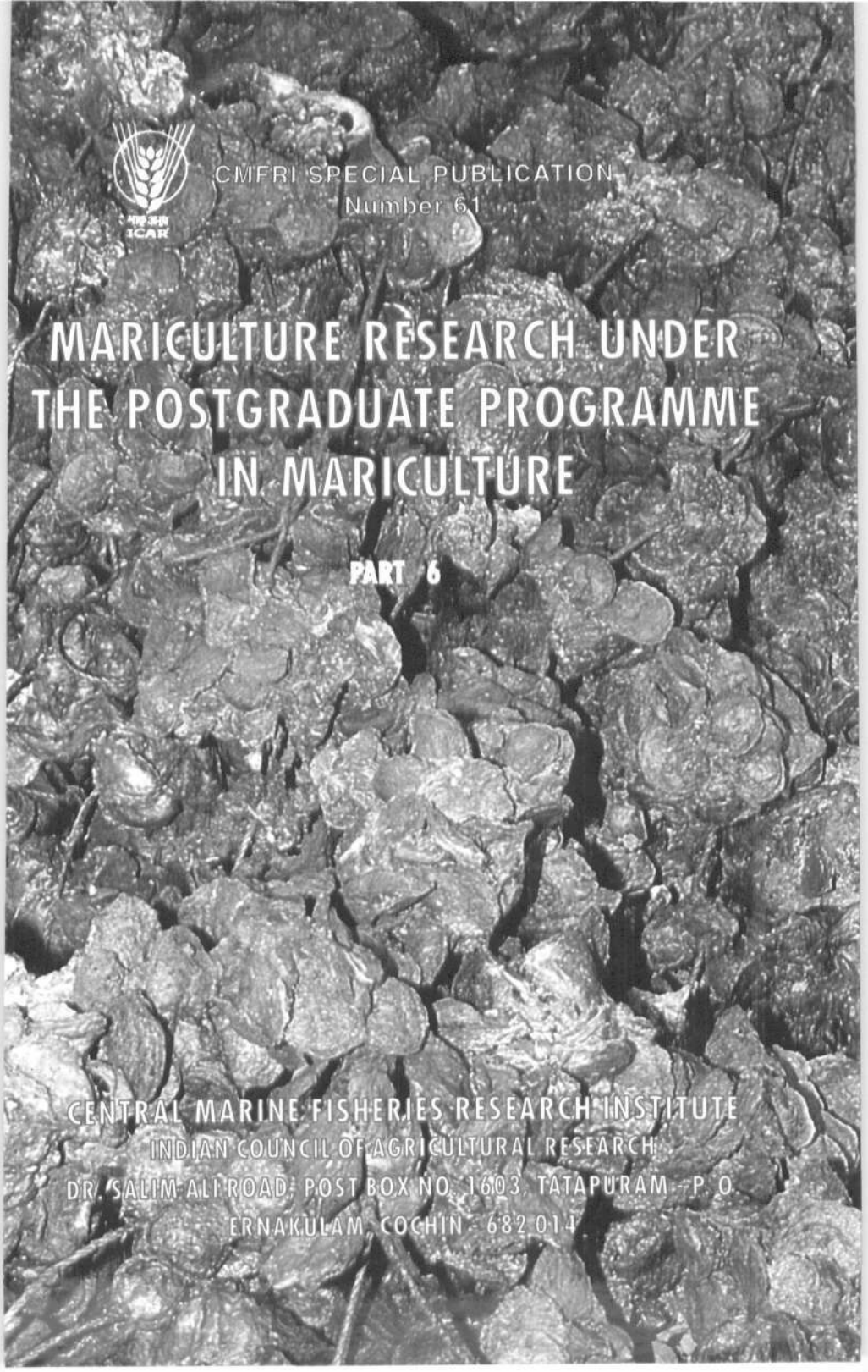

CMFRI SPECIAL PUBLICATION
Number 61

MARICULTURE RESEARCH UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE

PART 6

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 which 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 being 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 of augmenting fish production in 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 facilties, equipments and the lilerature;

— enhance the competence of professional staff;

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

— undertake collaboration progarmmes; and

— organise seminars and workshops.

The postgraduate courses are offered by the CMFRI, Cochin and M. F. Sc (Mariculture) and Ph. D. degrees are awarded by the Central Institute of Fisheries Education, Bombay, a deemed University under the Indian Council of Agricultural Research, New Delhi.

Front cover : Harvest of edible oyster *Crassostrea madrasensis* cultured at Dalavapuram near Quilon in Kerala.

Back cover : Collection of prawn seed in the canals of Vypeen Island near Cochin in Kerala.

Cover Photos by Shri P. Raghavan.

MARICULTURE RESEARCH UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE

PART 6

Edited by

Dr. K. Rengarajan

CMFRI SPECIAL PUBLICATION
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CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
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PREFACE

The Centre of Advanced Studies in Mariculture was instituted 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 being continued now as a regular **'Postgraduate Programme in Mariculture'**. Under this programme, postgraduate courses leading to M.Sc. and Ph.D. degrees were offered in collaboration with the Cochin University of Science and Technology since 1980 and now with the Central Institute of Fisheries Education, Bombay, a deemed University under the Indian Council of Agricultural Research, New Delhi since 1993. 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, sea-cucumber and seaweeds, management of mariculture and extension; instrumentation and research methodology and preparation of dissertation on the basis of a short-term research projects.

There is ever increasing demand for postgraduates in mariculture from this institute especially in the private sector aquaculture projects. The feed-back from the industry on their performance has been very encouraging. This is essentially due to their background knowledge in practical aspects of aquaculture which enables them to handle problems straightaway in the field. It is on the records, that our students occupy very high and key positions not only in leading aquafarms, but in all other Government organisations/agencies and research institutes as well. The research topics for their dissertations in partial fulfilment for the degree, are well

identified in priority areas such as nutrition, physiology, pathology, genetics, reproductive biology and physiology, and ecophysiology of cultivable marine organisms, culture systems, etc.

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

The first part of the results of 22 projects was included in Special Publication No. 19, issued in December 1984 followed by Parts 2 to 5 containing 110 research topics in 1993. This Special Publication covers 19 topics investigated by 10 (M. Sc.) Junior Research Fellows of the twelfth batch and 9 (Ph. D.) Senior Research Fellows of the PGPM.

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

I place on record my sincere thanks to Dr. K. Rengarajan for efficient screening, editing and printing of this Special Publication.

Cochin - 682 014,
June 1995.

M. Devaraj
Director,
Central Marine Fisheries
Research Institute

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1

PRESENT STATUS OF CORAL EROSION IN LAKSHADWEEP WITH SPECIAL REFERENCE TO MINICOY

K. A. NAVAS
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Introduction

Coral reefs are one of the most productive marine ecosystems found in tropical and sub-tropical seas. The International Union for Conservation of Nature (IUCN) has identified coral reefs as one of the essential ecological processes and life-support systems necessary for food production, health and other aspects of human survival. Coral reefs are inhabited by a variety of commercially important fishes, molluscs and crustaceans on which many coastal communities in developing countries depend. Coral reefs also provide nursery grounds for juvenile organisms. Reefs protect the coastline against waves and storm surges, preventing erosion. They help in the formation of sandy beaches and sheltered harbours. Tourism in many countries is also based on reef related activities. The aesthetic appeal and recreational value of reefs have increasing economic importance. Therefore the existence of corals and coral reefs is essential from the environmental, resource and social point of view.

However, coral reefs are extremely vulnerable to various forces of destruction. The agents of destruction of coral reefs can be dealt under different heads namely physical, chemical and biological. Though these forces act separately upon the reefs, they are intimately inter-related. The biological agents of destruction often weaken the substrate and make its more susceptible to physical and chemical erosion. The reverse situation also occurs where damage caused by physical or chemical erosion facilitates bioerosion. In addition, human activities on the reefs also influence the destruction of these fragile ecosystems. These call for the need of evolving effective measures for the rational management of these resources.

For the effective management of the coral reefs, a sound knowledge on the extent of coral degradation is essential. A proper understanding of the various agents of destruction acting on the coral reefs from time to time is also imperative. A systematic survey of the coral reefs is necessary for documenting information on the above aspects.

Objectives

The Lakshadweep atolls are no exceptions to the deterioration of the reefs. Of all the islands of Lakshadweep, the problems of coral bioerosion and degradation is most pronounced in Minicoy Atoll (Pillai, 1990, *Bombay Nat. Hist. Soc., Oxford Univ. Press*, pp. 267-276). Till date no study has been undertaken to investigate qualitatively and quantitatively the agents of destruction, and their possible role in the breakdown and erosion of calcareous materials in this island. Hence the present study was undertaken.

Material and methods

The various aspects studied during the research programme are detailed below.

I. Coral reef survey

During the two year period from January 1990 to December 1991, a survey was carried out in the Minicoy Lagoon. The objectives of the survey were (1) to determine the percentage of live and dead coral cover in the atoll, (2) to determine the species composition and species diversity of corals in the lagoon, (3) to collect coral samples for studying the different bioeroding agents causing coral destruction and (4) to estimate quantitatively the fauna associated with the coral reefs.

The rapid survey technique (Harger, 1984, *UNESCO Reports in Mar. Sci.*, 21 : 83-91) was adopted to study the extent of coral erosion in the lagoon. Prior to the actual survey, a pilot study was conducted to make a preliminary estimation of the distribution of coral communities in the lagoon. Based on the pilot study, the atoll was divided into 9 zones. Each zone had 5 sampling stations and at each

sampling station, 4 quadrate (1 m^2) were sampled at random by diving at low tide from an anchored boat.

