla terminalis, while a single medulla externa X-organ (MEGXO) was detected in the medulla externa. The neurohaemal organ in the Sinus gland was located between the medulla interna and medulla terminalis in the latero-longitudinal axis of the eyestalk.

Experimental evidence has been obtained in the role of neurosecretory system of eyestalk on moulting in *P. indicus* through extirpation of the eyestalk. Bilateral eyestalk ablation elicited precocious moulting with a significantly short moult cycle duration. But bilateraly ablated prawns which received an eyestalk extract injection (equivalent of two eyestalks) did not show any accelarated moulting, indicating the presence of moult inhibiting factor in the eyestalk. Unilateral eyestalk ablation has not showed any significant effect on the moult cycle.

The location and the structure of the Y-organ in *P. indicus* have been reported for the first time through the present study. The organ is situated between the mandibular and posterior dorsoventral muscle, in close association with the hypodermis at the junction of prebranchial and branchial chambers. The gland cells have rare cytoplasm with nuclei of oval to spherical shape. Changes were noted in the size of the Y-organ cells during different stages of the moult cycle. Y-organectomy

resulted in the failure of moulting processes indicating the necessity of Y-organ for the succesful completion of moult.

The mandibular organ was found and located using histological techniques in *P. indicus* for the first time. The organ was detected near the posterior central base of the adductor muscle of each mandible.

Changes in the biochemical constituents associated with different moult stages were carried out. General trend of metabolic variation observed was their minimum levels in the postmoult stages and an increase to the maximum concentrations in the premoult stages. Protein, RNA, Lipid, Glycogen and Glucose in different tissues such as haemolymph, muscle and hepatopancreas followed this trend. DNA values in muscle and hepatopancreas, Glucosamine in heamolymph, Chitin in cuticle and water content in muscle and hepatopancreas showed higher values in the postmoult and lower values in the premoult. The premoult increase of metabolites in body tissues can be due to the absorption from the food, synthesis and active resorption of the organic material from the chitino-protein complex of the exoskeleton, while postmoult fall of metabolites can be attributed to the utilisation of these in the energy cycle, chitin synthesis during the process of ecdysis and neocuticular synthesis.

Distribution and mobilisation of calcium, magnesium and phosphorus in relation to different stages of moult cycle were studied in the prawn *P. indicus*. Calcium is detected as the principal inorganic component of the exoskeleton (15.95%), while magnesium (1.19%) and phosphrous (1.2%) were relatively minor component of the exoskeleton. Different regions of the exoskeleton showed variation in calcium, magnesium and phosphrous concentrations. Variation of calcium, magnesium and phosphrous content in haemolymph, muscle, hepatopancreas and exoskeleton showed substantial difference between the moult stages of the animal.

All the three minerals in haemolymph gave the maximum values in late premoult stages, followed by a decline through the

postmoult to reach the minimum valus in intermoult and early premoult stages. In muscle, a gradual and linear increase of mineral (calcium, magnesium and phosphorus) accumulation was noted from the postmoult to the premoult stage. Hepato-pancreatic calcium and magnesium remained high in late premoult and early postmoult stages, while phosphorus gave lower values in late premoult stages. Minimum values for calcium and magnesium in hepatopancreas were recorded in stages C and Do, whereas minimum phosphorus values were obtained in stage A.

In the exoskeleton, the lowest concentrations of calcium and magnesium were recorded in stage A, which showed a rapid increase from stage B to stage C to touch the maximum values in intermoult and early premoult stages. A very sharp fall in the calcium concentration was noted between the stages D2 - 3 and A. In the case of exoskeletal phosphorus, values remained high in late premoult and early postmoult stages, and minimum in the intermoult stage C.

Effect of important environmental factors such as temperature, pH, salinity, light and other biological factors *viz.* autotomy and starvation on moult cycle of the prawn *P. indicus* was studied. Among the exogenous factors, temperature was found to have the major influence on moulting process. Salinity and pH also influenced the physiology of moulting, but light did not show any notable effect on the moult cycle.

Both lower and higher temperatures were found not to favour the occurrence of normal moulting. Prawns kept in high temperature of 37°C died in a few hours. A fast growth with short moult cycle duration was observed among the prawns exposed to 31°C (optimum temperature recorded in the present study), while low temperature (26±1°C) gave poor growth with considerably extended period of moult cycle.

In the extreme pH of 5±0.2 (acidic) and 10±0.2 (alkaline) all the experimental prawns died within 24 hrs. Prawns exposed to a pH of 6±0.2, and 9±0.2 became very weak and 50% of the

animals died during their effort in exuviation. In the present experiment, the optimum pH which favoured normal moulting and good growth was found to be at 8±0.2.

Prawns were allowed to moult in a wide range of salinities *i. e.* from 5‰ to 45‰. 15‰ was found to be ideal for the animals with shortest moult cycle duration and fast growth. In 5‰ and 35‰ the moult cycle duration was extended and growth was poor when compared to 15‰. Most of the animals kept in 3‰ and 45‰ salinity failed in their moulting efforts.

Experiment with different light periods has not revealed any significant effect over the moult process of the species.

Starvation experiments conducted in the present work indicated the importance and prime necessity of proper feeding for the successful moulting process. Prawns made to starve showed considerably extended moult cycle. Starving condition blocked the progress of moult, finally leading to the death of the organisms. In the present study autotomy has not revealed any significant effect on the moult cycle of the prawn.

It is well known that moulting in crustaceans is the indirect version of growth process. Hence to achieve faster growth rate and higher production of animals in aquaculture techniques, a good knowledge of different aspects of moult process and its control is very much necessary. In this context the new informations generated in the present investigation on moulting physiology of *P. indicus* would certainly help the scientific prawn farmers in enhancing prawn production by better management.

Publication

K. K. Vijayan, K. Sunilkumar Mohamed and A. D. Diwan 1993. On structure and moult controlling function of the Y-organ in the prawn *Penaeus indicus* H. Milne Edwards. J. *World Aquacult.* (in Press).

CMFRI Spl. Publ., 1993; 56 : 73 - 76

NUTRITIONAL REQUIREMENTS OF THE FRY OF GOLD-SPOT MULLET *LIZA PARSIA* (HAMILTON)

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Objectives

The aim of the study was to (i) quantify the requirement of the nutrients, (ii) identify some suitable protein and lipid sources and (iii) to determine the suitability of some compounded feeds, all for the successful rearing of the fry of *Liza parsia* in the nursery phase.

Material and methods

A series of statistically designed feeding experiments with *L. parsia*, in the laboratory preceeded a pond trial of a few compounded diets. The duration of the experiments ranged from 7 to 21 weeks and the abiotic factors were monitored regularly. Food was offered at 7% of the body weight to the fry maintained in the laboratory. The response of the fry to the diets were guaged from survival, growth, food utilization and biochemical indices.

Experiments, results and discussion

To determine the protein requirement, isocaloric purified diets incorporating graded levels of protein ranging from 0 to 60%, at intervals of five, were fed to the fry for a period of 10 weeks. Casein and gelatin were the main protein sources and the lipid component of the diet came from cod liver-oil and corn oil. Dextrin was the carbohydrate source. Mineral and vitamin mixtures were included to balance the dietary composition. The animals clearly elicited response to varying quantity of protein in the diet. Based on growth, food conversion, protein utilization, digestibility and nutrient retention, the optimum

level of dietary protein for the fry was found to be around 40%. It was also found that excretion of ammonia by the fry was directly related to the level of protein in the diet.

A ten week experiment was conducted to determine the optimum level of lipid required in the diet of *L. parsia*. Isocaloric, isoproteic diets were fed to the fry. The protein in the diet was fixed at 40% and the lipid levels ranged from 0 to 12 at intervals of 2%. Corn oil and cod liver-oil were used in equal proportions. A well defined gradation was observed in the weight gains as the lipid level in the diet increased. Conversion efficiency also showed a similar trend. The retention efficiencies and digestibilities were better in the range 4 - 8% lipid levels. Considering the cumulative effects of all the aspects studied, a dietary lipid level of 6 - 8% is suggested for mullet fry.

Two experiments were conducted simultaneously for twentyone weeks to determine the qualitative requirement of the major water soluble vitamins and to determine the optimum level of incorporation of the vitamin mixture in the diet. The deletion technique was adopted to determine the essentiality of choline, inositol, ascorbic acid, nicotinic acid, pantothenic acid, riboflavin, thiamine and pyridoxine. Survival was poor when riboflavin and niacin were deleted. Niacin deletion also resulted in poor growth. The food conversion was low when pyridoxine and niacin were excluded. Protein efficiency ratio was maximum affected when pyridoxine was not included in the diet. Body composition and nutrient retention efficiency data also prove the essentiality of the vitamins. A host of clinical symptoms associated with vitamin deficiency was recorded. They included anorexia, erratic movements, photophobia, fin degeneration, body lesions, haemorrhagic damage, etc. The characteristic deficiency symptoms observed were corneal opacity for riboflavin, gill damage for pantothenic acid and scoliosis/ lordosis in the case of ascorbic acid. Histopathological and haematological observations were also made.

The optimum level of vitamin mixture to be included in the fish diet was also determined by incorporating the mixture

at 0.5, 1, 1.5, 2 and 2.5% in the diet. Data on growth response, conversion efficiency and nutrient retention indicate vitamin levels of 1.5 to 2% is adequate in the diet. It is recommended that the vitamin level in the purified diet should not be below 1%.

A seven week experiment was conducted with diets containing selected protein sources. In all, three plant protein sources (groundnut oil cake, soyabean meal and algal meal) two animal protein sources (fish meal and prawn waste) and three combination of sources were tested. The protein content of the diets were fixed at 40%. Groundnut oil cake was identified as better among the plant sources and fish meal among the animal sources. The combination diets offered to the mullet proved to be superior to the diets based on individual feed stuffs. It is therefore suggested that equal proportion of plant and animal proteins (especially fish meal) be included when compounding diets.

The nutritive evaluation of a variety of lipid sources was completed in a seven week experiment. The plant oils tested were from gingely, soya, groundnut and sunflower. The animal oils were from sardine, shark liver, cod liver and beef tallow. The mixed oil diets were of the following combination : sardine oil, groundnut oil, soyabean oil; shark liver-oil, sardine oil, groundnut oil; and gingely oil, soyabean oil, groundnut oil. Cod liver-oil and sardine oil were found to be better among the individual sources followed by plant oil from soyabean. The diet containing the mixture of sardine oil, groundnut oil and soyabean oil, was the best among the diet tested. it is recommended that a mixture of plant and marine fish oil should be used as lipid sources, while compounding diets for mullet fry.

The pond trial was conducted over a period of seven weeks to test the efficacy of five formulated diets. The mullet fry were reared in velon hapas fixed in brackishwater ponds. Diets were tested in duplicate and the fishes were offered a daily ration of 4% body weight. The ingredients used in the diet preparation were groundnut oil cake gingely oil cake, coconut

oil cake, rice bran, mangrove leaves, fish meal and prawn waste. Tapioca powder and wheat flour were mainly intented as binders. The protein content of the diets was kept at 35% except for a lower protein diet were it was 28%. The diet incorporating all the mentioned ingredients, maintaining protein level at 35% provided the best weight gain. Exclusion of minerals and vitamins from diets applied in field condition did not seem to drastically affect growth. When plant ingredients alone was provided to the fry it was equally accepted under pond conditions. Lowering of protein in the diet induced a drop in weight gain of the fry in the pond. When no supplementary feed was provided, hardly 1/3 rd growth was recorded as compared to the wholesome diet with the protein level of 35%. This outlines the importance of artifical feeding in the nursery phase of *Liza parsia.*

CMFRI Spl. Publ., 1993; 56 : 77 - 82

STUDIES ON THE REPRODUCTION OF INDIAN SAND WHITING *SILLAGO SIHAMA* (FORSKAL) (SILLAGINIDAE, PERCOIDEI)

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Introduction

'Sand whitings' belonging to the family Sillaginidae are esteemed food fishes occurring in shallow estuarine and near coastal waters in the West Indo-Pacific region. They hold good potential for coastal aquaculture. *Sillago sihama* is the most widely distributed species (Mckay 1985, *Mem. Qld. Mus.*, 22 : 1-73).

Some information on their biology notwithstanding, a comprehensive knowledge on maturation and gametogenesis in sillaginids is lacking. Further, their taxonomic status is largely conjuctural. Keeping these lacunae in view, investigations on different aspects of reproduction in *S. sihama* along with a systematic study of the family Sillaginidae from the Palk Bay and Gulf of Mannar were taken up.

Objectives

i. Systematic study of sand whitings from the Palk Bay and Gulf of Mannar.

ii. Histological, histochemical and biochemical studies on gonadal maturation.

iii. Preliminary studies on the induced maturation and spawning.

Material and methods

Samples were drawn from selected landing centres in Ramanathapuram District of Tamil Nadu (79°10'E - 09° 10'N) during April 1984 to March 1986. Sampling was done from gears

such as shore seines, trawl nets and a type of stake net locally known as 'Kalamkattivalai'.

Taxonomic study was based on the analysis of selected morphometric and meristic characters as described by Mckay (1985, *loc. cit.*). Histological and histochemical studies were carried out using standard techniques (McManus and Mowry 1960, *Staining Methods - Histologic and Histochemic.* Harper & Row, New York; Pearse 1968, *Histochemistry - Theoritical and Applied.* J & A Churchill Ltd; Coolidge and Howard 1979, *Animal Histology Procedures.* NIH No. 80-275).

Biochemical composition of muscle, liver, ovary and testis was estimated (Proteins - Lowry *et al.* 1951, *J. Biol. Chem.*, **193** : 265-275; Lipids - Folch *et al.* 1957, *J. Biol. Chem.*, **226** : 497-505; Total carbohydrates - Dubois *et al.* 1956, *Anal. Chem.*, **28** : 350-356; Glycogen - Caroll *et al.* 1956, *J. Biol. Chem.*, **220** : 583-593; Cholesterol - Varley 1962, *Practical Clinical Biochemistry.* John Wiley & Sons; Glucose - Nelson 1944, *J. Biol. Chem.*, **153** : 373-380; Somogyi 1945, *J. Biol. Chem.*, **160** : 69-73).

Preliminary studies on induced maturation and spawning were conducted at Narakkal, collecting the specimens from Cochin Backwater. A total of 9 females and 2 males were subjected to experiments during October and November 1985.

Results and discussion

Fishes of the family Sillaginidae from the Palk Bay and Gulf of Mannar : Six species were collected and very briefly their field identification characters are given below :

Sillago (Sillaginopodys) chondropus (Bleeker, 1849)

Cheek scales in 3 rows; first ventral fin ray modified into a laterally compressed club-shaped structure; swimbladder reduced.

Sillago (Sillago) sihama (Forskal, 1775)

Cheek scales in 2 rows; minute discrete black spots along the flanks below lateral line; swimbladder with a pair of anterior extensions, a pair of lateral extensions and another pair of

posterior extensions of unequal length; a median duct-like process present.

Sillago (Parasillago) indica Mckay, Dutt & Sujatha, 1985

A dark band on each flank; very stout swimbladder bearing highly convoluted third pair of tubular extensions.

Sillago (Parasillago) vincenti Mckay, 1980

Cheek scales in 2 rows; second dorsal with 5-7 rows of black spots; anterior extension of swimbladder short and bulbus; single postcoelomic extension.

Sillago (Parasillago) argentifasciata Martin & Montalban, 1935

A well pronounced silvery band along each side of the body; swimbladder lanceolate with median tubular duct-like process absent.

Sillago (Parasillago) soringa Dutt & Sujatha, 1983

Lanceolate swimbladder with a median finger-like extension and a pair of recurved extensions at the anterior end.