II. Ecological characteristics of the environment

The ecological studies were conducted for a period of two years from 23.1.1990 to 26.12.1991. Water temperature, air temperature, salinity, dissolved oxygen and nutrients (nitrate, nitrite, silicate and phosphate) were compared on the leeward and windward side of the lagoon. Ecological parameters of samples collected from live coral areas, dead coral areas, sea-grass bed and sandy bottom were also compared during the study.

The gross sedimentation at 4 sampling stations in the lagoon was also studied (Cortes and Risk, 1985, *Bull. Mar. Sci.*, 36 : 339-356). These stations were selected in such a manner that the rate and pattern of sedimentation in the lagoon could be studied with the knowledge of the current pattern.

Current pattern in the lagoon was studied using drift technique (Maragos, 1978, *Coral reefs : Research Methods, UNESCO,* pp. 353-360). The compass survey method was adopted to locate the position of the floats, the distance covered and the direction of movement of the floats.

In the Kalpeni Lagoon, water samples collected from four stations representing live coral, dead coral, sand and seagrass bed were analysed for chlorophyll content.

Meteorological observations such as pressure, relative humidity, wind speed and direction, cloud cover and rainfall in Minicoy Atoll were also noted during the period of study.

III. Biological destruction of coral reefs

Coral samples collected during the survey from the windward and leeward side of the island were analysed qualitatively and quantitatively for the agents of destruction (sponges, bivalves, sipunculids, polychaetes, crustaceans and echinoderms). The objectives of the study were (1) to determine the faunal groups which are responsible for the bioerosion of reefs in Minicoy, (2) to deducce time dependent changes in some of the faunal composition, (3) to study the distribution

pattern of the bioeroding agents, (4) to estimate the nature and quantum of coral destruction and (5) to understand the relation between coral eroding fauna and environmental parameters.

IV. Influence of human interference on coral reefs at Minicoy

The influence of human activities like blasting and dredging in the lagoon for deepening navigational channels, removal of coral stones for construction, hand picking of live corals, fishing activities, pollution and destruction of natural vegetation on the coral reefs at Minicoy were studied.

Results and discussion

Coral reef survey

Except the deeper zones in the lagoon, in all the remaining areas the percentage of live coral cover was poor. On the lagoon reef flats, the major part of the substratum was constituted by dead corals. The index of species diversity in the lagoon did not show any predictable pattern with the depth of water. There was no significant change in the species diversity although there was considerable change in the pattern of live and dead coral cover. In general the coral reefs of Minicoy is characterised by low species diversity and live coral cover except for a few monospecific species in the deeper zones.

Ecological studies

The variations in temperature, salinity, dissolved oxygen and nutrients in the lagoon and reefs were well within the limit of lethal levels determined for corals and hence was not considered as major factors that cause mortality for corals. The sedimentation rate observed in the lagoon was similar to normal rates experienced in other reefs and hence Minicoy cannot be considered as a reef under stress. During premonsoon, the westerly flowing southwest and northwest currents dominated while during monsoon, the northeast currents dominated. The southeast and northeast currents equally dominated in the postmonsoon season. The velocity of currents in the lagoon varied between 0.011 and 0.633 knots/hour.

Biological destruction of coral reefs

The major faunal groups that caused erosion of calcareous substrate included fishes, echinoids, sponges, polychaetes, sipunculids, bivalves and cirripeds. The density of coral feeding fishes were higher in the deeper areas where there was good live coral cover. The diadeimid sea urchin *Echinothrix diadema* was the most abundant coraline algae grazing echinoid on the reefs while on the lagoon shoal, the rock boring *Echinometra mathei* was the most common echinoid species. The much dreaded crown-of-thorns starfish *Acanthaster planci* is not considered a serious threat in view of their limited occurrence. The damage caused to corals by boring species of sponges was widespread. The number and biomass of polychaetes varied between habitats and substrates with higher density did not necessarily have a higher biomass of individuals. A greater diversity and density of polychaetes, sipunculids and bivalves was observed in the numerous lagoon shoal. The boring cirriped was found only on the reefs. In order to estimate the volume of calcareous material eroded by the bioeroding agents in the case of sipunculids, cirripeds and bivalves, an exponential relationship was estabilished between the size of the animal and volume of the burrow.

Human interferences on coral reefs

Blasting of the reef flat and the lagoon shoals and dredging of the lagoon bottom were found to have detrimental effects on coral growth in Minicoy Atoll. Dredging caused stirring-up of the bottom sand and transportation of this sand along with the water current towards the southern end of lagoon resulted in a 0.5 cm thick additional deposit of sand. This also resulted in mass mortality of corals near the northern end of the lagoon. Blasting of the reef flat and subsequent dredging of deepening of the channel led to greater influx of water into the lagoon at high tide resulting in increased rate of erosion of the lagoon beaches. Hand picking of live corals contributed significantly to the destruction of branching forms of corals from the lagoon and reefs in Minicoy. Removal of coral rocks and coral shingles for construction purposes led to the exposure of the loose beach sand beneath to waves which led to an increased removal of beach sand during monsoon on the seaward beach side.

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2

EFFECTS OF SOME HEAVY METALS COPPOR, ZINC AND LEAD ON CERTAIN TISSUES OF *LIZA PARSIA* (HAMILTON-BUCHANAN) IN DIFFERENT ENVIRONMENTS

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K. RENGARAJAN
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Introduction

India is a developing country and it is tremendously advancing towards the industrial progress and developments. This development results to some level of pollution, both in atmosphere and hydrosphere, the latter has been blessed by Mother Nature providing a long coastline of over 6100 km with excellent marine wealth in both east and west coasts, and a number of rivers flowing on both directions into the sea forming reasonably broad estuarine systems. The brackishwater area available in the country is estimated to be about 1.4 million hectares. The conservation of estuarine and backishwater environments is of paramount importance and their monitoring of pollution is highly essential.

In view of the above an attempt has been made to study the extent of heavy metal (copper, zinc and lead) pollution in estuaries along the east and west coasts of India, the concentration of the above mentioned heavy metals in water, sediment and tissues of *Liza parsia* as the experimental animal for two years.

In addition ot the above field observations, an attempt was made in the laboratory exposing the test animal *L. parsia* to media with different concentrations of Cu, Zn and Pb for bioassay, accumulation of metals and Bioaccumulation Factor. This was felt essential to fully understand the effect of heavy metals on our coastal exploitable fishery resources as also cultivable finfishes and shellfishes for better management and formulation of policies.

Materials and methods

Eighteen stations in six centres *viz.* Korapuzha Estuary, Calicut; Cochin Backwater; Tuticorion Bay; the Gulf of Mannar and Palk Bay, Mandapam; Ennore Creek, Madras and Rusikulya Estuary, Ganjam; each one is unique in its nature were selected along the east and west coast of India for two years from January 1991 to December 1992. Monthly study was conducted in Cochin Backwater for a period of 12 months from June - 1991 to May 1992.

Parameters studied and their estimation

(a) Physical parameters

Temperature of water	:	Mercury thermometer.
pH of water	:	pH paper.
Depth of water	:	Graduated rope with weight.
Colour and nature of soil	:	Visual observation.
Organic carbon in soil	:	Black's rapid titration method (Walkley and Black, 1934, *Soil. Sci.*, 37(1) : 29-38).

(b) Chemical parameters

Salinity in water	:	Classical Mohr titration method (Strickland and Parsons, 1968, *Bull. Fish. Res. Bd. Canada*, **167** : 1 - 311).
Total hardness in water	:	Titration with EDTA (APHA-AWWA-WPCF, 1980, *American Public Health Assn.*, **15**th Edn., pp. 194- 199).
Cu, Zn and Pb in water	:	As per guidelines of Brewer *et al.* (1969, *ASTM STP.*, **443** : 70-77) and Sengupta *et al.* (1978, *Indian J. Mar. Sci.*, 7 : 295-299).
Cu, Zn and Pb in sediment	:	Digestion method of Lithner (1975, *FAO Fish. Tech. Paper*, **137** : 41-68).

Cu, Zn and Pb in tissues	:	Digestion method of Dalziel and Baker (1983, *FAO Fish. Tech. Paper*, **212** : 14-20).
Moisture content	:	Drying (Szefer *et al.*, 1990, *Mar. Pollut. Bull.*, **21** (2) : 60-62).

(c) Biological parameters

Length and weight of *L. parsia*	:	By standard methods.
Bioaccumulation Factor	:	As per the formulae of Buikema *et al.* (1982, *Wat. Res.*, **16** : 239-262) and Connell and Schuurmenn (1988, *Ecotoxicol. Environ. Safety*, 15 (3) : 324-335).
Tissues selected		Liver, gills, kidney, intestine, ovary, skin and muscle.

Seasons selected

The seasons selected were Premonsoon, Monsoon and Postmonsoon (Qasim and Gopinathan, 1969, *Proc. Indian Acad. Sci.*, **69** B(6) : 336-348).

Data analysis

The obtained data were analysed for mean, standard deviation, level of significance by applying ANOVA, interrelationship with other parameters studied with the help of correlation study (Snedecor and Cochran, 1967, *Statistical Methods*. Oxford and IBH Publishing Co., pp. 1-593).

Laboratory study

(a) ***Range-finding bioassy*** : This was conducted as per the guidelines of APHA-AWWA-WPCF (1976, *American Public Health Assn.*, **14**th *Edn.*, pp. 800-869) for selection of the experimental concentrations of heavy metals for definite acute toxicity studies (Bioassay).

(b) ***Bioassay*** : This was conducted to find out the LC50 values along with LC16, LC84, slope function, 95% confidence limits and 95% fiducial limits for heavy metals (individually and their combination). The method followed here was

designed by Reish and Oshida (1987, *FAO Fish. Tech. Paper,* **247** : 1- 62).

Toxic unit : It was calculated as per the formulae of Ward and Parrish (1982, *FAO Fish. Tech. Paper,* **185** : 1-23).

Chronic exposure studies : Two sublethal concentrations (1/10th and 1/100th of 96 hr LC50 for combined toxicants) along with controls were selected for chronic exposure experiments. In these the experimental animals (*L. parsia*) were experimented for 21 days. From the experiments the Bioaccumulation and Bioaccumulation Factors were estimated/calculated in 1st, 2nd and 3rd week in different tissues of *L. parsia* for different heavy metals such as copper, zinc and lead. The obtained data were analysed for level of significance between treatments and exposure periods; % of variation from control tissues; mean; standard deviation and correlation between Bioaccumulation and Bioaccumulation Factor.

Results and discussion

Field studies

Copper : The very high significant differences were observed for copper in water in different centres, seasons and, surface and bottom collections. Comparing the mean values (over two years of collection) with that of WHO standard (3.0 ppb), all the centres were found polluted but, with that of EPA safe limit (25.0 ppb) they were found below the level.

The highly significant differences were noticed for copper content in sediments of different centres, stations and seasons. The copper in sediment of Cochin Backwater and Ennore Creek were found above the "MESS-1" and "BCSS-1" standards.

In *L. parsia* the highest content of copper was recorded in liver and the lowest in muscle. Seasonal variations were noticed in ovary, skin, intestine and kidney of *L. parsia* from Cochin Backwater. The centrewise differences were seen in the case of liver and intestine. No good relations were obtained between the content in tissues and water; tissues and sediment. Good and significant relations were obtained between intestine and liver; ovary and gills; skin and kidney; skin and intestine; skin and ovary; muscle and gills; muscle and kidney; muscle

and intestine; muscle and ovary, and muscle and skin. Comparing with the Standard Reference Material (SRM) of IAEA/Monaco for copper (4.5 + 0.3 ppm), in all the tissues copper contents were above the standard. The contents in tissues were seen positively correlated with the Bioaccumulation Factor (KB). For calculation of KB the moisture values were utilized.

Zinc : The significant differences were seen for zinc in water in different centres, seasons and, surface and bottom collections. The negative relations were obtained for zinc in water with that of salinity and total hardness. Comparing with that of WHO standard zinc of 5.0 ppb, all the centres were seen polluted. Except few collections at Cochin Backwater and Ennore Creek, the mean zinc concentration in respective centres were found below the safe level of EPA.

The significant (0.1% level) centrewise, seasonwise and stationwise differences were observed in zinc contents in sediments. Zinc in water and sediment were seen related at 1% level with coefficient 'r' 0.774. The sediment in Cochin Backwater was found polluted when compard to that of "MESS-1" and "BCSS-1" standards. The Cochin Backwater along with Ennore Creek recorded higher zinc in its sediments than the USGS rock standards.

The ovary and muscle of *L. parsia* recorded highest and lowest content of zinc respectively. The seasonal differences were seen for zinc content in gills, kidney, intestine and skin at Cochin Backwater. In all the centres the seasonal differences were recorded for liver and intestine. The centrewise differences were seen in the case of ovary, skin and muscle. The relationships were established between liver and gills; liver and intestine; liver and skin; lover and muscle. In Cochin Backwater gills, intestine and skin of *L. parsia* showed significant relationships with sediment. It is linked to the browsing feeding of *L. parsia* in sediment. All the tissues recorded higher content of zinc than the IAEA/Monaco standard. The centrewise differences in Bioaccumulation Factor was noticed for liver, kidney, intestine, skin and muscle. The KB and content of zinc in tissues seen positively related in all the tissues.

Lead : In water the lead content did not vary significantly between seasons, centres, and surface and bottom water samples. But in Cochin Backwater the monthly and seasonal variations were observed from the monitoring programme. The yearly variation was also seen in centres for lead content in water. Except certain collections at Ennore Creek, all the mean values of lead in water in centres found below the EPA safe limit (100 ppb).

The yearly and seasonal differences were seen for lead in sediment. The centres and stations differed among themselves at 0.1% F-value. The Cochin Backwater sediments recorded higher lead in it than the USGS, MESS-1 and BCSS-1 standards.

The lead in gills was seen higher than the other tissues of *L. parsia* studied. the 10% significance was seen between seasonal contents of lead in liver in six centres and, in intestine in Cochin Backwater. For none of the tissues the centrewise difference was noticed for lead content. Very highly significant and good relation was observed between intestine and water; intestine and kidney; skin and liver; skin and gills; muscle and liver; skin and gills; muscle and liver; muscle and skin; muscle and gills; and kidney and water. In Cochin Backwater, in muscle and ovary of *L. parsia* lead content seen related to that the lead in water. For muscle and overy in Cochin Backwater the Bioaccumulation Factor seen related to the lead in water. The Bioaccumulation Factor showed the positive relationship with the lead content in tissues. The Bioaccumulation Factor and the content in tissues were found more for zinc followed by copper and lead. The exceptions for metal contents were for ovary (lead followed by copper and zinc) and gills (zinc followed by lead and copper).

Laboratory studies

Bioassay : From bioassay based on 12 hr LC50, the toxicities of different metals on *L. parsia* were found in the order : Cu > Zn > combination > Pb and based on 96 hr LC50 : Zn > Cu > combination > Pb.

The toxic unit calculated for the metal was 1.3489 which is greater than the unit showing the synergism of the metals

of *L. parsia*. Based on Gaddum's diagram the toxicities of the metals on *L. parsia* were seen "less than additive".

Chronic exposures

Copper : The highest accumulation of copper was seen in liver and the lowest in muscle. The higher the concentration of copper in medium, higher the bioaccumulation of it in tissues were found. In gills and kidney the accumulation of copper was seen as a function of the exposure period. The copper content in tissues showed negative relationships with that of Bioaccumulation Factors.

Zinc : The differential accumulation of zinc over time was noticed in all the tissues of *L. parsia*. The accumulation in tissues was seen as a function of the zinc concentration in medium. The maximum zinc detected in liver and the minimum in muscle. Except skin in all other tissues in 1/10th 96 hr LC50 the 14th day recorded the maximum concentration of zinc. The accumulation of zinc in tissues and Bioaccumulation Factors seen negatively correlated.

Lead : The highest content of lead was estimated in gills and the lowest in kidney. The accumulation of lead was seen many folds in tissues in experiments than the field centres. It is because, the lead is an non-essential metal. The bioaccumulation and Bioaccumulation Factor was seen negatively correlated.

For all the metals the Bioaccumulation Factors were seen decreasing from control towards the higher metal concentrations in medium. The correlation of metal content in tissues with Bioaccumulation Factor in field centres was combined with that in experiment for the particular tissue to get the composite graph in computer. Such graphs were obtained for copper in kidney, zinc in liver, lead in gills and kidney. This type of graphs indicated the maximum accumulation (more than proportionately) of a metal in a particular tissue upto a limit where the Bioaccumulation Factor started declining. After that stage the accumulation was seen less than proportionately.

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3

STUDIES ON THE BIOLOGY, ECOLOGY AND FISHERY OF THE SEA-CUCUMBER *HOLOTHURIA (METRIATYLA) SCABRA* JAEGER FROM SOUTHEAST COAST OF INDIA

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Introduction

Sea-cucumbers or Holothurians are the most important groups among the echinoderms which belong to the family Holothuriidae. Among the holothurians, *Holothuria scabra* is commonly available in the Gulf of Mannar, Palk Bay, Andaman and Nicobar Islands and they are used for the preparation of *Beche-de-mer*. *Beche-de-mer* is the product of the degutted, dired or smoked holothurian rich in protein content. This product is exported to Singapore, Hongkong and other Southeast Asian countries for use as an item of food. Annually India is earning a foreign exchange of about 20 lakhs rupees by exporting this product. Thus there is a good scope to develop the processing industry and to step up the production of *Beche-de-mer* in this country.

Objectives

The biology of the Sea-cucumber *H. scabra* is not known in detail. In order to fill up this gap and to provide the basic information for the industry for its expansion and rational utilisation of these resources, the present research work on biology, ecology and fishery was undertaken on *H. scabra* along the southeast coast of India.

Materials and methods

Holothuria (Metriatyla) scabra Jaeger were collected by Tallu valai and skin diving from Tuticorin, Keelakarai and Tirupalakudi in the southeast coast of India, Tuticorin as main centre, from March 1988 to February 1990. Food and feeding, feeding intensity, length-weight relationship, biochemical composition of bottom sediments and the gut, organic carbon

from gut materials, size frequency distribution, reproductive biology, age and growth, animal behaviour and sea-cucumber fishery, etc. were analysed/studied during this investigation following standard procedures.