Maturation and spawning : Five maturity stages *viz.* Immature, Maturing, Mature, Ripe/Oozing and Partially spent were recognized. Ripe ovary contained three major modes (0.23, 0.38 and 0.88 mm) of ova. The maturing and mature groups of ova pass in succession to advanced stages, when fresh batches of maturing ova from the immature stock take their place. The fish spawns more than once in the spawning season. No completely spent female or male could be collected in the present study.

Occurrence of advanced maturity stages and monthly GSI values indicate a prolonged breeding season in *S. sihama*, extending from about July to February with peak spawning in November. Females and males mature for the first time at 179 and 159 mm respectively. Fecundity in 17 individuals of length range 150-210 mm varied from 6,956 to 48,373. Overall female to male ratio was 1.1:1.0 ($X^2 = 4.86$; $P < 0.05$). Males dominated till 170 mm and females above this length.

Histology and histochemistry of gonadal maturation : Previtellogenic phase in oocyte growth is constituted by primary oocytes and vacuolated oocytes, while the vitellogenic phase by the yolk granule oocytes.

Zona radiata of vitellogenic oocytes has a bipartite structure with an inner finely striated zona radiata interna (ZRI) and an outer basophilic, homogenous layer zona radiata externa (ZRE). Carbohydrates and RNA are more in ZRE and it is sudanophilic, while ZRI does not contain lipids. ZRI of yolk granule oocytes show undulations indicating the micropynocytotic activity associated with yolk deposition at the oocyte surface.

Of the yolk bodies, lipid yolk appears first in the perinuclear ooplasm of vacuolated oocytes followed by carbohydrate-rich yolk vesicles and protein-rich yolk granules in the cortical ooplasm. Toward the end of the vitellogenesis, the oil droplets coalesce and yolk appears as a homogenous mass.

Monthly mean percentage frequency distribution of oocyte classes indicate prolonged breeding season in *S. sihama*. Study of relative volume of various oocyte stages suggest that yolk granule oocytes and hyaline oocytes are relatively more important in the mature and ripe ovaries respectively.

Histological examination of testes reveals that they belong to the 'lobular' type, since each testis is made up of a number of seminiferous lobules and to 'unrestricted type' since each lobule consists of cysts of different spermatogenic cells. The interlobular space is made up of connective tissue with few Leydig cells and blood vessels. Six types of spermatogenic cells *viz.* primordial germ cells, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are identified. The type of spermatogenesis is 'cystic' (Lofts 1968, *In : Perspectives in Endocrinology - Hormones in the lives of lower vertebrates*. Academic Press, London, pp. 239-251), with few cysts in the same phase of development occurring synchronously inside the lobules. One of the morphological changes

associated with spermeogenesis *viz.* shrinkage of nucleus was evident.

Biochemical changes in muscle, liver, gonads and blood plasma : In *S. sihama*, the inverse relationship of moisture content with protein and lipid exists only in the ovary throughout the reproductive cycle, while in the muscle and liver such a relationship was not constant in all the maturity stages. In male, except in liver, the inverse relationship of moisture content with other components was not apparent in other tissues.

Increase in liver protein content of female in stage II followed by a gradual decline till stage IV suggests initial accumulation of yolk precursors in liver and their subsequent translocation to the ovaries in the advanced maturity stages. The increase in ovarian protein content between stages II and III is corroboratory of the pattern of protein translocation in the liver.

Cholesterol contents in both ovary and testis exhibited increase during the early maturing stages and plummeted in the final stages of maturation and spawning. This explains its utilization for the synthesis of steroids. Blood glucose level in both sexes gradually increased with advancement of maturation which may possibly be due to active feeding during the pre-spawning period.

Protein, lipid, glycogen and cholesterol contents in female suffered comparatively more depletion than those in male in the advanced stages. Suggesting more energy expenditure in the female for spawning.

Preliminary experiments on induced maturation and spawning : Results reveal that ovarian maturation can be induced by major carp pituitary gland extract (60, 100, 120 and 150 mg/kg body weight), HCG (3000 & 5000 IU/kg body weight) or a combination of both (60 mg p.g + 1000 IU HCG/kg body weight). Female was found to spawn after receiving either 150 mg/kg of p.g extract or 3000 IU HCG/kg. Males in 'non-seminating' condition, developed to 'oozing' stage by administering 280 mg p.g extract or 1000 & 2000 IU HCG/kg.

Conclusions

The results of the present work have cleared the confusion about the taxonomy of sand whitings from the Palk Bay and Gulf of Mannar, contributed new information about the finer aspects of reproduction in *Sillago sihama* and showed that this species could be induced to spawn in the laboratory.

Publication

1. On the sillaginid fishes of Palk Bay and Gulf of Mannar with an account on the maturation and spawning in Indian sand whiting *Sillago sihama* (Forskal). *Indian J. Fish.* (In Press).

CMFRI Spl. Publ., 1993; 56 : 83 - 87

STUDIES ON THE DIGESTIVE ENZYMES OF THE CULTIVABLE GREY MULLET *LIZA PARSIA* (HAMILTON-BUCHANAN 1822)

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Objectives

The present investigation was carried out with a view to understand and suggest solutions to the following problems : a. What kind of qualitative and quantitative distribution profiles the digestive enzymes envince in *L. parsia*; b. How does the digestive enzymes respond to the changes in the physiochemical factors prevailing in the habitats; c. Are the digestive enzymes linked up with the size, food and feeding habits of the fish, and d. Is there any change in the relative contribution of various digestive enzymes in response to changes in the dietary composition.

Programmes

The present study included the following programmes of work : a. Assessment of the distribution pattern of digestive enzymes along the various regions of the alimentary canal; b. Characterisation of digestive enzymes in relation to different physicochemical parameters; c. Studies on the relationship between digestive enzyme activity and size of the fish; d. Observations on changes in the digestive enzymes in relation to feeding habit; e. Investigation on the changes in digestive enzyme activity after different hours of feeding, and f. Assessment of the effect of dietary composition on the digestive enzymes.

A thorough review of literature revealed that works on nutrition and digestion of mullets are rather scanty and limited to few reports from abroad and in India.

Material and methods

The mullet *L. parsia* were collected from Cochin Backwater nearby Vypeen Island using Chinese dipnets and the fishes were transported to the laboratory and subjected to further studies. During feeding experiments, they were maintained at room temperature in FRP tanks filled with filtered sea water. The relative length of gut for the adult mullet was 2.4 (±0.32) times of the length of the fish.

The crude enzyme extracts of the digestive tract was prepared using the different regions, oesophagus, cardiac stomach, pyloric stomach, pyloric caeca, liver, gall bladder, spleen, anterior intestine and posterior intestine. The crude extract from each region was assayed for the various carbohydrases, proteases, lipases and phosphotases. Of all the regions, pyloric caeca followed by the intestine were the major areas of enzyme activity.

Results and discussion

Carbohydrases assayed in the crude enzyme extract of the digestive tract included maltase, dextrinase, sucrase, trehalase, alpha-glucosidase, raffinase, melibiase, cellobiase, salicinase, beta-glucosidase, betagalactosidase, amylase and cellulase. However maltase dextrinase, sucrase, trehalase, alpha-glucosidase, raffinase, cellobiase, salicinase, beta-glucosidase, beta-galactosidase, and amylase were present in the digestive tract. Of all the carbohydrases, amylase was the major enzyme found in the various regions.

Proteases assayed in the present study included acid protease-pepsin, total alkaline protease, endoproteases-trypsin, chymotrypsin and elastase, exoproteases-carboxypeptidase A, carboxypeptidase B and leucine aminopeptidase. All the enzymes were present in various regions of digestive tract. Pyloric caeca and intestine were the major areas of enzyme activity. Pepsin was the major enzyme in the cardiac stomach region. Elastase and carboxypeptidases A and B were found only in meagre quantities. Acid phosphatase, alkaline

phosphatase and esterase activities varied in their intensities in different regions.

The different group of digestive enzymes such as amylase, pepsin, total alkaline protease, trypsin, chymotrypsin and leucine aminopeptidase were characterised with respect to pH, temperature, chloride ion concentration, effect of inhibitors and metal ions. Amylse activity was optimum at pH 6.6 and temperature 52°C. Chloride ion was found to have no significant effect. Pepsin activity was optimum at pH 2.0. Total alkaline protease activity was optimum between pH 7.5 and 8.5 and there was also a smaller peak between 9.4 and 10.0. The optimum temperature was between 47°C and 60°C. Trypsin and chymotrypsin had optimal pH at 8.0 and 7.5 respectively.

Effect of inhibitor substances on the activity of alkaline protease, trypsin and chymotrypsin was conducted using PMSF, TLCK, TPCK, EDTA and SBTI. PMSF inhibited total protease, trypsin and chymotrypsin activities. TLCK inhibited total protease activity partly and trypsin completely, but not chymotrypsin. TPCK inhibited total protease moderately and chymotrypsin significantly. Whereas trypsin was not inhibited. EDTA had no effect while SBTI inhibited all the three activities. a. Effect of various metal ions on the activities of protease, trypsin and chymotrypsin was studied. Hg^{2+}, Cu^{2+}, CO^{2+} and Ni^{2+} strongly inhibited the enzyme activities, and b. Leucine aminopeptidase had a pH optimum of 8.4 and temperature optimum of 52°C.

The different digestive enzymes such as amylase, alpha-glucosidase, beta-glucosidase, beta-galactosidase, pepsin, total alkaline protease, trypsin, chymotrypsin, carboxypeptidase A and B, leucine aminopetidase, esterase, acid and alkaline phosphatases, were analysed in different size groups of *L. parsia* ranging from 1-2 cm to 19 cm in total length. Activities of amylase, alpha-glucosidase, beta-glucosidase, beta-galactosidase, and pepsin, were found to increase with increasing size; and activities of total protease, trypsin, carboxypeptidase A and B,

leucine aminopeptidase and acid phosphatase decreased as the size of the fish increased.

Studies on intestinal feeding index showed that the fish feeds only during the day with high intensity during the midday. The digestive enzymes, amylase, alpha-glucosidase, beta-glucosidase, beta-galactosidase, protease, trypsin, chymotrypsin and leucine aminopeptidase assayed from the anterior and posterior intestines exhibited a positive correlation with intestinal feeding index indicating that the active phase of digestion is in tune with feeding intensity.

The sequential changes in the digestive enzyme activity in the digestive tract of juveniles of *L. parsia* after feeding was investigated by monitoring the pepsin in the stomach tissue and contents and the amylase, alpha-glucosidase, beta-glucosidase, beta-galactosidase, total protease, trypsin and chymotrypsin in the intestinal tissue and its contents. All the enzyme activities tested were higher in the tissues of the organs than that of their respective lumen contents. All the enzymes recorded a decline at five hours after feeding in the intestinal tissue while recording a peak activity in the intestinal contents indicating that these enzymes are secreted into the lumen of the alimentary canal in response to the ingested diet. Active digestive process takes place around five hours after feeding. *L. parsia* is physiologically equipped to utilize infrequent and irregular meals effectively and has rapid digestive enzyme secretory response and has no intrinsic digestive enzyme cycle.

Effect of dietary composition on the relative contribution of various digestive enzymes was studied by feeding diets with different relative composition of protein, carbohydrate and fat in different groups of juveniles of *L. parsia* maintained in triplicates. The level of digestive enzymes were found to be adapted to the dietary composition.

Conclusions

The results of the present investigation embodied in the Thesis, besides enhancing our knowledge on the digestive

physiology of mullets, throws more light on the following aspects when this species is desired as a candidate species for aquaculture. *L. parsia* have full complement of various digestive enzymes, which can hydrolyse a variety of substances, and increased RLG which would increase the gut passage time and maintain an active phase of digestion over a longer duration. They posses the biochemical mechanism to adapt the secretion of the digestive enzymes to the composition of the diet. Since *L. parsia* has no intrinsic digestive enzyme cycle, other than that induced by the ingestion of diet, and have a rapid digestive enzyme secretory response, optimum ration and feeding frequencies can be established purely on the basis of maximum growth and most favourable food conversion ratios.

CMFRI Spl. Publ., 1993; 56 : 88 - 94

STUDIES ON THE DIGESTIVE ENZYMES OF THE INDIAN WHITE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

In intensive prawn culture operations, one of the foremost requirements is the availability of proper formulated practical feeds for the different stages of the prawn in the cultured ponds. These practical feeds should contain adequate levels of the essential nutrients to provide maximum growth, survival and best conversion efficiencies. The efficacy of a diet not only depends on its nutrient composition and nutrient balance, but also on the effective utilisation of the nutrients by the animal. In the utilisation of the dietary nutrients, the digestive enzyme play the vital role of catalysing the hydrolytic reactions splitting the macromolecules into simple absorbable molecules. The activity of these biocatalysts is regulated by so many biological, chemical and physical factors and thus any shift from the optimum conditions necessary for these enzymes may affect their activity, thereby correspondingly modify the digestibility of the nutrients supplied to the animals. Therefore, information on the digestive enzymes and their preferential conditions of activity would greatly help in rationally adjusting the quality and quantity of feed supplied to prawns during the different stages in their life cycle.

Objectives

The present investigation has been carried out on the Indian white prawn *Penaeus indicus* with reference to the following objectives.

(i) To delineate the morphology, histology and histo-

chemistry of the digestive system, as well as the ultrastructure of hepatopancreas of the adult prawn.

(ii) To survey the activity of digestive carbohydrsaes, proteases and lipases.

(iii) Detailed studies were undertaken on the biochemical characterisation of alpha amylase and activity pattern with respect to pH, temperature, substrate concentration, enzyme concentration and incubation time, and also the influence of certain metallic ions, amino acids and vitamins. Isolation and purification of ∝-amylase using gel filtration chromatography and disc gel electrophoresis was also carried out.

(iv) Independent experiments were conducted to determine the effect of diet, starvation and eyestalk ablation on the activity of digestive enzymes.

(v) Digestive enzyme activity in relation to size groups like postlarvae juveniles and adults was investigated.

Material and methods

The specimens for the study were collected from Marine Prawn Hatchery Laboratory (MPHL), Narakkal and transported alive in polythene bags and plastic tubs to the laboratory and maintained in plastic pools well aerated for 2 days prior to dissection. Aqueous extracts of hepatopancreas were made from a specific number of glands, ground well using a glass tissue homogenizer in ice with required volume of cold double distilled water. The extract was centrifuged at 20,000 rpm at 0° - 5°C for 30 min in refrigerated centrifuge and the clear supernatent taken for the estimation.

Enzyme assays : Amylase activity was measured according to the method of Bernfeld (1955) using starch as substrate. Protease was assayed by Anson's colorimetric method using casein. Lipases was estimated by the method of Cherry and Crandall (1932) using olive oil as substrate. Protein was determined by Lowry *et al.* (1951) using Bovine serum albumin as standard.

For histological preparations of the digestive glands and tissues adult prawns were dissected in ice and tissues transferred to Bouin's fixative. Standard procedures were followed and the sections were cut at 12 μ thickness and stained in Heidenhain's Iron haematoxylin counter stained in Eosin. For histochemical studies small tissue pieces were fixed in 10% neutral buffered formalin. Standard routine tests to detect protein, carbohydrates, lipids, DNA and RNA were conducted.

Ultrastructural studies were carried out using small pieces of the hepatopancreas fixed in chilled glutaraldehyde at 4°C (3.5% in phosphate buffer at pH 7.0) for 4 hours. After post-fixation for another 2 hours in buffered OsO_4 (1%), the tissues were dehydrated in ethanol and embedded in Spurr medium. Thin sections were stained for 15 mins in uranyl acetate and for another 5 mins in lead citrate. Electron microscopy was performed with Carl Zeiss EM 109 R electron microscope.

Results and discussion

In *Penaeus indicus*, the alimentary canal shows three distinct regions : the foregut, the midgut and the hindgut. The mouth is a big opening on the ventral surface of the cephalic region and is bound by portions of the mouth parts. Leading from the mouth is the buccal cavity. The oesophagus is a very short tube running vertically downwards and leads into the stomach. The stomach has cardiac and pyloric regions. The grooves on the floor of the cardiac stomach serves for the passage of the digestive secretions from the gland which gets mixed up with the food ingested. The posterior pyloric stomach is partly embedded in the digestive gland and is divided into a dorsal channel which leads directly into a long simple midgut and a ventral filter press which allows only fine particles to pass into the digestive gland. The midgut is a long narrow tube with slender walls, stretching from the thoracic region to the abdomen. The hindgut is a very short tube with thick muscular walls, connecting the midgut and the anus. The anus is a slit-like opening, with muscular walls.

The brownish-red coloured hepatopancreas is made up of loosely arranged hepatopancreatic tubules, each tubule in cross section showing a characteristic four rayed shape. The lobulations are called 'acini' and each acinus has a lumen. Different acini joined together to form the duct of hepatopancreas. The outermost layer of connective tissue is the tunica propria with muscular fibres and also the basement membrane.

Under light microscopy, the epithelial layer showed three different types of cells, secretory, absorptive and embryonic cells. In the embryonic cells, the nucleus was prominent and comparatively large with granular chromatin material distributed evenly in the nucleoplasm. Nucleolii ranges from 1 to 3 and has a basophilic, homogeneous cytoplasm. Secretory cells were large swollen cells with prominent vacuole towards the distal end.

Showed positive reaction to all protein, lipid and carbohydrate tests. The absorptive cells showed slight traces of acid mucopolysaccharide material in the cytoplasm. They are with large deposits of lipids and with thick brush borders.

Ultrastructural studies revealed 4 cell types classified as E, F, B & R cells according to the scheme of Jacob's (1928). E (Embryonic) cells are with few mitochondria, centrally located nucleus with golgi complexes and rough endoplasmic reticulum. Cytoplasm posses abundant free ribosomes and vesicles of smooth endoplasmic reticulum. R - cells (Absorptive cells) are prominent by the presence of lipid material and cytoplasm with large number of mitochondria. F - cells (Storing cells) contain abundant rough endoplasmic reticulum, the cisternae of which are filled with a flocculent material. Cytoplasm with abundant free ribosomes. B-cells (Secretory cells) are characterised by large number of vesicles with electron dense material. The distribution of cells in the tubules showing the embryonic cells at the blind end of the tubules, middle region occupied by absorptive and secretory cells, the distal end containing secretory cells corresponds to the distribution of cells in many other crustaceans. The cell types found are similar to the type

reported in other natantians. This marvellous organ serves for secretion, absorption, storage and utilisation of nutrients.

From the study of the properties of the hepatopancreatic extract of *P. indicus* it appears that carbohydrates, proteins and fats are effectively digested. The existence of separate enzymes in the digestive glands conforms to that of many other crustaceans. The digestive fluid is able to digest all the three types of energy yielding nutrients and thus is related to the omnivorous feeding habit of the animal. It is evident that the extensive carbohydrate complement in the extract is able to act on a variety of carbohydrates like starch, glycogen, sucrose, maltose and lactose.

The high amylase activity indicates the important role of this enzyme in carbohydrate digestion. From the data obtained, it has been confirmed that amylase has a pH optima at 7.0 and was fairly active between the range 6.8-7.2 and the optimum temperature recorded was at 40°C and showed maximum range between 40°-50°C. It was found to be stable in cold and frozen conditions for more than a week. It may be suggested that the diet formulated for culture operations should also be of the same pH for effective synthesis of this enzyme and utilisation of nutrients. Optimum substrate concentration for amylase activity was at 1 mg starch/ml of hepatopancreatic extract. It had a K_m value of 1mg starch/ml. The optimum enzyme concentration was found to be 0.7mg hepatopancreatic protein/ml of enzyme extract. In the first 15 to 20 minutes there was a sharp increase in the activity reaching the maximum at 50 minutes thereafter it remained steady more or less without showing any drop in the activity. Thus the optimum incubation of the enzyme activity was determined.

Certain metalic chlorides, vitamins and amino acids seem to affect starch hydrolysis. Statistical data has shown that mercuric chlorides markedly induce the activity by 98% even at 0.0001 M concentration, whereas stannous chloride also showed 85.15% inhibition at 0.0001 M and ferrous chloride at 0.05 M concentration and showed about 88.04% inhibition. However

all other metallic chlorides showed optimum activation at 0.05 M concentration.

Amino acids such as alanine, phenylalanine, ornithine, leucine, cysteine nor leucine, valine, cysteine monohydro chloride showed activation. Alanine and Dihydroxy phenyl-alanine was active at all six tested concentrations with maximum activity at 0.0001 M to 0.05 M respectively. For phenylalanine, ornithine and cystine optimum was at 0.0001 M. Nor leucine and valine recorded optimum at 0.0005 M, leucine showed an optimum at 0.0005 M. An increase in the activity of the enzyme was observed when compared to the control. However proline, leucine, histidine, aminobutyric acid and tryptophan inhibited the enzyme activity even in the lowest concentration tested.

Vitamins such as vitamin C, carotene, folic acid, choline and riboflavin activated the enzyme at 0.001 M concentration. The increase in activity was only about 5% when compared to the control. At other concentrations tested, the activity was greatly reduced. Relatively total inhibition by calciferol, inositol menadione, nicotinic acid and tocopherol was recorded at all the tested concentrations.

In the present study, purification of amylase by gel filtration chormatography has been undertaken and the purity of the enzyme established by polyacrylamide disc gel electro-phoresis. The electrophoretic pattern has indicated the presence of two closely associated bands. Thus the presence of isoenzymes of amylase needs further studies for their detailed characterisation.

Digestive enzyme complement during the developmental stages reflect a varied picture. In the experiments conducted it was shown that amylolytic activity increased steadily from postlarva to adult stage, while proteolytic activity recorded a sharp increase from postlarva to adult stage and lipolytic activity shot up from juvenile to adult stage.

During starvation, a sharp increase in the amylase activity and steady decline in proteolytic activity was detected with apparently no change in the pattern of lipolytic activity.

Neuroendocrines play a definite role in the secretion of the enzymes. Eyestalk ablation resulted in enhanced starch hydrolysis when compared to the intact animals. Activity was found to be 15% more in the bilaterally ablated prawns and 10% more activity recorded in the unilaterally ablated ones than the control.

In experiments conducted with varying levels of starch in the diet, an enzyme activity optimum was recorded at 20% starch in the diet, beyond that level activity was greatly reduced. Irrespective of the quantity of starch supplied in the diet, its utilisation for activity was reduced by 55% when compared to the optimum level. At 40% starch (the highest level used), starch utilisation was only about 25-30% of the amount supplied, the rest of the starch supplied is excreted out along with the faeces. Thus further studies on digestibility of proteins and lipids should be carried out so that a nutritionally well defined diet could be designed based upon a better understanding of the physiological abilities of the organisms.

CMFRI Spl. Publ., 1993; 56 : 95 - 98

ROLE OF TRACE ELEMENTS ON THE GROWTH AND PHYSIOLOGY OF SELECTED MICROALGAE

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Introduction

The results of a comprehensive study on the importance of trace elements namely copper, manganese and zinc on *Isochrysis galbana* and *Synechocysts salina* which are of nutritive value and used as live food for rearing the shellfish larvae and for developing mass cultures in the hatchery systems, are presented here.

Material and methods

The flagellate and the blue green alga isolated from the coastal region off Cochin, were grown in enriched seawater and maintained in the logarithmic growth phase of the life cycle.

The impact of varying conditions of environmental parameters such as salinity, pH and temperature and their role on the rate of metal uptake have been investigated. The physiological activity of the algae has been expressed by their growth kinetics in terms of cell concentration, quantities of chlorophyll and carotenoid pigments and rate of photosynthesis has been measured by the radioisotope technique.

The concentration of the three trace metals Cu, Mn and Zn in extracted seawater samples and algae has been determined by Atomic Absorption Spectrophotometry. Differential Pulse Anodic Stripping Voltammetry was employed to study the existence of copper complexes in the two species.

Experiments to assess the effect of interaction of metals in different combinations on *I. galbana* and *S. salina* have been conducted in order to understand the phenomenon of synergism and antagonism.

Transmission Electron Microscopy was undertaken using Carl Zeiss TEM to investigate the response of these microalgae to metals at cellular level.

Statistical analysis of data was done by analysis of variance using split-plot design method.

Results and discussion

All the three metals exhibit toxic effects on growth when their concentrations in the medium increase, but the important point is that the organisms react in different ways and reveal variation in their tolerance. Of the two species, *I. galbana* has been found to be more tolerant to copper and zinc and *S. salina* shows a greater tolerance to manganese. In all the experiments toxicity has been indicated by a general delay in growth. The optimum concentration of Cu, Mn and Zn which enhances the growth rate of *I. galbana* and *S. salina* has been shown to differ under the influence of salinity, pH and temperature.

Manganese has been found to play a more important role than copper and zinc in accelerating the growth of the flagellate and the blue green alga. Inhibition due to copper has been found to be higher than the other two metals even at a lower concentration. Moderately higher concentration of Mn and Zn does not affect the growth of the two species

The influence of trace metals on growth measurements of the two microalgae indicates that they exhibit peak growth and activity from four to twelve days of inoculation and thereafter the growth rate declines gradually within fourteen to twenty days when the two species attain senescence.

Quantitative evidence has been found for the complexation of Cu, Mn and Zn in the culture media by extracellular ligands produced by algae. An increase in metal complexation correlates well with a decrease in metal toxicity and a decrease in metal uptake in the culture medium. The detoxification of trace metals may represent an important physiological function of extracellular products.

The pigments chlorophyll *a*, chlorophyll *c* and carotenoids are found to increase and they even exceed that of control in lower concentrations of copper and zinc and in higher concentrations of manganese.

The rate of carbon fixation by the flagellate and the blue green alga is affected by the action of different concentrations of Cu, Mn and Zn. The rate of ^{14}C uptake is comparatively higher in optimum concentrations of manganese than in copper or zinc for both *I. galbana* and *S. salina*.

The rate of copper, manganese and zinc uptake by the algal cells determined by AAS shows that the weight of the metal per mg dry weight of the alga increases with an increase of metal in the medium and generally decreases with the age of the culture.

Anodic stripping voltammetry has confirmed the presence of copper complexes in both species exposed to different photoperiods with interspecies difference.

Electron microscopy has illustrated the extent of intracellular disorganisation induced by sublethal dose of copper on *I. galbana* and *S. salina*. Drastic changes have not been observed. Discontinuity of the cell membrane and photosynthetic lamellae have been noticed at certain regions of the cell in *S. salina* whereas appreciable disruption in the cellular structure is not visible in *I. galbana*. The mechanism of cellular detoxification by an increase in the number of lipid inclusions in *I. galbana* and an increase in the size of polyhedral bodies in *S. salina* may be of importance in reducing the toxic burden of the metal to algae.

Investigations of metal interactions indicate that with changes in metal ion combination there is an alteration in the algal response. Synergistic and antagonistic behaviour of metal ions has been detected in the two species.

The modification of metal toxicity is controlled by environmental factors such as salinity, pH and temperature. The optimum salinity level for the two species differs with the flagellate growing best at 25 ppt and the blue green alga shows

better growth at 15 ppt. A linear relationship exists between pH and toxicity of copper in *I. galbana,* but in *S. salina,* they exhibit an inverse relationship. The inverse relationship between pH and toxicity is also applicable to manganese and zinc for both the species.

The optimum temperature required for the activity of metal ions to maximise the rate of carbon production varies from 25°C - 30°C for *S. salina* and *I. galbana.*

The above findings indicate that interactions between trace metal toxicity and nutritional metals could play a significant role in regulating phytoplankton growth in natural systems.

CMFRI Spl. Publ., 1993; 56 : 99 - 104

STUDIES ON THE REPRODUCTIVE ENDOCRINOLOGY OF THE PENAEID PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Aquaculture of prawns has gained tremendous importance. Among the penaeid prawns used for culture in our country, *Penaeus indicus* is the most ideal species because of its short life-span, fast growth and excellent survival rates.

Aquaculture technology of penaeid prawns has developed remarkably and at present these prawns are cultured on a large scale in captivity. Controlled breeding of these prawns is done in some prawn hatcheries by using the technique of eyestalk ablation or extirpation. This technique now used worldwide, leads to the acceleration of gonadal maturation. However, informations on the physiological changes occurring in the neuroendocrine system of the animals are limited, although neuroendocrine factors are known to control the process of gonadal maturation.

The present investigation attempts to understand the fundamentals of the various aspects of reproductive physiology in relation to the changes occurring in the neuroendocrine system of *P. indicus* and to know about the mechanism involved in the technique of eyestalk ablation for induced maturation.

The following aspects were studied.

1. Oogenesis and spermatogenesis, and the mechanism of spermatophore formation in the vas deferens.
2. Identification of the neuroendocrine centres and distribution, and mapping of the neurosecretory cells and their chemical characteristics.

3. Histological investigations on the neuroendocrine control of reproduction during eyestalk extirpation, eyestalk extract injection and central nervous system extract injection to correlate the architectural changes in the neurosecretory cells during the different phases of maturation.

4. Investigations on the biochemical changes during gonadal development in different tissues such as ovary, hepatopancreas, muscle and haemolymph.

Material and methods

Penaeus indicus were collected from off Cochin or from the perennial prawn farms in Vypeen, Cochin. The studies were carried out using standard histological, histochemical, electron microscopic and biochemical methods. The salient findings of the present study are listed below.

Results and discussion

The maturation of the ovary was found to be accompanied by distinct colour changes as well as an increase in the Gonado Somatic Index (GSI) and diameter of the oocytes and nuclei. Based on these characters five different maturity stages (I to V) were identified. The manner in which the oocytes accumulated yolk and the concurrent changes in the ooplasm and cytoplasmic organelles, were considered to describe the five vitellogenic phases.

Histochemical studies revealed the yolk to be a glycolipoprotein complex deposited sequentially in the yolk complex followed by carbohydrates and lipids. Among proteins, tryptophanyl, aromatic and amino end groups were the first to be formed. Thiol-disulphide end groups were incorporated into the yolk platelets from external sources. The cortical bodies which finally forms the jelly layer of the spawned eggs were found to be rich in sulphur containing amino acids (cystine and cysteine) and sulphated and carboxylated AMP. Their probable functions such as controlling the permeability of water, protec-

tion against bacterial attack and catalysing cytokinesis in the eggs of penaeids are discussed.

Electron microscopic studies revealed the presence of nuclear pores through which RNA rich nucleolar material is extruded into the cytoplasm, mitochondria and rough endoplasmic reticulum during pre and early vitellogenic phases. The presence of these organelles indicated the autosynthetic capability of the ovary during the initial phases. During the late vitellogenic stages the oocytes were decidedly heterosynthetic as evidenced by the presence of micropinocytotic vesicles along the oolemmal wall and the absence of cellular synthetic machinery. Folliculogenesis was observed to be accomplished during the early stages of vitellogenesis. Ultrastructural studies showed that the follicle cells had considerable protein synthetic ability and histochemically the follicle cells were major source for the carbohydrate moiety of the yolk.