Results

The results of the present study are summarised as follows :

1. The distribution of *Holothuria (M) scabra* indicated its availability all along coastal areas on the Palk Bay from Rameswaram to Mallipattinam and along the Gulf of Mannar from Pamban to Ervadi and Tuticorin at 4-20 m depth.

2. The major fishing for holothurians was done by skin diving at all the centres. The Tallu valai was operated at Tuticorin and Vedalai and trawlers were operated at Rameswaram.

3. The food of *H. (M). scabra* consists of organic matter which contains mud, sand, shell debris, bivalves and algae. Observations indicated that the species seems to be a non-selective feeder.

4. Quantitatively, *H. (M.) scabra* preferred the particles of below 500 μ size which constituted 62.15% of the gut content. This confirmed that the above species prefers the muddy-sandy substratum.

5. There is no significant difference between the size of individual and size of the particles in the gut contents of *H. (M.) scabra*. Also, there is no relationship between total length, total weight and gutted weight of the animal with the total length and total wet weight of the digestive tract.

6. It was observed the *H. (M.) scabra* feeds actively during breeding seasons *viz.* March-April and November-December.

7. The analysis of the biochemical constituents *viz.* organic carbon, organic matter, carbohydrate, nitrogen and protein from the sediments where the individual lives as well as from the digestive tract showed that the assimilation efficiencies from oesophagus to anus to be 16.29% of organic

carbon and organic matter, 18.53% of carbohydrate and 32.41% of nitrogen and protein. From the sediment to faeces the assimilation efficiency was found to be 7.56% of organic matter, 30.38% of organic carbon, 26.53% of carbohydrate, 21.75% of nitrogen and 21.77% of protein.

8. The assimilation efficiency from sediment to faeces indicated that the faecal pellets of *H. (M.) scabra* were semidigested.

9. The length - weight relationship was found to be : for indeterminate $W = 1.047887\ L^{1.0527}$ ($r = 0.79$); male $W = 0.0091878\ L^{2.3648}$ ($r = 0.86$); female $W = 0.000591583\ L^{2.4460}$ ($r = 0.89$); and for (pooled) male and female $W = 0.0007392524\ L^{2.4049}$ ($r = 0.88$). The exponent value was significantly different from 3. This indicated that the length-weight relationship corresponds to allometric growth.

10. The reproductive cycle of the present species showed five stages of maturity *viz.* immature, maturing, early mature, late mature and spent based on the macroscopical and microscopical observations of the gonads.

11. The gonad index (GI) values were found maximum in males during April - June and October - November while in females during April-May and November. This indicated a high gonadial maturity in these months. A negative correlation was found between gonad index and salinity and no relationship was noted between gonad index and temperature.

12. The size at first maturity of the species indicated that the male attains maturity at 21.0 cm and the females at 21.3 cm. The mean total weight (TW) recorded in male was 290 g and in female 310 g, and the mean gutted weight observed was 125 g for the pooled data.

13. Two spawning seasons were observed in *H. (M.) scabra* one in March - April and next in November - December. Juveniles (3 - 5 cm) was recorded in March 1989 from Pamban (Chinnapalam) along the Gulf of Mannar Coast which would have been recruited some time in November - December of the earlier year.

14. Young ones of *H. (M.) scabra* prefer algal ground to bury themselves into the muddy-sandy substratum.

15. The fecundity observed in *H. (M.) scabra* ranged from 1,04,688 to 10,04,160 ova. A significant relationship was noted between the fecundity and gonad weight by logarithmic regression ($\log_e F = 9.3892 + 1.6768 \log_e G$; $r^2 = 0.82$).

16. A multiple relationship was fitted between total length, total weight, gutted weight, gonad weight and maturity stages were found significant.

17. The ratio of males to females, on an average was found to be 1 : 0.89 and during the breeding season ratio was found to be 1 : 1. Significant chi-square values were obtained during May, July, December 1988 and January 1989 ($P < 0.05$).

18. Results on age and growth indicated $L\infty = 40.37$ cm and $k = 0.327$ (annual) and $L\infty = 39.6$ cm and $k = 0.42$ (annual) respectively. The maximum length recorded was 40 cm. The growth parameters $L\infty$ and k as obtained in both the methods did not show much variation. Based on these, a life span of 10 years is indicated for the species. The growth rate was 8 cm in the first year which decreased to 3 cm in the fifth year. From the sixth year onwards the growth was uniform with an average growth rate of 1-2 cm.

19. The burrowing behaviour and locomotion of *H. (M.) scabra* was observed in the laboratory. The experimental work showed that light plays a vital and controlling role in burrowing habit of holothurian.

20. The fishing season for holothurians commences from October to March along the Gulf of Mannar Coast and from March to October along the Palk Bay Coast.

21. The species fished in the southeast coast include *Holothuria scabra, H. spinifera, H. atra* and *Bohadschia marmorata*. Of these, *H. (M.) scabra* was dominant.

22. The total catches recorded from Tuticorin, Keelakarai, Vedalai, Rameswaram and Tirupalakudi centres were 33.35, 31.15, 12.66, 15.26 and 134.24 tonnes respectively. Of the five

centres, Tirupalakudi forms the major centre for holothurian fishing.

23. The percentage of catches recorded by skin diving, trawlers and Tallu valai were 80.04%, 10.27% and 9.69% respectively. Skin diving contributes to the maximum catch.

24. Of the total catch, 83.85% of the catches by skin diving comes from the Gulf of Mannar and 74.32% from Palk Bay. Tallu valai contributes to 16.15% from the Gulf of Mannar. Trawlers contribute to 25.69% from Palk Bay.

25. The holothurians landed all along the Gulf of Mannar and the Palk Bay Coasts constitute 25.6% and 74.4% respectively. This showed that Palk Bay Coast is more productive.

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STUDIES ON THE EFFECT OF STEROID HORMONES ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF THE MULLET *LIZA PARSIA* (HAMILTON-BUCHANAN)

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Objectives

The present study was conducted to determine the efficacy of selected steroid hormones, *viz.* 17 Lα - methyltestosterone (MT, diethylstilbestrol (DES, 17 β - estradiol (ES) and thyroid hormone (T3), and to find out their optimum dosages, which elicit maximum anabolic effect in the fry of the mullet *Liza parsia*. As a part of the study, the interaction of selected dosages of T3 + MT was also examined to find out if growth promotion could be achieved through a combination of hormones. Through another experiment the protein - sparing effect of MT also studied with a view to improving the dietary protein utilization and reduce the protein levels in the diet without affecting the performance of the fish. Besides this, estrone and MT were administered intramuscularly to adult immature mullets to see the change in the ovary, if any, associated with these exogenous hormones.

Materials and methods

The duration of the feeding experiments ranged between 45 and 60 days and injection experiments 30 days depending upon the objectives of the study. All the feeding experiments were conducted in the laboratory selecting a randomized block design with three replicates for each treatment. Isocaloric and isonitrogenous compounded diets were used for most of the experiments except for the protein sparing experiment. Compounded diets were prepared from locally available feed ingredients such as fish-meal, groundnut oil-cake [lipid - free], tapioca, rice bran, corn oil and cod liver oil. Vitamin and mineral premixes were added in appropriate quantities as recommended by John Halver for fish. Graded levels of

hormones were used for determining the optimum hormone requirement in the diet. Fish were fed on a restricted ratio of 7% of the body weight twice a day.

Environmental parameters (salinity, ammonia, pH and dissolved oxygen contents of water) were monitored regularly, and most of them were found to be within normal range.

Sampling of the animals for obtaining growth data were carried out at regular intervals and based on this, feeding rates were adjusted.

Response parameters considered included specific growth rate, condition factor, survival rate, food conversion ratio, digestibility coefficient, gross food conversion efficiency, protein efficiency ratio and proximate composition of the fish.

Standard procedures were followed for biochemical analysis. Data were processed with suitable statistical procedures.

To find out the optimum levels of MT two experiments were conducted. The first experiment was conducted for 60 days with dietary MT doses ranging from 0 to 60 mg with an interval of 10 mg. Based on the findings of this experiment, another experiment using 0 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg and 15 mg doses, was conducted for 45 days to find out the optimum dietary levels of MT.

Results and discussion

Heavy mortalities in the fish groups receiving diets with MT levels above 40 mg compelled the discontinuance of the treatments after 40 days and the remaining fish groups were reared upto 60 days. The response of the fish fry to the dietary doses of MT was found to be dose - dependent. MT doses exceeding 10 mg/kg diet induced poor growth in *Liza parsia*. Moderate gains in weight over control was found from 2 to 8 mg MT and, therefore, doses from 2 to 8 mg seems to be anabolic, whereas MT doses above 10 mg results in a negative growth. Diets containing MT levels exceeding 30 mg has deleterious effect on the fish and induce heavy mortalities. The survival, growth, digestibility, conversion efficiency, protein efficiency ratio, food conversion rate and body

composition indicate that 2 mg is the optimum dietary level of MT for the fry of the mullet *Liza parsia*. There is also no advantage by using more than 2 mg MT in the diet. MT doses exceeding 10 mg/kg diet has a growth depressing effect on the fish fry.

Since diethylstilbestrol [DES] gave contradictory results in teleosts, one experiment was performed for sixty days to find out the efficacy of this steroid. In this experiment dietary levels of DES ranged between 0 to 1.8 mg, with an interval of 0.3.

The response achieved by the fish suggest that DES is not a anabolic steroid for the fish fry, within the dosages tested during this experiment. Only marginal weight gain was observed in the fish groups receiving 0.3 mg/kg DES; above this level weight gain was found to decrease steadily as dosages increased, indicating the growth depressing effect of DES. Further DES is known to be a carcinogenic steroid and hence not recommended in the diet of *L. parsia*.

To determine the anabolic effect of 17 β - estradiol one experiment was conducted for sixty days using graded levels of this hormone *viz.* 0 mg, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg and 10 mg/kg. The results revealed that relatively low dosages upto 4 mg/kg, promote growth and improve the feed and protein conversion efficiency in *L. parsia*.

One set of experiments was conducted to test the efficacy of thyroid hormone [T3] and also to study the synergetic effect of MT and T3, if any. In this experiment selected levels of individual hormones T3 (0 mg, 3 mg, 6 mg and 9 mg/kg) and MT (0 mg, 2 mg and 4 mg/kg) and their mixtures (3 mg T3 + 2 mg MT, 6 mg T3 + 2 mg MT, 9 mg T3 + 2 mg MT, 3 mg T3 + 4 mg MT, 6 mg T3 + 4 mg MT and 9 mg T3 + 4 mg MT per kg diet) were fed to *Liza parsia* fry for 45 days. A hormone-free control diet was also kept.

Among the individual hormone dosages 2 mg MT showed the best anabolic effect and of the T3 dosages, 3 mg/kg showed moderate response. Based on the survival and growth indices, the dose of 9 mg T3 + 2 mg MT proved to be the most effective in promoting growth during the first 30 days of rearing. However, at the termination of the

experiment, after 45 days, the concentration of 2 mg MT/kg gave superior growth than all other treatments. Therefore, for the short rearing periods the combination of 9 mg T3 + 2 mg MT is more effective, while for the longer duration 2 mg MT is the best anabolic agent for *L. parsia.*

After noting the efficacy of MT at the dosage of 2 mg/kg diet, one experiment was performed for sixty days to observe the protein sparing action of this hormone for the gold-spot mullet fry, if any. Compounded diets, with protein levels of 35%, 30% and 25% and for each protein level three different MT dosages (1 mg, 2 mg and 3 mg), were prepared and fed to the fish.

Results obtained for growth, food conversion, digestibility, protein utilization and body contents showed that the diets containing 30% protein with 1 mg, 2 mg and 3 mg levels of MT, are better than the 35% and 25% diets. However, the dose of 30% protein + 2 mg MT/kg have the best results, thus showing protein could be better utilized and that significant savings can be made in the protein content of the diet by incorporating MT at optimum levels in *Liza parsia.*

Two experiments were conducted to study the changes in the ovary associated with the injections of MT and estrone. Adult immature fish were used to test the efficacy of these steroid hormones through injections. The experimental fish were fed on a isocaloric compounded diet containing 35% protein during the experimental period.

A total of ten injections were given intramuscularly to each of the fish at the rate of 1 injection/3 days and the doses were 0, 0.5, 1.0 and 1.5 mg MT/kg and 0, 1, 2 and 3 mg estrone/kg body weight.

Histological observations indicate that both the hormones have induced the ovary development. All the MT treated fish and the fish injected with 1 mg and 2 mg estrone were observed in central nucleolus stage of development. The 3 mg estrone treated ovary was in perinucleolus stage as compared to the oogonia stage of control ovary. The results suggest that estrone at a dosage of 3 mg/kg has significant positive influence on ovarian maturation in *L. parsia.*

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HETEROTROPHIC BACTERIAL ACTIVITY IN SELECTED AQUACULTURE SYSTEMS NEAR COCHIN

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Introduction

Success of aquaculture operation depends mainly on the techniques and on the fertility of aquaculture pond. Increase in bacterial productivity will help in economising the culture operation through reduced costly inputs like artificial feed and fertilisers. Microbial food webs are in integral part of all aquaculture ponds and have a direct impact on productivity, even where intensive artificial feeding is practised. There are three major reasons for this. Firstly, in all the ponds, respiration is predominantly due to microbes. Hence the oxygen content of the water is governed by bacterial activity. Secondly, in all extensive, semi-intensive and some intensive aquaculture systems, bacteria also contribute significantly to the food-web. They may be eaten directly by the target species (*e.g.* tilapia or mullet) or by small animals on which the target species feed (*e.g.* larvae and juveniles of penaeid prawns). Thirdly, through the activity of the heterotrophic decomposers, nitrogen and phosphorus are recycled to stimulate primary production and zooplankton which in turn will increase the production of fish and prawns.

In this context it is important to elucidate the dynamic relationship between phytoplankton, bacteria and zooplankton and the extent to which such interactions are controlled by extracellular organic carbon released by phytoplankton.

If we can quantify the heterotrophic activity of bacteria, we can then make informed judgements about the above three functional roles of bacteria and thus improve pond management and productivity.

Materials and methods

The study on "Heterotrophic bacterial activity in selected aquaculture systems near Cochin" was carried out based on the data collected in perennial and seasonal pond from January 1989 to December 1990 to know the seasonal and vertical pattern of bacterial chemo-organotrophy by measuring glucose and acetate utilisation. ^{14}C Glucose and acetate were used as tracers to study the uptake and incorporation of glucose and acetate by natural bacterial populations in the aquaculture ponds. The technique was useful in measuring glucose and acetate incorporation rates as low as 0.5 n ml^{-1} The incorporation followed saturation kinetics. The relationship of environmental variables to bacterial chemo-organotrophy was also found out to understand the factors controlling microbial processes and the total energy flow in this ecosystem.

Results and discussion

The heterotrophic activity was generally affected mainly by monsoons and tides when the organic and inorganic matter were brought down to aquaculture pond and is of considerable importance in the maintenance of microbial life in the perennial as well as seasonal pond. The monsoon was characterised by general decline in heterotrophic activity and postmonsoon season was found to be productive over the two year study period, when fertiliser input can be reduced considerably.

The substrate turnover time [T_t] varied seasonally; substrate concentration S_n ($k_t + S_n$) varied little; V_{max} was directly proportional to the concentration of transporting enzyme (*e.g.* heterotrophs). The values of V_{max} tended to increase with total aerobic heterotroph, but the high variability of number and V_{max} values in different seasons suggested quantitative and qualitative changes in the bacterial population. The concentration of total aerobic heterotrophs (plate counts by pour plate techniques) was not related to heterotrophic uptake parameters and found negatively correlated with dissolved oxygen in both the ponds. The activity parameters were somewhat more variable than bacterial numbers and biomass. The kinetic parameters of glucose and acetate utilisation did not vary considerably between surface water and bottom water. T_t, V_{max} and ($K_t + S_n$) increased for both

the substrates in premonsoon months. This probably reflected the general increase in activity of the estuarine biological community during the period and also can be attributed to higher temperature.

T_t of acetate was found to be positively correlated with chlorophyll 'a' indicating direct dependance of bacteria on DOM coming from phytoplankton. Kinetic parameter and total heterotrophy appear to be restricted in their distribution by temperature rather than salinity probably because the salinity changes are not sufficient to alter them. Total number of heterotrophs obtained by plate counting techniques was less than expected, but the data showed distinct seasonal succession of bacterial types and that there was consistant differences between seasonal and perennial ponds. Psychrophilic bacteria dominated during monsoon and psychrophilic and mesophilic bacteria dominated in postmonsoon season. All these results demonstrate that the microbial parameters in both the ecosystem are controlled more by a concentration gradient.

The chemo-organotrophic microbial community responded very rapidly to changes in temperature, substrate concentration and oxygen concentration by altering the bacterial population quantitatively and qualitatively. Glucose and acetate are produced through phytoplankton excretion or dissolution of phytoplankton after death and because of high rate of glucose and acetate utilisation as observed in the present study it is to be concluded that they are very important substrates for the maintenance of fertility of the pond by the chemo-organotrophic community.

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HISTOPATHOLOGICAL STUDIES ON ZINC TOXICITY IN *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Aquatic pollution which is a menace in both the natural and culture ecosystems, is undoubtedly one of the major factors that poses a threat to the sustenance of aquaculture systems. Heavy metals which enter the aquatic ecosystems through industrial discharge, are considered to be serious pollutants since they are often incorporated into food chains and are concentrated by aquatic organisms to a level which affects their structural and functional integrity. The heavy metal zinc, though indispensable to life in trace amount, becomes toxic at higher concentrations and presents the problem of being accumulated and biomagnified at various trophic levels.

Bioassay studies aim at determining the range of toxic levels which effect mortality in the animal populations in the ecosystem. In order to discern the damage caused by the toxicant on the cellular architecture and the functional physiology of the animals, detailed investigations at the histological, physiological and biochemical levels following sub-lethal exposure are resorted to. Histopathological studies are particularly useful in assessing the extent of disturbance within the organ systems concerned.

The Indian White Prawn *Penaeus indicus* is one of the most important decapod crustaceans of considerable commercial value, contributing notably to both capture and culture fisheries. Juveniles of *P. indicus* inhabit estuaries and backwaters along the coasts of India, supports a rich brackish-water fishery and also offers considerable potential for aquaculture. However, the hazards arising from harmful effluents discharged from several major industries situated along the stretches of these ecosystems, are more than likely to have an impact on *P. indicus* seed resources in these areas.