No specific maturity stages were ascribed to the process of spermatogenesis, which include spermatogonia, spermatocyte, spermatid and spermatozoa stages. The sperm in *P. indicus* was of the altered vesicular type with a spherical main body, that partially encompassed by a morphologically diverse cap region containing the acrosomal complex, from which extended a single spike. Histochemical studies revealed that the spermatozoa had abundant glycogen and basic proteins like arginine and lysine which are presumably used for the transmoult maintenance of the spermatozoa in the thelycum of the female.

The spermatophores were formed in the vas deferens with the aid of the glandular epithelial cells lining the duct. The electro-ejaculated spermatophore was oval in shape with a parachute-like wing at the apical end of the body. Sperms were packed inside the spermatophores in lobules and encompassed by two structurally diverse layers composed mainly of acid mucopolysaccharides.

The different neuroendocrine centres were identified by the presence of neurosecretory cells (NSC) in the optic,

supraesophageal, tritocerebral, subesophageal, thoracic and abdominal ganglia. Based on cytomorphological differences, 4 different types of NSCs were observed *viz.*, giant neuron (GN), A-cells, B-cells and C-cells. All the four types of NSCs undergo cyclic changes in their perikarya in relation to the synthesis of neurosecretory material. On the basis of the appearance of secretory granules, vacuolisation and involvement of extracellular glial cells and capillary plexus, three phases were identified *viz.* quiescent (Q), vacuolar (V) and secretory (S).

In the eyestalk, 3 NSC groups *viz.* the MTGXO 1 and 2 and MEGXO were identified. The neurohaemal organ on the sinus gland, was situated between the medulla externa and interna lobes. Totally 11 NSC groups were recognized on the dorsal and ventral sides of the supraesophageal ganglion, 1 NSC group in the tritocerebral ganglion, 15 NSC groups in the suboesophageal ganglion and 4 NSC groups each in the thoracic and abdominal ganglia.

The neurosecretory material was constituted mainly by a protein rich in cystine and a carbohydrate, mainly of glycogen. Electron microscopic studies explained that the neurosecretory material was composed of a haloed dense core of granules measuring 1400 to 1600 Å in diameter.

In the eyestalks of immature females more than 75% of the NSCs in the MTGXO and MEGXO were in the V and S phases. In mature females the majority of the NSCs were in the Q phase. However, in the supraoesophageal, suboesophageal and thoracic gangalia of immature females almost 65% the NSCs were in the Q phase while in fully mature females, active NSCs dominated. Histochemical studies showed significant fluctuation in the cystine content in the eyestalks, indicating that A and B-cells were the most active cell types in relation to reproduction, whereas in the brain and thoracic ganglia, GN, A and B-cells were the most active cells towards gonadal maturation. The pyriform C-cells were not invovled in the process of maturation.

Both unilateral and bilateral eyestalk ablation significantly enhanced the GSI and ova diameter leading to precocious

maturation of the ovary and this process was blocked in ablated prawns injected with an extract of the eyestalk, thus confirming the inhibitory nature of the neurosecretory principles contained in the eyestalk. Extracts of the central nervous system injections enhanced the GSI and oocyte diameter although full gonadal maturation was not achieved. A temporal relationship exists between gonadal maturation and moult cycle. Gonadal maturation usually occurred during early premoult period of the moult cycle. However, this pattern was altered in bilateral ablation in females.

The various biochemical components like protein, carbohydrate, lipid, cholesterol, moisture, carotenoids, RNA and DNA showed significant variation and accumulation in the haemolymph, ovary and hepatopancreas during the different maturity stages. The biochemical composition of muscle tissues did not show any significant variation to the maturation process. In general, the ovary showed accumulation of protein, carbohydrate, lipid, cholesterol and carotenoids and loss of moisture, RNA and DNA during vitellogenesis. High RNA content during the early stages of vitellogenesis indicated an autosynthetic mode of yolk synthesis. However, in the later stages significant amount of protein, lipid, carbohydrate, cholesterol and carotenoids were mobilised from the hepatopancreas to the ovary through the haemolymph. The yolk in *P. indicus* was predominantly composed of water. Protein, lipid, carbohydrates and cholesterol were the major organic reserves. Carotenoids, DNA and RNA were found only in small quantities.

The information generated from the present study clearly establishes the neurosecretory control of reproduction in the penaeid prawn *P. indicus*. The precise cellular factors controlling reproduction has been identified and their roles were established. The neurosecretory mechanism involved in the technique of eyestalk ablation for induced maturation has been explained. The observation that the ovary in *P. indicus* had only limited autosynthetic capabilities indicated that the brood-stock nutrition has an important role in the effort to achieve induced maturation in penaeid prawns. The basic knowledge on the

structure of the male gamete acquired from this study can help future research on artificial insemination in these prawns. The findings of the present investigation should help in streamlining the hatchery production of prawn seed by endocrine manipulations. It should also pave the way for future research in penaeid hormones using modern separation techniques.

Publications

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CMFRI Spl. Publ., 1993; 56 : 105 - 107

STUDIES ON THE PATHOBIOLOGY OF PENAEID LARVAE AND POSTLARVAE

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Summary

This study covers the pathogens of larvae and postlarvae of penaeid prawns of India, diagnosis and characteristics of diseases, and in the case of bacterial diseases, isolation methods, characterisation, pathogenic mechanism and control of pathogens.

The present investigation was carried out from 1985 to 1988 and it was initiated by undertaking a survey to record the common diseases occurring in the penaeid larvae and postlarvae in the hatcheries. Seven cases of diseases and abnormalities such as ciliate infestation, *Nitzschia closterium* infestation, parasitic protozoan infection, parasitic dinoflagellate infection, appendage necrosis, heteromorphic eye and abnormal eggs and deformed nauplii encountered during the survey are presented and discussed. A bacterium, isolated from the infected larvae of *Penaeus indicus* from the Prawn Hatchery Laboratory, Narakkal near Cochin, was found to be a new isolate of *Vibrio*. This bacterial pathogen and the infection caused by it were selected for detailed investigation since it caused high mortalities in the larvae of penaeid prawns.

A study on the total heterotrophic bacteria associated with eggs, larvae and postlarvae of *P. indicus* were carried out to obtain an insight into the bacterial disease problem. The results revealed a gradual increase of bacterial flora from egg to postlarvae. *Vibrio* was found to be dominant in eggs, larvae and postlarvae followed by *Pseudomonas, Alcaligenes, Aeromonas* and *Flavobacterium*. In the rearing medium, Alcaligenes was predominant followed by *Vibrio, Pseudomonas, Aeromonas* and *Flavobacterium*.

Taxonomy of the new isolate of *Vibrio* (coded as *Vibrio* sp. 024210), responsible for appendage necrosis in the larvae of *P. indicus* was studied. The morphological, biological, physiological and biochemical characters of this isolate are enumerated and compared with the related vibrios and the results discussed.

Pathogenicity of *Vibrio* sp. 024210 was tested on the larvae and postlarvae of *P. indicus*, *P. monodon* and *P. semisulcatus* by immersion method, and in the adult *P. indicus* by intramuscular injection and oral route. *Vibrio* sp. 024210 was found to be intrinsically pathogenic to larvae and postlarve of all the three species of prawns as well as to the adult *P. indicus*. The LC_{50} values of *Vibrio* sp. 024210 for nauplius, protozoea, mysis and postlarvae of the three species indicated that the susceptibility to the infection varied with the developmental stages of larvae and postlarvae. The larvae and postlarvae of *P. indicus* were found to be the most tolerant to *Vibrio* sp. 024210, whereas the larvae and postlarvae of *P. monodon* appeared to be the most sensitive and in *P. semisulcatus*, it was intermediate between *P. indicus* and *P. monodon*. *Vibrio* sp 024210 was found to be the most virulent to the larvae of *P. indicus* as compared to *V. alginolyticus* and *V. parahaemolyticus*.

Histopathological investigation carried out on the organs such as hepatopancreas, heart, haemotopoietic tissue, gut, muscle and gill of the uninfected and experimentally infected adult *P. indicus* and larvae of *P. indicus* and postlarvae of *P. monodon* with *Vibrio* sp. 024210 revealed moderate to marked destruction of most of the vital organs.

Eleven antimicrobial compounds were tested against the marine vibrios to study their sensitivity towards these compounds. Chloramphenicol was found to be effective to control *Vibrio* sp. 024210, *V. alginolyticus* and *V. parahaemolyticus*. Subsequently, minimum inhibitory concentration of chloramphenicol, oxytetracycline and tetracycline to the *Vibrio* sp. 024210, effect of chloramphenicol and oxytetracycline on the larvae and postlarvae of *P. indicus* and on the mixed phytoplank-

ton used to feed the larvae, were studied and the different aspects of disease control measures particularly those relating to bacterial diseases in the hatcheries are discussed in detail.

CMFRI Spl. Publ., 1993; 56 : 108 - 111

STUDIES ON THE ECOLOGY AND PRODUCTION OF ALGAE IN PRAWN CULTURE SYSTEMS

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Introduction

Successful planning and utilization of the vast brackish-water areas, inland bays, tidal pools, lakes and backwaters along the entire coastline of India for cultivation of prawns under controlled conditions requires very urgent consideration in the context of increased food production in the country. Although a small portion of these areas, especially those in Kerala, are being utilized for capture of prawns by an indigenous process called filtration, vast stretches of these backwaters and estuaries still remain unutilized. These areas in most cases provide biologically potential environment for healthy growth of many species of fishes and prawns. This realisation paved way for a series of investigations and developmental activities in the field.

Background

Existing method of culture is largely based on empirical knowledge. Lacking of scientific basis, leads often to wasteful efforts and poor results. Modern methods of fish and prawn culture based on scientific research, have revolutionised the industry in recent years and not only extended its scope to cover the whole country, but led to increased fish and prawn production. An understanding of the biological capability of the water in the perennial and seasonal culture ponds, and the nature and extent of the influence of the abiotic factors on the production of organisms in the primary level of food chain would contribute to effectively implement management measures in the stocking strategies and in the evaluation of economics of production of prawns. It is against this back-

ground, the present "Studies on the ecology and production of algae in prawn culture systems" was carried out.

Material and methods

Investigations were carried out on the hydrological characteristics, seasonality in the concentration of nutrients, algal production and nutrient enrichment aspects in prawn culture systems during the premonsoon, monsoon and postmonsoon period of 1986 and 1987. A perennial prawn culture pond at the Central Institute of Brackishwater Aquaculture (referred to as CIBA pond in the text, area : 0.6 ha), perennial canals in the coconut grove where prawns are farmed (referred to as COCO field in the text, area : 1.0 ha), and a seasonal culture pond locally called Pokkali field (area : 0.8 ha), all situated at Narakkal near Cochin, were selected for the present study.

Spatial and temporal variation in the distribution of hydrological properties such as salinity, dissolved oxygen, temperature, hydrogen-ion concentration (pH) and redox potential (Eh) in the three culture systems were investigated. The pattern of distribution of the available nutrients such as reactive phosphate (PO_4), nitrite (NO_2), nitrate (NO_3) and ammonia (NH_4) of water in the ecosystems are discussed.

The magnitude of gross primary production (GP) and net primary production (NP) was estimated. Nature and extent of variation in the concentration of Chlorophyll *a*, *b*, *c* and carotenoids and their possible ratios were also estimated. Results of observation on the seasonal numerical variation of algal cells during 1987 are explained. Results of computer analyses of the correlation between ecological and productivity parameters, and the significance of correlation by multiple regression analysis are highlighted. An assessment of the status of production at primary level has been made through NP : GP ratio, assimilation number and ratio of Chlorophyll pigments.

In order to delineate the effect of fertilization on the primary production in the culture ponds, nutrient enrichment experiments were conducted under *in situ* during pre- and

postmonsoon seasons and *in vitro* during monsoon season. Nutrients such as nitrogen, phosphorus and silica and trace metals such as zinc, manganese, molybdenum, cobalt, copper, iron and EDTA were employed in the experiment individually and in combination. ANOVA was used to test the significance of the effects of treatments. The results of the experiments and statistical interpretation of the findings elaborately discussed. The interrelations between abiotic environmental factors and productivity has been attempted and a critical evaluation of the implication of primary productivity on the economic production of prawns under farming presented and both the above aspects discussed in detail.

Results

The present observations clearly indicate that the ponds are highly influenced by environmental factors such as tidal influence, seasonality, run off, agricultural and industrial activities, etc. On the basis of the overview of these investigation salinity varied from 5-20‰, dissolved oxygen from 3-5 ml/l, temperature 28-33°C, pH from 7.5-8.0 and Eh from 130-250 mV are found to be favourable environmental conditions and conducive for culture practices.

The annual ranges in nutrient concentration clearly show the richness of water in the culture systems. Nutrients in general were high during monsoon season. Ammonia varied from 4-16 µg-at/l, phosphate from 1-3 µg-at/l, silicate from 7-40 µg-at/l, nitrate from 1-5 µg-at/l and nitrate from 5-25 µg-at/l were accounted for.

The annual range of gross primary productivity ranged from 2000 to 15000 mg C/m^3/day, concentration of chlorophyll *a* from 7-45 mg and the total counts clearly establish that these culture ponds are high productive systems.

Statistical analysis revealed that each culture system were independant. The contributing parameters also varied on account of the dynamic nature of the estuary.

Enrichment of the culture ecosystems during the period of active operation such as premonsoon and postmonsoon ensured enhanced primary production.

It was the first time that an attempt has been made to compute the potential fishery yield from primary production. A critical assessment of the primary productivity and its implication on the economic production of prawns in the culture pond was also discussed. These encouraging results of this research programme offer considerable scope for improvement through planned scientific methods.

CMFRI Spl. Publ., 1993; 56 : 112 - 118

STUDIES ON THE HAEMOLYMPH OF *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

The Indian white prawn *Penaeus indicus* H. Milne Edwards, is widely distributed along east and west coast of India and is one of the important candidate species for aquacutlure in India. Eventhough techniques for induced breeding and larval rearing have been perfected, methods for large scale culture operations are not fully explored. This is mainly due to various management problems in the culture systems . Water quality, nutrition and disease problems are some of the main factors leading to heavy mortality of stocked prawns.

Penaeid shrimps live in a wide variety of environments. Marked alterations in the physico-chemical characteristics of the medium are reflected in the composition of the haemolymph. During the moulting and reproductive cycles also, the haemolymph constituents show variations quantitatively and qualitatively. Exposure to polluted waters can also affect the haemolymph in various ways.

In order to understand the factors affecting the growth (moulting), maturation and breeding of shrimps, it is necessary to know the composition or alterations in the biochemical constituents in the haemolymph. These change are reflected in the haemocytes also. The studies on the haemolymph would provide an insight into the factors affecting the normal growth and thus the successful culture of the species.

Studies on the haemolymph of *Penaeus indicus* H. Milne Edwards was taken up in order to obtain more comprehensive information on the qualitative and quantitative aspects of haemolymph of the species. The study was undertaken under

three main aspects (a) Biochemical (b) Electrophoretic and (c) Cytological.

Material and methods

Penaeus indicus was collected from the growout ponds of Marine Prawn Hatchery Laboratory at Narakkal. Immediately these prawns were transported alive to the wet laboratory of the Central Marine Fisheries Research Institute, Cochin. After a period of 12-16 hr acclimation in 23‰ - 25‰ seawater the haemolymph was extracted, using 3% Sodium citrate as anticoagulant.

Results and discussion

Biochemical studies

Variations in the biochemical constituents of the haemolymph of *P. indicus* in relation to the following were studied.

a. Sex (male and female).

b. Size groups Group I (60-80 mm), Group II (80-100 mm) Group III (100-120 mm) and Group IV (120-140 mm).

c. Moult cycle (A, B, C, D_{σ}, D_1', D_1'', $D_{2\text{-}3}$ according to Drach and Tchernigovtzeff.

d. Maturity stages - Ist, IInd, IIIrd, fully mature or IVth and spent (Vth) stages.

The biochemical constituents in the haemolymph estimated are total protein, total free amino acids, glucose, total lipids, cholesterol, copper, calcium, iron, zinc and manganese. Haemolymph samples from specimens of similar moult stages and sex was pooled together for all estimations for the smaller size group *i. e.* 60-80 mm. Standard methods were followed for all biochemical estimations with necessary modifications.

The mean total protein content with haemolymph in the four size groups was 47.79 mg/ml in group I (60-80 mm), 49.72 mg/ml in group II (80-100 mm), 66.32 mg/ml in group III (100-120 mm) and 72.56 mg/ml in group IV (120-140 mm). The total free amino acids (TFAA) in the haemolymph in group I was

1.63 mg/ml, in group II 2.71 mg/ml, 3.56 mg/ml in group III and 3.893 mg/ml in group IV. The mean total lipid content recorded in the haemolymph in size group I was 0.836 mg/ml, in group II 1.34 mg/ml, group III 1.49 mg/ml and 1.67 mg/ml in group IV. The mean total free cholesterol content recorded in the haemolymph in group I was 0.434 mg/ml, 0.5917 mg/ml in group II, 0.637 mg/ml in group III and 0.99 mg/ml in group IV.

The mean glucose content recorded in the haemolymph in the four size groups was 0.267 mg/ml in group I, 0.504 mg/ml in group II, 0.504 mg/ml in group III and 0.592 mg/ml in group IV.

The mean total iron content recorded in the haemolymph in group I was 364.16 µg/ml 391.01 µg/ml in group II, 495.12 µg/ml in group III and 713.38 µg/ml in group IV. The mean total zinc content recorded in the haemolymph in the first two size groups was 193.22 µg/ml and 291.62 µg/ml. The mean total manganese content recorded in the haemolymph in the four size groups was 270.57 µg/ml in group I, 347.38 µg/ml in group II, 454.27 µg/ml in group III and 619.97 µg/ml in group IV.

There was no significant difference in the level of any of the biochemical constituents in the haemolymph of either sex. Analysis of variance between the four size groups indicated significant difference in the haemolymph constituents between the four groups. There is significant rise in the level of the biochemical constituent with increase in size of the species.

During the moult cycle, all the parameters except calcium reflected a similar cyclic pattern. While calcium also reflected a cyclic pattern, it registered a different pattern. The content of protein, free amino acids, lipids, cholesterol, glucose, calcium, copper, iron indicated significant differences between the moult stages. The calcium content in the haemolymph was maximum just prior to ecdysis, minimum during intermoult and early moult. The calcium content during early postmoult was significantly higher than the intermoult and premoult stages. The protein, amino acid, lipid, cholesterol, glucose, copper, zinc

content in the haemolymph was minimum during the period just after ecdysis and gradually increased thereafter and reached a peak in early and mid premoult stages and declined suddenly during premoult. Manganese and iron content in the haemolymph did not refelct the cyclic pattern as in other cases.

The biochemical constitutents in the haemolymph registered a sharp increase during the vitellogenic process. The protein, free amino acids, lipids, cholesterol, glucose, calcium, copper, zinc, iron and manganese contents in the haemolymph increased from stage I to stage IV fully mature stage and declined immediately after spawning (spent stage).

Electrophoretic study

Haemolymph of *P. indicus* was analysed electrophoretically to identify the species-specific haemolymph proteins. The haemolymph protein of both male and female, of different size groups and moulting stages (of size group 100-120 mm), and maturity stages was traced and compared with normal human serum as standard. Specific staining techniques was used to identify the copper protein fractions-hemocyanin, (Rubeanic acid method), calcium binding fractions (Alizarin red), glycoproteins (PAS) and lipoproteins (Oil red O). Determination of molecular weight of the copper protein fractions - Hemocyanin was also done electrophoretically using standard molecular weight markers.

The polyacrylamide gel disc electrophoresis (PAGE) method as given by Subhashini and Ravindranath (1981, *CMFRI Spl. Publ.*, **7** : 1-172) was followed with necessary modifications.

Electrophoresis of haemolymph of *P. indicus* revealed a species specific pattern. Wide intra-individual variation in the haemolymph pattern was observed. Generally, 10-16 protein fractions are observed in the haemolymph, the major fractions being the copper-bearing proteins - the fast and slow hemocyanins, heteroagglutnin and fibrinogen. Apart from these important fraction several unidentified simple protein fractions are also observed in the haemolymph. These vary greatly with

respect to size, sex, moult stage and reproductive cycle and nutritional stage.

Haemolymph protein pattern of four size groups (60-80 mm, 80-100 mm, 100-120 mm and 120-140 mm) and both sexes was studied electrophoretically. No significant variation in the qualitative pattern was observed in either sex, the basic pattern does not vary. The quantitative difference in the protein content is represented by the fast hemocyanin fraction. More protein fractions are observed in the largest size group studied (120-140 mm). This reflects the progressive alteration of the nature of the protein in the haemolymph to suit the functional requirement of the species as size increases. The haemolymph protein pattern does not vary with respect to the moult cycle. No major polypeptide seems to be involved in the moulting process.

Haemolymph protein fractions separated from individuals in different stages of ovarian maturity presented interesting features. The female specific protein or vitellogenin could be identified in stages I, II and III and are glycoproteins. The copper bearing fractions exhibit peculiar pattern.

The copper bearing fractions - the fast and slow hemocyanin fractions are the major components in the haemolymph of *P. indicus*. The molecular weight of the two fractions was determined by calibration with known molecular weights namely *Limulus* hemocyanin and bovine serum albumin. The method yielded values of 280,000 daltons and 137,184 daltons as molecular weights of the fast and slow hemocyanin fractions respectively.

Cytological studies

In order to identify and classify the circulating haemocytes present in the haemolymph of *P. indicus*, light as well as electron microscopy studies were carried out. Haemolymph smears were prepared according to the procedure described by Mix & Sparks (1980). However, this procedure causes distortion of the haemocytes and revealed ambiguous staining properties. Also

structural details could not be clearly visualised. Ultrastructure study of haemocytes was therefore, adopted to clearly distinguish differences in the morphology of haemocytes in the haemolymph. The ultrastructural details of haemocytes in the haemolymph of *P. indicus* was studied with the help of Carl Zeiss transmission Electron Microscope (TEM).

Ultrastructural study of circulating haemocytes in the haemolymph of *P. indicus* aided the identification of three types of haemocytes based on the presence and abundance of granules in the cytoplasm of the haemocytes. The three type of haemocytes are agranulocytes, semi-dense granulocyte and dense granulocyte.

The agranulocyte are the smallest type of haemocyte found in the haemolymph. The average size of these haemocytes is 3.36 µm x 4.76 µm. They are ovoid to spherical with nuclear chromatin dispersed. Granules are lacking and these haemocytes show little differentiation.

The dense granulocyte are all differentiated and posses large number of dense granules. Generally ovoid and larger than the other haemocytes, the average size is 4.72 µm x 5.86 µm.

The semi-dense granulocyte are intermediate type of haemocytes. They are ovoid or spindle shaped, sometimes irregular, with an average size of 4.16 µm x 7.18 µm. They are characterised by the presence of round shaped cytoplasmic granules, more numerous than in the agranulocytes. But these cytoplasmic inclusions are smaller than those found in the dense granulocytes.

Apart from these circulating haemocytes an entirely different type of haemocyte is noticed in the haemolymph of females with developing ovary. This haemocyte is atleast three times larger than the other haemocytes and possess large granules of different nature. The presence of 'cyanocyte' - probably hemocyanin containing hemocyte, is also reported in the haemolymph of *P. indicus*. The functions of the haemocytes is discussed.

This study has provided valuable information on the haemolymph composition and also provides the baseline information required for further research, particularly on the defense mechanism of *P. indicus* to invasive and pathological organisms.

CMFRI Spl. Publ., 1993; 56 : 119 - 125

NATURE AND ECOLOGICAL SIGNIFICANCE OF NUTRIENT REGENERATION IN DIFFERENT PRAWN CULTURE FIELDS

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Introduction

Nutrient regeneration in its broadest sense, covers the entire field of biological oceanography, as it links the two bio-ecological processes such as the primary production and remineralisation. As such, all the major unresolved questions in quantitative marine ecology can be traced back to this central issue. No general agreement exists about such basic questions as the structure of food webs, the interaction of their component populations or whether current methods successfully measure rates of ecosystem processes. Critical appraisal of this situation have been made from a variety of sub-disciplines of which some are phytoplankton, microbial, zooplankton ecology, nutrient regeneration, magnitude of primary production and pelagic/ benthic coupling.

Recently in many parts of the world the possibility of improving aquaculture methods in enclosed environments, has become a point of serious consideration. Studies concerned with the culture system in the past were concerned mainly in determining the identity of the system, abundance of fauna and their relationship to environmental variables. More recent investigations have been concerned with subjects such as productivity, faunal diversity and ecophysiology of individual taxon.

Only limited studies have been made on the distribution and seasonal variation of phosphates, nitrate and silicate, fractions and circulation of phosphorus and nitrate, nutrients and productivity relations in the prawn culture fields. In the context rapidly developing coastal aquaculture in the country and

considering the importance of inter-relationships between environmental parameters, nutrients, metals and productivity of the culture ponds, the present investigation was taken up to study the spatial and temporal distribution, seasonal abundance and regeneration of nutrients, and primary productivity in certain prawn culture fields of Narakkal near Cochin.

Material and methods

The different types of prawn culture farms under three broad categories were selected for sampling of water and sediment at fortnightly intervals from January 1986 to December 1987. They are perennial fields (Station I and II), canals in coconut fields (Station IV and V) and Pokkali field (Sation III and VI). The investigation was carried at Narakkal (76°14'E; 10° 03'N) about 10 km northwest of Cochin in Kerala. The water samples were analysed for temperature, pH, salinity, dissolved oxygen, total alkalinity, total phosphorus, organic phosphorus, particulate phosphorus, total nitorgen, total inorganic nitrogen, dissolved organic nitrogen, nitrate nitrogen, nitrite nitrogen, silicate, primary productivity, chlorophylls, copper, zinc and iron. Sediment samples were analysed for copper, zinc and iron.

Results

The temperature value of the water showed fluctuations from 28.0 to 31.8°C in different prawn culture systems. The hydrogen ion concentaration of the water showed fluctuations between 7.4 and 9.1 in the prawn culture fields. During the premonsoon season, especially during March-April, the salinity at all stations exhibited considerable increase, while during monsoon values showed decrease. Dissolved oxygen values were between a minimum of 2.50 ml/l and a maximum of 6.80 ml/l during August and May respectively. The total alkalinity values showed fluctuations from 0.07 to 2.72 meq/l in different prawn culture fields.

The total phosphorus of water in all the prawn culture fields fluctuated between 1.06 and 13.76 µg at/1. In the perennial field maximum total phosphorus value (7.72 µg at/1)

was recorded in July and minimum (1.06 µg at/1) in January. In the coconut grove canal, the total phosphorus was fluctuating between 2.79 and 13.76 µg at/1. In the pokkali field it was seen fluctuating between 2.14 and 12.40 µg at/1. Inorganic phosphorus of water in all the prawn culture fields showed fluctuation between 0.16 and 8.80 µg at/1. In the perennial field, maximum value (4.20 µg at/l) was recorded during June and minimum value (0.16 µg at/l) during January. While in the coconut grove canal the maximum value (8.80 µg at/l) and minimum value (1.00 µg at/l) during the months of July and May respectively. In the Pokkali field, relatively high values were recorded throughout the period as compared to other fields.

Organic phosphorus of the water showed fluctuation between 0.40 and 3.68 µg at/1. The concentrarion of the organic phosphorus of the water in the perennial fields, canals, in the coconut grove and in the pokkali field was varying between 0.40 and 3.40; 0.43 and 3.22; 0.49 and 3.68 µg at/1 respectively. Maximum value was recorded in June-July and minimum in January-April. In general the higher values were recorded during monsoon period, the lowest during premonsoon season. During monsoon season organic phosphorus at all the stations exhibited considerable increase while during postmonsoon season value showed decrease. Particulate phosphorus of the water in all the prawn culture fields showed an upward trend from January to July, but it declined to reach lowest value during November. In the perennial fields maximum particulate phosphorus value (0.86 µg at/l) was recorded in June and minimum. (0.16 µg at/1) during January. In the coconut grove canals the concentration was seen fluctuating between 0.24 and 1.16 µg at/1 and in the pokkali field maximum value (1.39 µg at/l) was recorded in June and minimum (0.60 µg at/l) during August. The particulate-P was at its maximum during the monsoon months, extending upto August, after which it began to decline.

Total nitrogen of the water in all the prawn culture fields were seen increasing gradually from January to reach a

maximum value 94.54 µg at/1 in July. The range of variation of total nitrogen varied from 15.01 to 94.54 µg at/1. The range in the concentration was between 15.01 and 71.72 µg at/l in the perennial field; 16.20 and 87.87 µg at/l in the coconut grove canal and 18.06 and 94. 54 µg at/1 in the pokkali field. Maximum value was recorded in the pokkali field during July and minimum value in the perennial field during January. Total inorganic nitrogen of the water in the perennial field, canals in the coconut grove and in the pokkali field was varying between 3. 63 and 26.68; 3.89 and 30.55; 6.76 and 32.90 µg at/l respectively. There was a gradual increase in the concentration from February to July followed by a fall during August - September. Maximum value was recorded in the pokkali field during July and minimum value was noted during January. In the coconut grove canals relatively lower values were recorded throughout the period as compared to the other fields.

Dissolved organic nitrogen value of the water showed fluctuation from 6.9 to 52.64 µg at/1 in different prawn culture fields. The concentration in the perennial field, in the coconut grove canal and in the pokkali field was ranging between 8.37 and 40.02; 8.61 and 51.24; 6.90 and 52.64 µg at/1 respectively. The nitrate nitrogen of the water in all the prawn culture fields were seen increasing gradually from January to reach a maximum value in July, thereafter showed decrease in the concentration. Nitrate nitrogen of the water in all the prawn culture fields showed an upward trend from March to July, but it declined to reach the lowest value in October. The range in nitrite nitrogen value was between 0.1 and 2.4 µg at/1 in the perennial fields; 0.20 and 3.60 µg at/l in the coconut grove; 0.1 and 4.20 µg at/1 in the pokkali fields. The range in particulate nitrogen value in all the prawn culture fields was between 1.60 and 12.04 µg at/1. In the perennial field maximum value (10.35 µg at/1) was recorded during June and minimum value (1.05 and 1.60 µg at/1) during January and November, while in the coconut grove canal the concentration was seen fluctuating between 1.70 and 11.40 µg at/1.

Primary productivity of the water fluctuated from 642.00 to 1246.00 mg C/m^3/day in the perennial field; 636.00 to 1113.75 in the coconut canal field and 618.50 to 1403.75 mgC/m^3/day in the pokkali field. In the perennial field maximum production was recorded during May - June and minimum recorded in December - January. Chlorophyll 'a' concentration of the water in the perennial fields, canals in the coconut grove and in the pokkali field was varying between 0.36 and 6.69; 0.68 and 11.70; 0.10 and 9.42 mg/m^3 respectively. Chlorophyll ' b' concentration of the water showed fluctuation from 0.01 to 4.95 mg/m^3 in all the prawn culture fields. The range in chlorophyll 'c' concentration value was between 0.06 and 6.98 mg/m^3. Chlorophyll 'c' concentration of the water in the perennial field, canals in the coconut grove and in the pokkali field was varying between 0.06 and 6.98 ; 0.10 and 5.82 ; 0.06 and 6.71 mg/m^3 respectively.