Objectives

This study was aimed at assessing the extent of harm done by the heavy metal zinc to *P. indicus*, through lethal toxicity tests and through histopathological observations on the hepatopancreas and gills of the animals following exposure to sub-lethal concentrations of the metal. The histopathological effects of starvation of the hepatopancreas were also worked out for a comparative study, as aquatic animals often have to endure starvation either due to the scarcity of food or when subjected to some other stress, extrinsic or intrinsic by nature.

Materials and methods

Juveniles of *P. indicus* [40 - 50 mm length, measured from the tip of the rostrum to the tip of the telson] collected from the prawn culture ponds at Puthuvyppu near Cochin were acclimatised for 5 days in 30 lt plastic tubs holding 25 lt of well aerated seawater of 20 ± 1 ppt salinity at room temperature [28 ± 2°C]. Raw clam meat was given as feed and water exchange was done at the rate of 80% every day.

Static renewal bioassay, with 100% water exchange once in every 24 hrs was conducted following APHA [APHA-AWWA-WPCF 1980, 15th Edn., pp 194-199]. Zinc sulphate [$ZnSO_4$. $7H_2O$, M.W. 287.55; Analytical Grade] was used as the source of zinc for the experiments. Test concentrations were decided after performing range-finding tests. Ten animals each were exposed to 25 lt of the test solution, all concentrations being run in triplicate. A control in triplicate, with no toxicant, was also maintained. Uniform aeration was maintained in all the tubs throughout the experiment which was run for 96 hrs. The animals were not fed during this period. Animals behaviour and mortality in each concentration were recorded every 12 hrs. Dead specimens were removed immediately; the criterion for death was taken to be the lack of movement even on gentle Prodding. The mortality data obtained was subjected to probit Analysis to estimate the 96 hr LC_{50}. The ET_{50} and toxicity curves were obtained through graphical representation.

During the chronic exposure studies, the test animals were subjected to two sub-lethal concentrations [100 and

300 ppb] of zinc for a period of 20 days. Both the concentrations and the control were run in duplicate with 100% water exchange daily. Uniform aeration was maintained in all the tubs and the animals were fed with raw clam meat every evening. Animals belonging to the intermoult stage were sacrificed after 10 and 20 days of exposure and the hepatopancreas and gills were fixed immediately in Aqueous Bouin's Fixative for 24 hrs. The tissues were processed through a graded series of tert. Butyl Alcohol, cleared in chloroform and embedded in paraffin wax [M.P. 58 - 60°C]. The sections [7μ] were stained in Harris' haematoxylin - Eosin and mounted in DPX.

In order to make a comparative study on the effect of starvation, the test animals were subjected to starvation for a period of 10 days, stocking them individually [as a measure to avoid cannibalism] in 20 1t of filtered seawater. Each run was accompanied by a control in which the animals were fed *ad libitum*. Water exchange was done at the rate of 80% every day. On closure of the experiment, animals belonging to the intermoult stage were sacrificed and their hepatopancreas were processed for histopathological studies, as mentioned earlier.

Results

Eposure of juvenile *P. indicus* to various concentrations of zinc over a period of 96 hrs revealed a marked difference in the response of the animals to low and comparatively higher concentrations. Mortality was first recorded in 3200 ppb and 4200 ppb test solutions in just 12 hrs of exposure. Maximum mortality [90%] was observed in 4200 ppb test concentration. The 96 hr LC_{50} was worked out to be 1668.16 ppb by Probit method. A regular feature noticed during the 96 hr assay was the development of a distinct blackened appearance of the gills of the animals exposed to zinc. Consistant development of blackened gills was noticed in doses above 1000 ppb. This phenomenon was less consistant in animals exposed to 500 ppb of zinc. There was no development of black gills in any of the animals exposed to 100 ppb of the metal.

The hepatopancreatic tissue of juvenile *P. indicus* exposed to sub-lethal concentrations of zinc showed marked

deviation from the normal structure. Excessive vacuolation of cells, loss of homogeneity of vacuolar contents, reduction in cellular inclusions, occurrence of pycnotic nuclei, delamination of epithelial cells, clumping of cells in the tubular lumen and rupture of tubules as a whole, were among the histopathological changes encountered. The occurrence of clumps of epithelial cells was more common in the case of animals exposed to 100 ppb of zinc. Exposure to 300 ppb of zinc resulted in a widespread occurrence of cellular rupture and tubular damage, along with an increase in the number of vacuolated B-cells.

The gills of juvenile *P. indicus* showed drastic changes following zinc exposure. Haemocyte infiltration and distension of secondary lamellae were noticed in the gills of prawns exposed to both 100 and 300 ppb of zinc. Sloughing of primary and secondary gill filaments, accompanied by the occurrence of pycnotic nuclei was conspicuous in the animals exposed to higher concentration of the metal. A peculiar feature noticed was the occurrence of necrotic lesions in the gills of zinc-abused animals, leading to blackening of the gills in animals exposed to 300 ppb of zinc.

Starvation for a period of 10 days brought about a high degree of degenerative changes in the hepatopancreas of juvenile *P. indicus*. A general reduction in cellular inclusion, presence of pycnotic nuclei and shrinkage of the basal lamina were noticed widely. Most of the vacuoles looked empty and vacuolar contents, when present, showed little homogeneity. A significant feature noticed was the presence of residual contents in the tubular lumen, which was not noticed in the tubules of control animals. Rapid autolytic activities seem to lead ultimately to cellular atrophy in this vital organ.

Discussion

The capacity of a toxicant to effect death in an organism is best assessed by means of lethal toxicity tests conducted under laboratory conditions. The present study reveals that juvenile *P. indicus* can tolerate zinc concentrations to an extent; this is indicated by the high 96 hr LC_{50} value of 1668.16 ppb. However, one important aspect which lethal toxicity tests often tend to overlook, is the fact that though animal succumb

to toxic substances after exposure to specific concentrations for specific periods of time, the actual damage to the cellular structure and physiology is initiated at a much earlier stage by even meagre concentrations of the toxicant. This is evident from the results of the histopathological studies conducted, since concentrations as low as 100 ppb and 300 ppb are found to bring about destructive and deteriorative changes in the hepatopancreas and gills which are, among the most, vital organ systems of the animal. Histopathological alterations such as delamination of epithelial cells, clumping of cells in the tubular lumen and rupture of tubules, which occur as a result of heavy metal abuse, show the effect of this xenobiotic on the structural integrity of the organelles and organs of the animal. Destruction of the cellular architecture which assumes alarming dimensions will no doubt affect the functional integrity of the organ system. Excessive vacuolation in the epithelial cells of the hepatopancreas could be indicative of the hyperactivity of this organ which often plays a major role in detoxification, under pollutant stress. Important histopathological alterations noticed in the gills of *P. indicus* exposed to zinc included haemocyte infiltration, distension and distortion of secondary lamellae and the widespread occurrence of pycnotic nuclei. The formation of necrotic lesions and blackening of the gills which are indicative of destruction of the gill processes as a whole, suggest a possible impairment of the vital function of respiration; the gills can thus act as useful indicators of stress in the animals due to zinc pollution.

Based on these findings, it would be desirable to limit the 'safe level' of the metal to a very narrow range in the aquatic environment. The prediction of such a level should undoubtedly be of help in effecting proper regulatory measures in the management of pollution problems caused by zinc in culture systems and in the natural ecosystem. The results of the studies on starvation indicate that this stress brings about drastic changes in the structure of the hepatopancreas, which can thus be used as an indicator organ in the assessment of the nutritional status of the animal and any dietary stress it is subjected to.

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THE COMBINED EFFECTS OF RATION SIZE AND FEEDING FREQUENCY ON GROWTH IN POSTLARVAE, JUVENILE AND ADULT *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Feed management is the most critical factor in determining the profitability of a shrimp farm. Nearly 50% to 70% of the total operating cost goes to feed. Overfeeding causes wastage of feed, water pollution and in turn increases operating cost. Underfeeding on the other hand results in loss of production. For proper feed management, importance should be given to both ration size and to frequence of feeding.

Ration is considered to be a driving force and any restriction to it results in a lower metabolic rate [Brett, 1979, *Fish Physiology,* 8: 279-352]. In order to achieve an efficient feeding regime in an aquaculture situation, continual adjustment of the ration level is necessary to compensate in changing requirements [Sedgwick, 1979, *Aquaculture,* 16(4) : 279 - 298]. An optimum ration size is very significant when considering the reduction in the cost of operation and also in the environmental hygiene. Relevant data on ration size for postlarva are limited.

The frequency with which animals consume feeds, has also been shown to have substantial effect on metabolism [Cohn and Joseph, 1959, *Am. J. Physiol.,* **196** : 965-968; Cohn *et al., 1963, Am. J. Physiol.,* **209** : 153-157; Kekwick and Pawan, 1966, *Metabolism,* **15** : 173-180; Fabry, 1967, *Handbook of Physiology,* Sect. **6**, 1 : 31-49]. In intensive farming operations, feeding frequency has an important role in improving overall yield and reducing the cost of operation. There is a dearth of literature as regards the feeding frequency of *Penaeus indicus* which along with *Penaeus monodon* are the commercially important species on the Indian continent. Information,

regarding the feeding frequencies in shrimp nutrition is based upon the recommendation of the commercial shrimp manufacturers and pertains mainly to *Penaeus monodon*.