Copper concentration of the water fluctuated from 3.44 to 12.64 ppb in all the prawn culture fields. In the perennial field maximum value was recorded during August and minimum value was recorded during March. Zinc concentration of the water in the perennial fields, canals in the coconut grove and in the pokkali field was varying between 4.16 and 9.88 ppb, 4.50 and 11.96; 7.80 ppb and 14.40 ppb respectively. Maximum concentration was recorded during August - September in the pokkali field and the minimum concentration was recorded during May - June in the coconut grove canals. Iron concentration of the water was fluctuating from 1.12 to 4.95 ppb in the perennial fields; 1.26 to 5.85 ppb in the coconut grove fields; 2.10 to 7.20 ppb in the pokkali fields. Zinc concentration of the sediment showed fluctuation from 32.08 to 58.29 ppb in the perennial fields; 36.09 to 75.04 ppb in the coconut grove canals; 60.75 to 92.80 ppb in the pokkali field. Copper concentration of the sediment in the perennial field, canals in the coconut grove and in the pokkali field was varying between 28.40 and 55.27 ppb; 31.95 and 71.50 ppb; 53.25 and 88.00 ppb. The range in iron concentration was between 0.72 and 4.18 ppb in the perennial fields; 0.81 and 4.94 ppb in coconut grove canals; 1.35 and 6.08 ppb in pokkali fields.

Conclusion

In general, phosphorus values were at their maximum during the monsoon months, extending upto September after which they began to decline during the premonsoon and postmonsoon months. The maximum values observed here was due to the transport of phosphate by bubbles rising in the surface, decomposition of particulate organic matter, rapid regeneration with microbial precipitation, the excretion by phytoplankton, horizontal and vertical transport of water and advection. The positive correlation between primary productivity and phosphorus, nitrogen and silicon in the present study proved to effect the nutrient regime upon the dynamics of population growth and photosynthetic process in the ecosystem. Once nutrients have been absorbed, it should be fed into the photosynthetic cycle. The cycle would then proceed until it is blocked by a shortage of some necessary ingredients.

Nitrite production was observed to be high which may be due to the biological processes like excretion of nitrogenous compounds by plankton decay of vegetation. It may be noted that nitrite is recycled here relatively repeated number of times than other compounds. Nitrite is an intermediate product in the regeneration of nitrogen compounds by bacterial action. Changes in concentration of nitrate are the net effect of nitrification, nitrate reduction and assimilation. Nitrogen is considered as a limiting nutrient for marine primary production and the recycling of nitrogen is, therefore, an important factor in the regulation of primary production.

The total nitrogen/phosphorus ratio of the prawn culture fields ranged from 4.48 to 40.40 ; inorganic N/P ratio from 1.08 to 22.33; organic N/P ratio from 7.96 to 160.21; particulate N/P ratio from 3.01 to 28.53 throughout the period of study. During the premonsoon period the ratio was below 11 and hence nitrogen limitation of phytoplankton biomass was observed. But during the monson period the ratio was above 20 and hence phosphorus is limiting.

The present result reflects the year to year variability of the physical environment, in which the impact of the horizontal advection is more important for the regulation of the physical structure of the water column for the import or export of nutrients. Phosphorus cycling in the prawn culture systems is influenced by the river input in both dissolved and particulate form, contribution of sewage and intensive contact of water masses with the underlying sediments. The speed of phosphorus regeneration from the shallow bottom seems to be high enough to maintain the phosphate almost at a constant level. In the regenerating communities, the bulk of the primary production is based on short term recycling within the surface layers. The organisms comprising regenerating system will differ fundamentally in their mode of adaptation, both the environment and to each other from those of new system. New system can only arise where environmental transport of energy provide new nutrients for plant growth, regenerating system are dependent only on radiant energy for this maintenance. The present information on regenerating system indicates them to be much more complexly structured than expected; further, spatial and temporal variability appears to be much greater than initially assumed about the concept of nutrient regeneration.

CMFRI Spl. Publ., 1993; 56 : 126 - 128

POPULATION BIOLOGY AND ECOLOGY OF *ARTEMIA* FROM SALINAS OF THE SOUTHEAST COAST OF INDIA

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Introduction

For the successful rearing of larvae of marine fishes and crustaceans in hatcheries where intensive aquaculture is practised, the availability of suitable food is an essential prerequisite. The cyst of the brine shrimp *Artemia* forms one of the most important sources of food for the larvae. The demand for good quality *Artemia* cyst is far more than the present production level and the insufficient cyst supply sometimes is the major bottle-neck in the proper functioning of hatcheries the world over.

Artemia occurs in the natural salt lakes as well as in man-made salt pans. Efforts are being made to identify new natural habitats of brine shrimp, besides augmenting the production through extensive and intensive culture operations to meet the demands of the expanding aquaculture industries. Despite the availability of voluminous literature on *Artemia* in general, information on population, biology and ecology of brine shrimp in natural environment particularly from India, is extremely poor. Hence the present study was designed to collect information on the population biology and ecology of *Artemia* from salinas of the southeast coast of India.

Objectives

The population characteristics of *Artemia* and the effect of different environmental parameters on the different stages of *Artemia* in a salina at Tuticorin, southeast coast of India, are the main objectives of this study.

Material and methods

The present investigation was carried out from 1985 to 1987. The study was initiated by undertaking a survey to find out suitable *Artemia* habitats along the southeast coast of India and a perennial salina with an area of 0.25 ha was selected at Karapad (Tuticorin). Weekly samplings were made for two full calender years (1986 -1987) to collect the different stages of *Artemia* population as well as the different environmental parameters.

Results and discussion

The results and discussion give the characteristics of *Artemia* population in the salina, the seasonal variations of different environmental parameters in the salina and their effects on different stages of *Artemia* population. Description of an experiment conducted to show the sudden changes of salinity on different stages of *Artemia*, is also discussed.

The study on systematics, biology and distribution of *Artemia* gives an idea of the taxonomic position of *Artemia*, its biology and life cycle, types of *Artemia* habitats, distribution mechanisms, the places in Asia where *Artemia* occur and *Artemia* find-spots in India.

The population characteristics of *Artemia*, the occurrence and relative abundance of different stages of *Artemia* such as nauplii, juveniles, preadults, cyst bearing adults and nauplii bearing adults and their variations during the presummer, summer and post-summer seasons are the highlights and discussed elaborately. Analysis of variance (ANOVA) was conducted to find out the statistical significance.

The seasonal variations of different environmental factors such as hydrography (temperature, pH, salinity and dissolved oxygen); nutrients, (ammonia-nitrogen, nitrite-nitrogen; nitrate-nitrogen, inorganic phosphate and silicate); biological (gross primary productivity, number of algal cells and number of predatory insects) and meteorological parameters (rainfall, wind velocity and sunshine) in the salina and their influence on the

different stages of the *Artemia* give valuable informations. Correlation matrix was constructed to see the influence of the above mentioned parameters on different stages of *Artemia*.

An experiment was conducted in the laboratory and described the impact of sudden changes in salinity on different stages of *Artemia* population. ANOVA was conducted to find out the statistical significance of the actual observation.

CMFRI Spl. Publ., 1993; 56 : 129 - 132

STUDIES ON SOME ASPECTS OF THE REPRODUCTIVE PHYSIOLOGY OF THE FEMALE GREY MULLET *MUGIL CEPHALUS* L.

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Introduction

In the development of aquaculture, finfishes have been given top priority and among cultivable finfishes, mullets form one of the most important groups, which can be cultured in confined water such as estuaries, lagoons, etc. The grey mullet or the leaping mullet *Mugil cephalus* is one of the fastest growing and highly delicious table fish which is extremely tolerant to wide ranges of salinity and temperature and occupying the lowest position in the food-chain. This species is widely distributed and very popular world-wide, as a cultivable fish. For the purpose of mass culture programme of mullets, seeds are the prerequisite and the collection of mullet seed from wild is uncertain. Therefore, the method of raising seed by induced breeding is resorted to. Considerable information is available on the procedures adopted in the induced spawning of this species. However, there is a paucity of detailed information on certain aspects of reproductive physiology of this species especially in the local population.

Control of reproduction of the candidate species is one of the most important aspects of aquaculture management. Reproductive control has multiple significance in that, it helps controlled seed production and genetic improvement of the stocks on the one hand and on the other, it aids in the production of monosex population as preferred in certain species of finfish. In order to proceed with the artificial means of reproduction, the aquaculturists have to be fully aware of the gonadal maturation stages, spawning season and the nutritional status of the

breeders before, after and at the time of spawning. Moreover, it is necessary to have first-hand knowledge on the biology, physiology and biochemistry of the fishes that are to be cultured, so that the fish may be placed in the most suitable environmental conditions and be supplied with appropriate feed and energy. This in turn can efficiently enhance the growth of the stock with the resultant increase in the overall fish production. Further, the stress such as severe depletion due to phenomena like maturation, migration, etc. which most of the fishes undergo as a part of their life, have a profound effect on the composition of body. So the fish chemistry becomes essential and requires a special approach. It is against this background, the present study has been taken up, in the female grey mullet *Mugil cephalus*.

Material and methods

The materials and methods employed in different experiments, are dealt with under the following subheadings :

1. Collection of specimens, 2. Collection of blood from specimens, 3. Reproductive biology, 4. Histology, 5. Histochemistry, 6. Electrophoresis and 7. Biochemical analyses.

Results

Various aspects of the breeding biology which are essential pre-requisites for the successful management of both capture and culture fisheries were studied. The organisation and structure of female reproductive system of *M. cephalus* is described in detail and the ovary has been classified into five arbitrary stages of maturity based on its size, shape, colour and texture and microscopic structure of ova. The variations in the gonadosomatic index (GSI), the condition factor (K) and the hepatosomatic index (HSI) during the breeding cycle are determined. With a view to trace the development of ova from the immature stage to the ripe condition, the occyte size - frequency profiles are constructed at various developmental stages. The fecundity has been estimated and the values were correlated with the total length, total body weight and total gonad weight of the fish.

Histological (light microscopic and transmission electron microscopic) picture of oocyte development, ovarian atresia and post-ovulatory follicles are detailed. Various types of yolk inclusions (Yolk globules and lipid droplets) formed during vitellogenesis and the cortical alveolar layer were identified. A number of distinct developmental stages were delineated and the histological observations related to the macroscopic ovary maturity stages. The hepatocytes being the site of synthesis of vitellogenin undergo considerable changes during and after vitellogenesis. Hence the cyclic histological changes of hepatocytes were also studied.

Histochemical techniques are employed to know the chemical nature, function and characterisation of cells, organelles and cellular inclusions, without disturbing their normal structural organization. The histochemistry of oocytes and hepatocytes are presented. Histochemical reaction of proteins, carbohydrates, lipids, nucleic acids, enzymes (alkaline phosphatase, acid phosphatase, succinate dehydrogenase and Δ^5 3 β HSD) were studied and the results provide very useful and important informations.

Changes were observed in the electropherograms of fish serum proteins in relation to sexual differences and gonadal development. The female specific serum protein(s) (FSSP) or vitellogenin (Vtg), which appear(s) in serum of maturing and mature fishes is regarded as the immediate precursor of maturing and mature fishes is regarded as the immediate precursor of the egg yolk proteins. Polyacrylamide gel electrophoresis of egg protein and serum of immature, maturing, mature, ripe and spent females and mature male were carried out; the details of which have been given in detail in the Thesis.

Biochemical composition of fishes, which is an indicator of their nutritive value has been subject to variations depending on season, food intake, breeding and migration. The most important among these, is the "reproductive drain" of nutrients meant for somatic growth, to the gonads. Proximate composition analysis of fish during gonadal development could disclose

the energy banks of various nutrients in the body and trace out the path way through which they are mobilised to the gonad. From aquaculture point of view, a thorough understanding of the biochemical composition of the parent fish keeps us informed about their suitability to face the procreation and that of eggs helps us to assess their quality and hence the condition of young ones. In the present study, the biochemical composition of four tissues *viz.* muscle, liver, ovary and blood serum of *M. cephalus* have been analysed. Seven major parameters namely moisture, proteins, lipids, carbohydrates, cholesterol, carotenoid and ash have been estimated in relation to the maturation of gonad. The concentration of total calcium and iron in the serum and ovary has also been determined at different maturity stages. The analysis of variance (ANOVA), two-way with interaction, was carried out for each biochemical parameter to test the significant changes, *i.e.* (i) between different tissues at various stages of maturity and (*ii*) between different stages of maturity in various tissues.

CMFRI Spl. Publ., 1993; 56 : 133 - 136

SOME OBSERVATIONS ON THE ECOLOGY AND BIOCHEMICAL ASPECTS OF THE SEAWEEDS OF KERALA COAST

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Summary

Economically seaweeds have proved themselves to be a very significant group. Seaweeds are used as food, as the only raw material for phycocolloids like agar-agar, algin, carrageenan, etc. which are extensively used in various industries as gelling, stabilising and thickening agents; in medicine; as fodder; as manure and as a source of vitamins, energy and so on. In addition to their commercial importance, macroalgae play an important role in marine ecosystems. Devastation of seaweed beds has often lead to serious ecological imbalances which in turn has serious fisheries interactions. Thus ecologically, the algal communities of the sea shore lend themselves admirably to a detailed study.

Taking into consideration, the ever growing demand for protein-food for human consumption, it has become very essential to locate non-conventional resources of nutritive value. In this context, the food values of marine algae is currently gaining a lot of importance. Therefore in the present study it was thought worthwhile to investigate into the basic ecological aspects and biochemical constitution of the seaweeds of Kerala Coast.

For the convenience of study, the entire coast of Kerala, was divided into three zones 1. North zone 2. Central zone and 3. South zone. Stations of study were fixed in each zone. Once in every month, trips were undertaken to each zone for making ecological observations and for seaweed collection, for a period of two years.

During the period of study, 52 species of seaweeds were collected from Kerala Coast, out of which 20 species belonged to Chlorophyta, 10 to Phaeophyta and 22 to Rhodophyta. Thus Rhodophyceaen algae were most abundant along Kerala Coast, followed by Chlorophycean alage. Phaeophycean algae were relatively less along the Kerala Coast.

Commercially important seaweeds of Kerala Coast and the places of their availability are given. This included 3 species of agarophytes, 7 species of agaroidophytes and 9 species of alginophytes. Names of important edible seaweeds of Kerala are also given.

Zonewise and stationwise distribution pattern of seaweeds of Kerala Coast are described. Number of seaweed species was maximum in North zone (42 species), followed by South zone (37 species) and Central zone (9 species). Out of the 42 species of seaweeds recorded from North zone, 13 were exclusive to North zone. Out of the 37 species of seaweeds recorded from South zone, 8 were exclusive to South zone. Out of the 9 species of seaweeds recorded from Central zone, 1 was exclusive to Central zone. No Phaeophycean alga was present in the Central zone.

A definite zonation pattern was observed with regard to several species of seaweeds in the present study. Horizontal zonation pattern of seaweeds was observed at Saudi and Varkala.

Density of seaweeds was estimated to be 3971.25 gm/m^2 at Mullur, 2047.5 gm/m^2 at Varkala, 1832.29 gm/m^2 at Elathur, 1408.13 gm/m^2 at Thikkotti and 1387.5 gm/m^2 at Saudi (wet weight). Density of each species of seaweed, seasonal density of different divisions of alage and of some selected species of seaweeds at each station provide useful informations.

Along Kerala Coast, postmonsoon recorded the highest seaweed density followed by premonsoon. Monsoon recorded the lowest seaweed density. Green algal density was highest during monsoon, brown algal density during premonsoon and red algal density during postmonsoon.

Frequency of occurrence of each species of seaweed along Kerala Coast was studied and discussed.

Average standing crop of seaweeds along Kerala Coast (based on the stations studied) was estimated to be 2129.33 gm/m^2. Out of this, agarophytes constituted 13.5%, agaroidophytes 6.98% and alginophytes 9.06%. Thus 29.53% of the seaweed standing crop of Kerala is constituted by commercially important seaweeds.

Environmental data *viz.* atmospheric temperature, surface water temperature, dissolved oxygen, salinity, phosphate, nitrate and silicate of ambient waters were recorded from Mullur, Varkala, Elathur, Thikkotti and Saudi for two years. Statistical significance of seasonal variation in each environmental factor studied at Mullur, Varkala, Elathur, Thikkotti and Saudi are given. Comparison of environmental data recorded from different stations has been made. Statistical significance of the variation between stations, with regard to each environmental factor is also given. Correlation observed between environmental factors at each station are described.

Effect of environmental factors on the density of some selected seaweeds from each station was studied statistically and the results of this study are presented. Density of each species of seaweed studied, showed a correlation of some kind (positive or negative) with one or more environmental factors studied. From this we can conclude that each species of seaweed requires a specific combination of environmental factors for its biomass production.

The protein, carbohydrate and lipid contents and the corresponding calorific values of the seaweeds collected from Mullur, Varkala, Elathur, Thikkotti and Saudi are given. Along Kerala Coast, brown algae recorded the highest protein content of 11.3%. Green and red algae recorded protein contents of 10.2% and 10.3% respectively. Both green and red algae recorded high carbohydrate contents of 22.6% and 22.5% respectively and brown algae the lowest of 11.6%. Brown algae

recorded the highest lipid content of 5.6% followed by green algae with 4% and red algae lowest with 1.9%.

Seasonal variation in biochemical composition of some selected seaweeds from each station and their statistical significance, and stationwise variation in protein, carbohydrate and lipid contents of *Ulva fasciata, U. lactuca, Chaetomorpha antennia* and *Gracilaria corticata* are described and given.

Parameters showing correlation (positive or negative) with protein, carbohydrate and lipid contents of some seaweeds from each station were identified statistically.

Thus in the present study, observations on the ecology of seaweed flora of Kerala, their distribution and zonation pattern, monthly/seasonal density of seaweeds at each station, frequency of occurrence, standing crop, monthly/seasonal/station-wise data on physico-chemical characters of ambient waters at the stations and their influence on seaweed density have been made. This data will help us in the farming of economically important seaweeds, by providing information on the ideal conditions of seaweed biomass production. Biochemical observations on protein, carbohydrate and lipid contents of different species of seaweeds will give us an idea of their nutritive value. Seaweeds with high content of proteins, carbohydrates and lipids can be recommended for food and feed formulations after subjecting them to toxicological studies. The study on monthly/seasonal/station-wise variation in biochemical composition of seaweeds will provide necessary information on the appropriate time and place of harvesting the algal species for exploiting its constitutents.

CMFRI Spl. Publ., 1993; 56 : 137 - 144

STUDIES ON THE CORAL REEFS OF LAKSHADWEEP

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Summary

Lakshadweep is a group of coral islands situated in the Arabian Sea between 08°00' and 12°30'N and between 71°00' and 74°00'E. The archipelago consists of 27 islands and a number of sunken banks and open reefs. Of these, 10 islands are inhabited by man. Our knowledge on the distribution and availability of living marine resources, dynamics of the important physical, chemical and biological parameters in the lagoons, growth of corals, maintenance of the system and status of the environment is meagre. The present study, hence, attempted to widen our knowledge on the above aspects and results of which are summarised below.

Results of the faunistic survey conducted at Kavaratti, Kalpeni, Agatti, Bangaram, Amini, Kadmat and Chetlat Islands for corals and reef associated echinoderms, crustaceans, molluscs and fishes revealed the presence of a large number of species.

A total of 110 species of corals divided among 40 genera and 15 families have been recorded; out of this 22 species are new records to Lakshadweep. Genera such as *Herpolitha, Leptoseris, Oulophyllia* and *Pachyseris* have not previously been recorded from Lakshadweep. Maximum number of species were recorded from Kavaratti and minimum from Kadmat. Though certain islands harbour good number of species, their distribution is patchy and area of live coral-cover was found to be less. Twentytwo species were found to be common to all the islands surveyed.

Altogether 50 species of crustaceans, divided among 32 genera and 18 families have been recorded. Out of these, 41 were

crabs, 2 were lobsters and 7 were prawns. Kavaratti Island has the highest number of species (37) and lowest in Amini (20). Eight species were found to be common to all the islands surveyed. These islands were not found to possess any substantial resource of crustaceans which could be exploited on a commercial level. Sea ranching and culture programmes could improve the stock of lobsters and edible crabs.

Fourtysix species of echinoderms divided among 31 genera and 19 families were noted in the survey. Out of these, *Mithrodia clavigera* is a new record from Lakshadweep. Holothuroidea showed domination with 16 species. Maximum number of species were recorded from Kavaratti (42) and minimum from Bangaram (18). The starfish *Acanthaster planci* was found to occur in Kalpeni Lagoon. Thirteen species were found to be common to all the islands surveyed. Of all echinoderms, the commercially important forms from Lakshadweep are holothurians used in *beche-de-mer* industry. Four species of these were found to be available in substantial quantity. Since the exploitable area is limited, these islands may not withstand large scale commercial exploitation. There is possibility for culture and farming of holothurians, which could be tried to increase the production.

There were 230 molluscs divided among 87 genera and 60 families in the present survey, of this 37 species come under bivalves, 5 species under cephalopods and 188 species under gastropods. Total number of species was highest in Kavaratti (190) and lowest in Amini (70). Thirtyfive species were found to be common to all the islands surveyed. Gastropods ranked highest in all the islands. Micromolluscs and deep water forms were not covered and many more species are likely to occur. The survey indicated a remote possibility for large scale commercial exploitation. However, some species of gastropods, cephalopods and bivalves have potential for commercial farming.

There found to be 120 species of lagoon and reef associated fishes, belonging to 67 genera and 35 families. Out of this, two species - *Forcipiger flavissimus* and *Pygoplites*

diacantthus, were recorded for the first time from Lakshadweep. The family *Labridae* with 13 species was found to be dominating. Species abundance was highest in Kalpeni (105) and lowest in Amini (57). Fourty two species were found to be common to the islands surveyed. The survey indicated the availability of a large number of species of ornamental value.

Hydrobiological studies were carried out in Kavaratti Atoll, which is a perfect atoll, situated along 10°33'N and 72°38'E. The lagoon is 4,500 m long and 1,200 m wide, having a maximum depth of 1.8 m at low tide and 3.5 m at high tide.

Samples were collected from 5 stations inside the lagoon and one station outside the lagoon on fortnightly intervel for the studies on the hydrographical conditions. Productivity of phytoplankton and seagrasses was studied for one year and production from three species of corals for two years. Zooplankton samples were collected from 4 stations at day and one station at night for the entire period of study. Diurnal studies on hydrographical parameters and on the occurrence and abundance of zooplankton were carried out in one station.

Variation in water temperature between stations were insignificant. Between stations the temperature variation was between 29.32 and 29.63°C. Temperature decreased during monsoon due to the seasonal variation in atmospheric temperature. Temperature increased during day and decreased at night.

There was no variation in pH and salinity with location of stations. Average variation in pH was between 8.12 and 8.18 and that of salinity between 34.26 and 34.5‰. Both these parameters exhibited seasonal variation by a decrease during monsoon. Temperature, pH and salinity were positively correlated, which explains the diurnal variation in pH and salinity.

Dissolved oxygen was high in lagoon stations than the open sea station. The variation between stations was from 4.58 to 5.37 ml/l. The high photosynthetic activity in the lagoon by the benthic and symbiotic plant community accounts for this.

High photosynthetic activity during day increases oxygen concentration and intense respiration at night decreases the dissolved oxygen concentration.

Concentration of silicate, phosphate, nitrite and nitrate was very low. Except nitrate, all other parameters showed highest concentration in open sea, indicating their uptake in the lagoon. Nitrate was slightly higher in the lagoon due to the high rate of fixation in the form of nitrate by nitrogen fixing agents in the lagoon. Average variation in silicate between stations was from 3.50 to 4.54 µg at/l, phosphate 0.26 to 0.35 µg at/l, nitrite 0.54 to 0.71 µg at/l and that of nitrate from 0.11 to 0.13 µg at/l. Except silicate, all other parameters showed definite diurnal variation with an increase at night and decrease during day indicating the relation between light and photosynthesis related utilization of these nutrients in the lagoon. This suggests the role of seagrasses and algal communities in the recycling of nutrients within the lagoon community. Except nitrate all these parameters decreased during monsoon, which may be due to the relation between light, photosynthesis, assimilation and fixation.

The lower concentration of calcium in all the lagoon stations than the open sea station indicated the high rate of precipitation by calcifying organisms. The average range of variation between stations was within 422.56 to 433.97 mg/l. Since calcification is strongly light dependent, the lower light intensity during monsoon reduced precipitation of calcium which increased during monsoon. The day time decrease and increase at night of calcium also suggests the role of light in precipitation.

Temperature, pH, salinity and dissolved oxygen increased with decreasing tide and phosphate, nitrite, nitrate and calcium showed a reverse trend, whereas silicate did not show any relation with tide.

Gross primary productivity of phytoplankton varied between 0.62±0.01 to 6.09±2.48 mg $C/m^3/hr$ and net production between 0.20±0.13 to 1.46±0.85 mg $C/m^3/hr$. Highest production was during postmonsoon which amounted to 4.75±0.93 mg

C/m^3/hr (gross) and 0.593±0.49 mg C/m^3/hr (net). The lowest was during monsoon, the values being 1.03±0.33 mg C/m^3/hr (gross) and 0.405±0.21 mg C/m^3/hr (net).

Productivity of the seagrass *Thalassia hemprichii* ranged between 0.281±0.10 and 1.370±0.29 mg C/g/hr (gross), and 0.154±0.10 and 0.769±0.26 mg C/g/hr (net). Production was maximum during premonsoon 0.902±0.44 mg C/g/hr (gross) and 0.556±0.26 mg C/g/hr (net) and minimum during monsoon 0.405±0.11 mg C/g/hr (gross) and 0.225±0.06 mg C/g/hr (net).

Minimum and maximum gross and net production of *Syringodium isoetifolium* was 0.255±0.10 and 0.812±0.10 mg C/g/hr (gross) and 0.175±0.13 and 0.494±0.10 mg C/g/hr (net). Highest production was during premonsoon (0.575±0.16 mg C/g/hr (gross) and 0.321±0.11 mg C/g/hr (net) and lowest during monsoon (0.368±0.10 mg C/g/hr (gross) and 0.246±0.07 mg C/g/hr (net).

Production from corals was found to be maximum during postmonsoon, the values being 0.045±0.01 mg C/g/hr (gross) and 0.020±0.003 mg C/g/hr (net) from *Porites cylindrica*, 0.052±0.01 mg C/g/hr (gross) and 0.025±0.01 mg C/g/hr (net) from *Acropora formosa* and 0.081±0.02 mg C/g/hr (gross) and 0.048±0.01 mg C/g/hr (net) from *Pocillopora damicornis*. Lowest production observed was during monsoon and highest during postmonsoon seasons.

Productivity of phytoplankton was found to be limited by all parameters except nitrite and silicate in which the relation with salinity was significant ($r = 0.677$, $P \leq 0.05$). Productivity of *Thalassia* and *Syringodium* was limited by all parameters except temperature, pH and salinity. The significant correlations were with silicate and nitrite ($r = 0.677$, $P \leq 0.05$ for *Thalassia* and $r = 0.640$, $P \leq 0.05$ for *Syringodium*). Productivity of corals correlated positively with nitrite, silicate, dissolved oxygen, temperature and salinity, indicating the possible influence of these parameters on production. Significant relations were that of *Acropora* with salinity ($r = 0.486$, $P \leq 0.05$) and *Porites*, *Acropora* and *Pocillopora* with silicate ($r = 0.453$, $P \leq 0.05$; $r = 0.581$,

$P \leq 0.01$ and $r = 0.512$, $P \leq 0.453$, $P \leq 0.05$ respectively). However, the relation with silicate is expected to be more of incidental, because silicate is mainly metabolised by diatoms.

Major zooplankton groups observed in day time samples were copepods, the eggs, zoea, decapod larvae, ostracods, bivalve larvae, gastropod larvae and foraminiferans. Night samples, in addition to the above groups, contained doliolum, salps, euphausiids, tunicates and tanidaceae. Numercial abundance varied with location of stations, as well as over seasons. Nocturnal abundance was very high than that of day time abundance. Average density were 581.9/m^3 for station-2, 222.8/m^3 for station-3, 387.1/m^3 for station-5, 317.2/m^3 to station-6 and 2,622.3/m^3 for night station. Nocturnal zooplankton was distinct in their occurrence and seasonal variation, suggesting the presence of resident zooplankton as a component of the lagoon fauna. The sharp increase to very high density after 1800 hrs and the independence of abundance on tide also support this view.

Growth of corals was studied by tagging and 'Alizarin' staining methods in respect of monthly skeletal extension and weight of CaCO3 accretion in a period of 28 days.

The average colony extension of *Acropora formosa* during first year was between 5.03±1.72 and 8.06±1.88 mm/28d during second year it was 4.90±1.27 to 8.68±2.3 mm/28d. Since light and zooxanthellar photosynthesis directly enhance calcification rates, the apical branches which receive more light grew faster (7.30 mm/28d) than the lateral (6.98 mm/28d) and basal (5.95 mm/28d) branches.

Skeletal extension of *Acropora aspera* colony was between 3.08±0.69 and 4.17±0.96 mm/28d for the first year and between 3.42±0.71 and 4.69±0.69 mm/28d for the second year. Extension rate was highest on apical branches (4.47 mm/28d) and lowest on basal branches (3.77 mm/28d).

$CaCO_3$ accretion of *Acropora aspera* colony during the first year was between 9.76±1.33 and 11.97±1.52 mg/28d and during

the second year it was between 10.39±1.44 and 13.38±1.95 mg/28d. Average accretion rate was highest on apical branches (12.97 mg/28d) and lowest on basal branches (10.04 mg/28d).

Total average colony growth and growth on the three positions of the colony also exhibited seasonal variation with a decrease during monsoon season. The low light intensity, drop in many environmental factors, high current velocity (15.06 cm/sec), high amount of total suspended matter (9.95 to 14.65 mg/l) and very high rate of sediment resuspension (103.3 to 124 mg/m^2/day) create less favourable conditions for growth of coral during monsoon. Heavy monsoon wind induces extreme turbulence which agitate the settled sediment and the removal of coral boulders and rocks by people create land and beach erosion which also enhance sediment resuspension rate during monsoon.

Lakshadweep coral reefs are under the threat of deterioration due to natural and manmade causes. Healthy and apparently untouched reef fauna exist only in islands which are not inhabited by man such as Suheli and Bangaram and in some deeper areas of inhabited islands like Kalpeni, Agatti and Chetlat where man cannot easily reach.

Natural damage is not in any large scale at present. The presence of *Acanthaster planci* does not cause threat at present, because the population is thin.

Human interferences pose more serious threat than natural damages. This is mainly by the removal of live corals by local people and visitors, excessive human activity during low tides, destructive methods of fishing, removal of coral stones and boulders from the reef and beach for construction activities, dredging and deepening of jetty, ever increasing developmental activities, housing to accommodate the teeming population and oil pollution from mechanised vessels.