Nutritional studies conducted with shrimp have classically been confined to empirically designed dietary trials, while investigations of the bioenergetics and digestive physiology of the organisms have received less emphasis. Until recently, most investigations concerning the digestive enzymes of shrimp have been qualitative and focussed on the comparative aspects of digestion. Since shrimp are now being evaluated for commercial culture, the changes in enzyme activities during the life cycle and adaptation to new diets are being examined quantitatively.

Objectives

The objectives of this investigation are to :

i. evaluate complete biochemical and physical condition of the three commercial feed [starter, grower and finisher] used for feeding the different stages of prawns *viz.* postlarvae, juveniles and adults respectively;

ii. study the correlation, if any, between varying ration sizes and growth at the postlarval, juvenile and adult stages;

iii. evaluate the effect of different feeding frequencies on growth, food conversion ratio, protein efficiency ratio, gross and net conversion efficiency and survival at the three different stages in the experimental animals; and

iv. record the effect of the varying ration sizes on the enzyme activities of trypsin in the experimental animals.

Materials and methods

Three different stages *viz.* postlarvae, juveniles and adults of the Indian white prawn *Penaeus indicus* were used as experimental animals. Experimental feed used was a commercial shrimp feed procured from a company of international repute. Analysis of the three different feeds *viz.* starter, grower and finisher were carried out to ascertain their proximate composition [AOAC, 1970; 1975, *Assoc. of Anal. Chem., Washington, p. 1094]*. Water stability of the feeds was

also ascertained [Jayaram and Shetty, 1981, *Aquaculture*, 23 : 355 - 359]. The control groups were fed *ad libitum* with the meat of *Sunetta scripta*.

Experiments were carried out using plastic tubs of 5 1t, 15 1t and 25 1t capacity respectively for the different stages. Uniform aeration was maintained throughout the experimental period. Care was taken to prevent the escape of animals. Salinity of seawater was maintained at 30 ppt for postlarvae, while for juveniles and adults it was adjusted to 20 ± 2 ppt by diluting with tap water since juvenile *P. indicus* prefer lower salinities [Colvin, 1976, *Aquaculture*, 7(4) : 315 - 326; Paul Raj, 1976, *Ph. D Thesis, Univ. of Madras;* 241 pp; Paul Raj and Sanjeeva Raj, 1980, *Proc. Symp. Coast. Aquacult. MBAI*, 1: 236-243].

Postlarvae of *P. indicus* belonging to the same broodstock having an initial average length of 2.98 ± 0.21 cm and weight of 0.1313 ± 0.01 g were obtained from the Prawn Culture Laboratory of the Central Institute of Brackishwater Aquaculture, Narakkal, Cochin. The postlarvae were acclimatised to the laboratory conditions for a week before start of the experiment juveniles of *P. indicus* having an initial average length and weight of 6.353 ± 0.39 cm and 1.49 ± 0.28 g respectively were collected from backwater canals near Pallithode in Alleppey District.

Adults of *P. indicus* having an average initial length of 9.34 ± 1.3 cm and an average initial weight of 4.40 ± 1.13 g were collected from extensive culture ponds at Pallithode.

Experimental animals were acclimatised for a week, after which animals were visually selected and randomly distributed into the experimental tubs. The number of animals maintained was 14 per tub for postlarvae 7 per tub for juveniles and 7 per tub for adults. Two replicates were maintained for each treatment. Prior to start of the experiment the length and weight of animals were again recorded. Feeding was suspended and animals starved for 48 hours before the start of the experiment.

Ration size and feeding frequency : Four different ration sizes were selected for each stage based on earlier published

works [New, M. B., 1989, FAO (UN), Rome ADCP/REP/87/26; Sick *et al.*, 1973, *Prog. Fish. Cult.*, 35(1) : 22-26]. The four different ration sizes [expressed as % body weight] chosen for postlarvae were 2%, 12%, 22% and 32%. The four different ration sizes chosen for juveniles were 2%, 8%, 12% and 16%, whereas for adults it was 1%, 4%, 6%, and 8%. The different feeding frequencies adhered to are specified in Table 1.

TABLE 1. *The feeding frequencies adhered in the present investigation*

Frequency	Timing
One time morning	0800 hr
One time night	2000 hr
Two times	0800 hr and 2000 hr
Three times	0800 hr, 1400 hr, 2000 hr
Four times	0800 hr, 1200 hr, 1600 hr, 2000 hr

The timings were adjusted so as to provide uniform time interval between the different feeding frequencies. Left over food and faecal matter were collected daily and oven dried and then the dry weight was recorded. Daily one-fourth of the water in the tubs was siphoned out and replenished with an equal amount of fresh seawater. Every 10 days of the experiment, the animals were randomly selected from their tubs and their length and weight recorded. Experiment was terminated on the 30th day and length and weight recorded.

Physicochemical parameters such as temperature, pH, salinity, dissolved oxygen were monitored throughout the experimental duration.

The different parameters studied were survival rate, % growth in length and weight, food conversion ratio [FCR], protein efficiency ratio (PER), specific growth rate (SGR), gross conversion efficiency (K_1 %) and net conversion efficiency (K_2 %).

Optimum ration size for the three size groups were plotted using specific growth rate against various ration sizes for each feeding frequency and then by construction of asymptotic curve through the data [Sedgwick, 1979, *Aquaculture*, **19(4)** : 279- 198].

Results and discussion

Biochemical analysis : Results of the biochemical analysis carried out on the three feeds are given in Table 2.

TABLE 2. *Chemical composition (%) of the three commercial feeds*

Constituent	Starter	Grower	Finisher
Moisture	10.83	7.41	8.35
Dry matter (DM)	89.17	92.59	91.65
Crude protein[1]	36.09	34.45	29.53
Ether extract[1]	8.00	7.50	8.50
Crude Fibre[1]	2.34	3.20	2.80
Nitrogen free extract (NFE)[2]	53.57	54.85	59.17
Organic matter (OM)[3]	76.55	79.00	80.49
Ash[1]	12.62	13.59	11.16
Acid insoluble Ash	9.88	12.69	10.95
Energy value (KJg^{-1})*	20.85	20.48	20.47

1. Calculated on DM%.

2. NFE calculated by difference = 100 - (Moisutre % + CP % + CF % + EE % + Ash %)

3. OM = DM % - Ash %

* Energy values calculated as protein 23.4 KJg^{-1} fat 39.8 KJg^{-1} and carbohydrate 17.2 KJg^{-1} fibre was assumed to have zero energetic value (Cho *et al.*, 1982, *Comp. Biochem. Physiol.*, 73 : 25 - 41).

TABLE 3. Chitin and mineral content (%) of the experimental feeds

	Starter	Grower	Finisher
Chitin	1.58	1.32	1.28
Minerals :			
Sodium	0.62	0.52	0.52
Calcium	2.95	2.25	2.15
Available phosphorus	0.71	0.64	0.55

The three feeds used in the present study were also found to be highly water stable as after five hours of immersion in seawater only a marginal loss of 16% was obtained in the dry matter content.

Postlarvae

Survival of postlarvae was found to be statistically significant ($P < 0.05$) with regard to change in ration size and 100% survival was obtained at 12% ration size. Feeding frequency however did not reflect any statistically significant variations ($P > 0.05$). Variations in ration size and feeding frequency failed to reflect any significant difference ($P > 0.05$) in the performance of postlarvae in terms of FCR, PER, K_1 and K_2, though significant difference was observed in terms of SGR ($P < 0.01$) with regard to ration size. Optimum ration size of *P. indicus* postlarvae obtained by plotting the various ration sizes (in % body weight for day) against specific growth rate (in % weight per day) was 12%. Rations below this were termed maintenance rations, while values above this were termed maximum rations. Best FCR of 1.09, maximum K_1 value of 91.74 and K_2 value of 147 were also obtained at the 12% ration size with four times feeding.

Juveniles

High mortality was encountered in juvenile *P. indicus* at all the ration sizes survival values ranged from 16% to 83% for juveniles. Variations in ration size and feeding frequency failed to reflect any significant differences ($P > 0.05$) in the performance of juveniles in terms of SGR, PER, K_1 and K_2. FCR was seen to be highly significant ($P < 0.01$) with regard to ration size for juvenile *P. indicus* with the best values of 1.52 for FCR and maximum values for K_1 and K_2 being obtained at the 8% ration level with three times feeding. Optimum ration size obtained for juvenile *P. indicus* by plotting various ration sizes against specific growth for different feeding frequencies was 8% body weight per day.

Adults

Wide variation was seen in the case of % survival with the values ranging fron 25% to 100%. Significant differences ($P < 0.05$) was seen with regard to ration size. Similarly,

significant differences was observed in the case of SGR ($P < 0.001$), FCR and K_1 ($P < 0.05$) with regard to ration size. But PER and K_2 failed to elicit any statistically significant difference ($P > 0.05$) with regard to variation in both ration size and feeding frequency. The optimum ration size against specific growth rate was 4.5 % body weight per day. Best FCR value of 1.5 and maximum conversion efficiency as obtained at 4.5 % ration size with three times feeding.

Ration size and feeding frequency failed to elicit any statistically significant variations ($P > 0.05$) with regard to protein, lipid, ash and moisture content of the experimental animals.