Imposing strict ban on removal of corals, supplying the people with alternate materials for construction, scientific management of reef fishery, restriction on dredging, construc-

tion of proper seawalls, establishment of marine parks, creation of artificial reefs, advanced research on the environmental problems and educating people about the fragility of these ecosystem have to be initiated immediately, which would help protecting these island ecosystems.

CMFRI Spl. Publ., 1993; 56 : 145 - 148

STUDIES ON THE REPRODUCTIVE PHYSIOLOGY OF *LATES CALCARIFER* (BLOCH)

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Summary

Lates calcarifer, though distributed through out the Indian Coast, does not contribute much to the capture fishery. The fish has been recoginised to have vast aquaculture potential, but availability of seed still remains a bottleneck. The study is aimed at providing the necessary information pertaining to various characteristics of natural reproductive cycle. Despite considerable progress achieved in the artificial propagation techniques in several countries of Indo-Pacific, little attempt has been made towards exploring the basic mechanisms underlying various processes associated with the reproduction. With this background, the current work has been taken up to provide descriptive account of the process of gametogenesis and the biochemical changes associated with.

L. calcarifer displays a complex sexuality, where lower length classes are dominated by males and larger ones by females, but for a few exceptions. The majority of the male fish mature after attaining the length in the range of 600 to 650 mm. The length of majority of sex-inverting males was found to be 885 mm. *L. calcarifer* populations is digynous represented by two types of females; primary and secondary. The former develop directly from the immature fish whereas the latter category include those derived from males; constituting majority of the female population.

The sex ratio is in the favour of females, which appear to be the artefact in the population under study. Since, in a protandric hermaphrodite, males usually outnumber the females. This deviation probably indicate the selective loss of

males from the population. The imbalanced sex ratio may be responsible for the high proportion of gravid fish undergoing involution.

L. calcarifer exhibits seasonally synchronized reproductive cycle. Spawning appear to start after the onset of rains, the season extending from mid October to December. *L. calcarifer* is a highly fecund fish, the fecundity estimate ranging from 3.85 to 30.18 million eggs. *L. calcarifer* does not appear to undergo multiple spawning, as no partially spawned fish was encountered during the study. Moreover, this inference find support from histological and physiological evidences too.

The gonad maturation cycle has been classifed into six stages of maturity corresponding to changes in macroscopic as well as microscopic characteristics. The testes are paired strap-like organs, packed with seminiferous lobules containing cysts of germ cells and sertoli cells. On the basis of optical as well as electron microscopic studies, seven stages of spermatogenesis have been identified. The primoridal germ cells, mitotically dividing cells are found in clusters in the newly differentiating testes only and transform into spermatogonia type A. Spermatogonia type A appear to exist in two forms; type Aa (active cells) and type As (stem cells). The stem cells remain dormant in the lobules and become active to resume the spermatogenic cycle in the recovering spent stage for the next spawning season. Spermatogonia type A are individually surrounded by sertoli cell processes and divide mitotically to give rise to spermatogonia type B, organized in the germinal cysts. The primary spermatocytes formed after several divisions of spermatogonia type B, are destined to undergo meiosis. The first meiotic division yields secondary spermatocytes.which in turn produce spermatid after second meiotic division. Spermatid transform into spermatozoa, through spermiogenesis, a process of concurrently occurred events *viz.* condensation of nuclear chromatin; development of axoneme and migration close to articular fossa in the nucleus; relocation of mitochondria and extrusion of excess cytoplasm. The spermiogenesis of *L. calcarifer* appears to belong to type B class, due to lack of nuclear notation. Mature

spermatozoa has morphological features of a primitive type spermatozoa.

The ovaries derived from post-spawned testes are paired, cylindrical organs consisting of ovigerous lamellae, the site for oocyte development. The newly formed ovary has clusters of mitotically dividing primordial germ cells, which give rise to oogonia. The dynamics of cytological events during the oogenesis, from oogonia to egg, parallel to those found in other teleosts, in general. The primary growth phase is characterised by chromatin nucleolus, early perinucleolus and late perinucleolus stages. During this period, oocytes grow in size and display high nucleocytoplasmic interaction. The nucleolar material are extruded into the cytoplasm in the form of nuage, considered to be invovled with biogenesis of mitochondria and ribosomes. The nuage material was found to be major component of Balbiani's vitelline body. During primary growth phase, oocyte accumulates organelles, required for further growth of oocyte. A group of oocytes is recruited into secondary growth phase characterised by appearance of cortical alveoli, lipid droplets and vitelline envelope. Pinocytotic activity, probably related to the sequesteration of exogenous yolk precursor proteins. During vitellogenic stage, the yolk material is incorporated into yolk spheres. The fully grown oocyte (at the end of vitellogenic growth) is tightly packed with mature yolk spheres, concurrently vitelline envelope grows in width and become architecturely complex; differentiated into external and internal layers. Close to ovulation, the oocytes undergo maturation and are referred as egg. The processes associated with maturation involve resumption of meiosis indicated by break down of germinal vesicle; lipid droplets coelsc to form single oil globule, compaction of vitelline envelope. The oocytes grow significantly during this period, probably due to rapid uptake of water.

The cyclic nature of reproductive recrudescence is reflected in the biochemical composition of both somatic as well as gonad tissues. In general, the reproductive development occurs at the cost of metabolic constituents translocated from the somatic sources like liver and muscle. The transportion appear

to be carried out through blood circulation. The fluctuations in biochemical composition are more pronounced in females than males, due to larger investments required to accomplish yolk accumulation in the oocytes. The yolk precursor protein or vitellogenin appear to be synthesised in the hepatocytes. Vitellogenin is a calcium binding glycolphosphoprotein which appear as a band in the electrophoretic profile of serum proteins at stage 3 (Rf - 0.124), becomes diffused at stage 5 and finally disappear at stage 6. The sequestration of vitellogenin is evidenced by pinocytotic activity near the oocyte surface at ultrastructural level. Vitellogenin level in serum, indicated by protein bound phosphate and total calcium contents, appear to be maximum at stage 4 thereafter start decreasing, indicating the development of single clutch of oocytes to be shed simultaneously, as mentioned earlier.

CMFRI Spl. Publ., 1993; 56 : 149 - 152

ECOLOGICAL CHARACTERISTICS OF PRAWN CULTURE FIELDS IN THE COCHIN AREA

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Summary

Brackishwater aquaculture is expanding at a rapid pace in many areas along the Indian Coast. In view of the importance of these water bodies for large scale culture of fish and shell fish, a number of studies have already been made on the ecology and allied aspectes of culture systems adjacent to it. Most of these studies were confined either to short periods of time or in a limited area. But our understanding of the pond ecology in relation to prawn/fish production is still limited. Therefore, the present study is taken up to investigate more on the ecology and comparative productivity of different culture system.

The present investigation on the ecological characteristics of prawn culture fields in the Cochin area was carried out from December 1988 to November 1990 in perennial fields, seasonal fields and canals of coconut plantation.

The study area received a total rainfall of 2963 mm and 2371 mm during 1988-89 and 89-90 respectively. Atmospheric temperature ranged between 24° to 31.5°C in these culture systems. Water temperature closely followed the air temperature and the range was from 26.8° to 36°C in perennial fields, 26.5°C to 36°C in seasonal fields and 27° to 31.5°C in coconut groves. All systems showed high temperature values in premonsoon. pH fluctuated from 6.0 to 9.2 in perennial fields, 6.75 to 8.25 in seasonal fields and 6.1 to 8.35 in coconut groves. No seasonal trend was observed. High salinity values were recorded in premonsoon followed by postmonsoon and the lowest in monsoon. The variation was from 0.28 to 27.23‰ (perennial fields), 4.9 to 28.5‰ (seasonal fields) and 0.96 to

25.25‰ (coconut groves). Total alkalinity values were generally high with wide fluctuation in different systems. It ranged from 10-130 mg/l in perennial fields, 22.5-111 mg/l in seasonal fields and 24-185 mg/l in coconut groves. High values were generally recorded during premonsoon and monsoon. Dissolved oxygen concentration never exceeded 10.29 mg/l. In perennial fields peak concentration was noticed during monsoon, whereas in seasonal fields and coconut groves it was during premonsoon. Dissolved oxygen concentration ranged from 2.03-10.29 mg/l in perennial fields, 1.4-6.66 mg/l in seasonal fields and 1.65-5.59 mg/l in cocount groves. High Nitrate-Nitrogen concentration was noticed in monsoon and postmonsoon in perennial fields and coconut groves; but no such seasonal trend was discrenible in seasonal fields. Its concentration varied from 0.15-27.8 µg at/l (perennial fields), 0.15-22.5 µg at/l (seasonal fields) and 0.94-12.6 µg at/l (coconut groves). No seasonal trend was observed in Nitrite-Nitrogen concentration in the perennial fields. But in seasonal fields and coconut groves high values were recorded during premonsoon and monsoon respectively. Nitrite concentration varied between 0.002-3.38 µg at/l (perennial fields), 0.02-11.1 µg at/l (seasonal fields) and 0.003-2.03 µg at/l (coconut groves). Ammonia-Nitrogen concentration ranged from 1.88 to 164.93 µg at/l in perennial fields, 1.96 to 9.04 µg at/l in seasonal fields and from 1.01 to 93.5 µg at/l in coconut groves and seasonal fluctuation was irregular. Reactive phosphorus concentration was high during premonsoon in perennial and seasonal fields with values ranging from 0 to 15.5 µg at/l and 0.13 to 22.88 µg at/l respectively. Whereas in coconut groves high concentration was recorded during monsoon and postmonsoon and the range was from 0 to 11.23 µg at/l. Silicate-silicon concentration fluctuated from 3 to 160 µg at/l (perennial fields), 4 to 196 µg at/l (seasonal fields) and 12.5 to 112.5 µg at/l (coconut groves). Seasonal fluctuation was inconsistent in these culture systems. Organic carbon content of the sediment did not show any seasonal trend. Among the three culture systems studied, seasonal fields showed high organic carbon content (1.45-3.93%) followed by coconut groves (0.16-3.38%) and perennial fields (0.12-3.64%). In perennial

fields no seasonal trend was noticed in primary production; whereas the production was high during premonsoon in seasonal fields and in monsoon and occasionally during premonsoon in coconut groves. The production ranged from 156-9186 mg C/m^3/day (perennial fields), 250-6378 mg C/m^3/day (seasonal fields) and 100-3338 mg C/m^3/day (coconut groves).

Diatoms were the dominant in phytoplankton, forming about 80-85% in all seasons, followed by dinoflagellates (monsoon and postmonsoon), blue green algae (monsoon) and green algae (monsoon).

There were no seasonal variations of chlorophyll *a* values in perennial fields and coconut groves, whereas high values were recorded during premonsoon in seasonal fields. Both seasonal fields and coconut groves showed high chlorophyll *b* concentration during premonsoon, but the seasonal trend was irregular in the perennial fields. Chlorophyll *c* concentration was more during premonsoon in all systems. No definite pattern in the distribution of carotenoids were observed in perennial fields and coconut groves. On the other hand high carotenoids concentration was recorded during premonsoon in seasonal fields. Percentage composition of different plant pigments indicated that chlorophyll *a* was the dominant plant pigment followed by carotenoids. Chlorophyll *b* and *c* were the least abundant and occurred in more or less uniform percentages.

High zooplankton biomass was noticed during pre- and postmonsoon in these culture systems. Species composition of zooplankton showed that copepods were the dominant component of the plankton in all fields and throughout the seasons. Copepod nauplii, brachyuran zoea, rotifers, ostracods and cladocerans were recorded during monsoon and postmonsoon months.

In the total tertiary production of the perennial fields, prawns accounted for 95.04 to 73.96% and fishes 4.96 to 26.04%. Prawn production fluctuated from 270.87 to 1114.43 kg/ha, with

an average of 692.6 kg/ha. Fish species recorded were *Liza parsia, Etroplus suratensis, E. maculatus, Tachysurus maculatus* and *Ambassis* sp. In seasonal field about 25% of the tertiary production was contributed by fish, comprising of species such as *Tilapia mossambica, Etroplus suratensis, E. maculatus, Liza parsia* and *Ambassis* sp. The prawn production fluctuated from 1640.63 to 2931.88 kg/ha (average 2286.3 kg/ha).

In both perennial and seasonal fields, *M. dobsoni* (56%) was the dominant species of prawn followed by *P. indicus* (29%) and *M. monoceros* (15%).

Comparison of primary, secondary and tertiary production revealed that only less than 14% of the primary production was utilized in the secondary level in these culture systems. A high percentage of primary production (33.67 to 61.27%) was utilized in the tertiary level in seasonal field, but in the perennial field it was less than 9%.

Diurnal study conducted at the Edavanakkad perennial field showed that fluctuation in none of the environmental parameters followed tidal rhythm and the tidal incursion was fully controlled by the sluice gate.

CMFRI Spl. Publ., 1993; 56 : 153 & 154

STUDIES ON BIOCHEMICAL GENETICS OF THE GREY MULLET *MUGIL CEPHALUS* LINNAEUS

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Summary

The results of an investigation on the Biochemical Genetics of *Mugil cephalus* from Cochin, Madras and Orissa is presented based on separate data collected on : standardisation of experiments, species specific identity of *M. cephalus*, its ontogenetic variations, comparative zymogram patterns of 2 general proteins and 8 enzyme systems from Cochin, Madras and Orissa populations of *M. cephalus*, allele frequencies, average polymorphic loci, average number of alleles, average heterozygosity, observed and expected genotype frequencies present at 17 polymorphic loci from Cochin, Madras and Orissa populations and average genetic identity (I) and genetic distance (D) present at 20 loci from the three populations of *M. cephalus*, etc. are explained in detail in the Thesis.

The following original important findings are projected on the biochemical genetics of *M. cephalus*.

1. For the first time species specific biochemical genetic identity of *M. cephalus, L. parsia* and *V. cunnesius* has been clearly established applying zymogram patterns of fifteen enzyme systems and two general protein systems.
2. Precautions were taken to avoid possible size dependent ontogenetic variations while estimating data on genetic variations.
3. This is for the first time the biochemical genetics of populations of *M. cephalus* from Cochin, Madras and Orissa were studied, analysed and reported. Analysis of 21 loci in selected tissues of *M. cephalus* showed 17

polymorphic and 4 non-polymorphic loci. A detailed analysis revealed that there exists a very high degree of biochemical genetic variability in the species as expressed by average values of 0.67 polymorphic loci, 1.77 alleles per locus and 0.36 heterozygosity.

4. Allele frequencies for each of 21 loci were estimated from the observed genotype frequencies of sample populations from Cochin, Madras and Orissa. Allele frequencies at 14 out of 21 loci were significantly different among three populations. These differences were reflected either in the form of frequency of the same allele or different alleles or fixation of either of the alleles in one or other population. These significant differences at allelic frequencies of several loci suggest these three populations are genetically different.

5. Expected genotype distributions according to Hardy-Weinberg Law deviated significantly at nine out of 17 loci. These deviations were caused mainly by the occurrence of excess of heterozygotes. Excess of homozygotes also occurred in three loci at Cochin. Possible reasons for excess of heterozygotes/homozygotes have been discussed.

6. The range of genetic identity and distance values computed between populations of *M. cephalus* from Cochin, Madras and Orissa were 0.859 to 0.921 (I) and 0.095 to 0.233 (D) respectively. The averages of these values for the species were 0.891 (I) and 0.157 (D) respectively. These values suggest that populations of *M. cephalus* from Cochin, Madras and Orissa are experiencing some form of reproductive isolation and undergo certain genetic divergence.

7. Four important conclusions were drawn on the basis of the results of the present investigation.

8. Three recommendations were also made on the basis of evaluation of the results.

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.

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