Enzyme study

Total trypsin activity reported as µM-p-nitroanilide produced/minute/gram tissue was seen to be slightly lower (4.23 - 6.69 µM) in postlarvae compared to juvenile (7.24 - 8.92 µM) and adult (10.23 - 12.24 µM) *P. indicus* with regard to variation in ration size. In the case of starved animals *viz.* postlarva, juvenile and adult *P. indicus*, lower enzyme activity was recorded. In the case of specific activity reported as µM-p-nitroanilide produced/minute/mg no significant variation was seen with regard to both ration size and starvation.

Discussion

The proximate composition analysis of the three commercial feeds *viz.* starter, grower and finisher used in the present study are very much in agreement with the composition of eight brands of commercial shrimp feed of other manufacturers reported by New (1990, *Tech. and Eco. Aspects of Shrimp Farming. Proc. Aquatech '90 Conference, Kaula Lumpur, Malaysia,* pp. 69-73) and five brands of imported feeds by Sanhotra and Pereira (MS). The lipid contents of the three feeds used in the present study were found to be slighly higher (7.5 % to 8.5 %) than the values ranging from 2.8 % to 4 % usually encountered in other commercial feeds and reported by New (1990, *loc. cit.*) However, our values are more in agreement with those of Sanhotra and Pereira (MS) who reported values ranging fron 4.95 to 7.85 as the lipid %

in the five commercial feeds analysed by them. Results of the water stability experiment are in agreement with those of Sanhotra and Pereira (MS) who observed a 14 - 18 % loss in the dry matter content of the five feeds analysed by them. Generally the feeds were within the standards prescribed for penaeid shrimp and designed to meet the nutritional requirements of postlarval, juvenile and adult *P. indicus*.

Ration size

The importance of daily ration is clear as excessive feeding is wasteful and possibly deleterious to the water quality while inadequate rations limit growth rate. Research on compounded diets for the postlarvae have hitherto been rare. Forster (1972, *Proc. First Satl. Conf. on Aquaculture Nutr. Delaware, NOAA,* pp. 229 - 248) reported having fed large (70 mg) postlarvae of *P. monodon* with a compounded diet and obtained a good survival though slower growth as with fresh food. Khanappa (1977, *Q. Res. Rep. Aquacult. Dept. SEAFDEC,* **1** (1): 24 - 26) fed much smaller *P. monodon* postlarvae (5.5 mg) with a compounded diet based on fish meal and rice bran and found that growth and Food Conversion Ratio were best with 30% protein in the diet. Our results yielded overall good survival and FCRs at the four ration sizes for postlarvae of *P. indicus*, with the best results being obtained for 12 % rations wherein a FCR of 1.09 was obtained with maximum conversion efficiency at a feeding frequency of four times per day. The specific growth rate (SGR) of *P. indicus* postlarvae was also apparently maximum at this ration size.

Jones *et al.* (1979, *mar. Biol.*, 54 : 261-267) stated mortality as a common problem in feeding processed diets to penaeid larval and postlarval stages. The fact that moulting cycle is much shorter and that reserves are consumed faster in younger stages than in adults emphasises the importance of formulae with the best adapted ingredients and properly balanced calorie rations. However, in our study we did not encounter heavy mortality in any of the treatment groups in the postlarval stages maintained on the commercial feed and survival was better in comparison to the clam meat fed control animals.

In the present study, juvenile *P. indicus* recorded more mortalities and poor survival throughout most of the experimental period, at all the feeding rates on the pelleted feeds giving an overall survival ranging from 16% to 83%. The resulting cannibalism due to moulting and lower densities presumably allowed the few survivors to grow well. Similar was the case observed in adults though survival was slightly better than that observed in the case of juveniles and ranged from 14% to 100% being statistically significant ($P < 0.05$) with regard to ration size.

In the present investigation, adult and juvenile shrimp did not consume all the feed right after being fed, at the higher feeding rates, but fed intermittently throughout the full 24 hour day. The results show that penaeids are capable of consuming large amount of food. Sick *et al.* (1972, *Fish. Bull.*, 70(1) : 101-108) comparing a pelleted feed supplied at 0, 5, 10 and 15% of the total biomass illustrated that growth was directly proportional to an increase in feeding rate in *P. setiferus* and *P. aztecus.* Tacchanuruk and Stickney (1982, *J. World. Maricult. Soc.*, 13 : 63-72) studying the effects of feeding rate and feeding frequency on protein digestibility in freshwater shrimp *M. rosenbergii* neither feeding rate nor frequency of feeding to affect digestibility and stated a feeding rate of approximately 2% of body weight daily to be sufficient for *M. rosenbergii* (adults) greater than 13 grams.

The success of experimental diets has frequently been assessed in terms of FCR (Forster, 1976, *loc. cit.;* New, 1976, *Aquaculture,* 9 : 101-144). Efficient utilisation of feeds can arise under conditions of restricted food supply when rations are close to the optimum level.

Forster (1976, *loc. cit.*) suggested that an arbitrary FCR of 2 : 1 may be required in tropical prawns before economic viability can be approached FCR values measured for *P. merguiensis* close to maximum rations (approximately 1.35 : 1) obtained with small prawns by Sedgwick (1979, *Aquaculture,* 16(4) : 279-298) indicates that economic feeding is possible. Best FCR values of 1.09, 1.52 and 1.5 obtained in our study upon feeding juvenile and adult prawns with commercial

pelleted feed were also those at the optimum ration and are in close agreement with the results of Sedgwick (1979, *loc. cit.;* Sick *et al.*, 1972, *loc. cit.).*

Starved postlarvae, juvenile and adult *P. indicus* were not able to sustain their initial biomass level beyond two weeks. Cannibalism appeared to be prevalent among starved organisms and the decline in weight was undoubtedly moderate due to growth of animals preying upon dead shrimp, an observation also in agreement with Sick *et al.* (1972, *loc. cit.).*

Feeding frequency

A knowledge of the frequency of feeding can be favourably manipulated to avoid overfeeding and reduce wastage (Singh and Srivastava, 1984, *Bamidgeh,* **36** : 80-90). Caillouet *et al.* (1973, *Proc. World Maricult. Soc.,* **3** : 415-465) found that feeding penaeid shrimp *P. duororum* once or three times per night did not affect survival, growth or yield. In the present study, though slight differences were observed with regard to survival, growth and FCR in the one time night and morning feeding, it was not statistically significant ($P < 0.05$).

Sreekumaran Nair *et al.* (1982, *Mahasagar,* **15**(2) : 95-104) studied growth of *M. dobsoni* and *P. indicus* and concluded that though different levels of feeding did not exert any influence on the two coefficients of length-weight relation, it showed definite effects on the growth rate, the maximum growth rate being shown by prawns fed five times a day.

Comparison of the present values with those of others is difficult because of differences in stocking size, density, habitats, temperature and depths. In our present study no statistically significant correlation ($P < 0.05$) could be deciphered at the different feeding frequencies with regard to survival, growth and proximate body composition in postlarval, juvenile and adult *P. indicus.*

Enzyme study

The little variation observed in the present study in the total activity of trypsin amongst the three size groups shows

that variation may be related to size of the animal and also the amount of protein in the diet as ration size varied. This is in agreement with the findings of Lee *et al.* (1984, *Aquaculture*, 42 (3-4) : 225-239) who reported a differing proteolytic response to protein level and source as a function of size reflecting changes in digestive physiology as the shrimp grow. Moreover, total enzyme activities reflected differences associated with protein levels, but not the specific activities as also observed in the present study. Therefore the concentration of enzyme in the digestive tract changed in relation to the mass or wet weight of the tract (total activity), but these changes were not so great with regard to the soluble protein of the tract (Specific activity).

Conclusion

Good survival and growth of postlarval, juvenile and adult *P. indicus* can be achieved by feeding optimum ration three to four times a day. This feeding regime should be practical for commercial nurseries and hatcheries and can also serve as a standard against which development and performance of a suitable, water stable dry feed can be measured. Since the rearing system used in the present study differs considerably from those used in commercial ventures, some slight changes will have to be incorporated when applied in the case of commercial ventures. Feeding the optimum rations, four times a day for postlarvae and three times a day each for juveniles and adults is best suited for maximum growth and better conversion efficiencies in *P. indicus*.

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OBSERVATIONS ON THE ECOLOGY AND FOOD HABITS OF THE PEARL-SPOT *ETROPLUS SURATENSIS* (BLOCH)

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Introduction

Pearl-spot is an important cultivable fish which also forms a reasonable fishery in the estuaries and backwaters of India. Though several workers have studied several aspects on this fish, information on food habits and ecology of this fish is scanty. Hence a detailed study has been undertaken to ellucidate on the food and feeding habits and ecology of this fish for the present study.

Materials and methods

Three representative natural systems were identified for the present study *viz.* (i) a brackishwater system near the Matsyafed Fishfarm at Poothotta (Stn. I), about 72 km southeast of Cochin, (ii) a brackishwater system near Kannyvilakettu at Edavanakkadu (Stn. II) about 30 km north of Cochin and (iii) the feeder canal of Matsyafed Fishfarm at Narakkal (Stn. III) about 20 km north of Cochin. The fish, environmental water and sediment samples were collected from each station fortnightly. The water and sediment temperatures were recorded at the station itself using a standard thermometer.

Data on water temperature, dissolved oxygen, salinity, pH, nitrate, nitrite, phosphate and silicate were collected from all the three stations, along with sediment soil for benthos. Standard analytical techniques / methods were followed for respective estimations.

Results

The upper layer of soil weighing 250 g was collected for a study of the benthos and identification of the algal composition. Alga included *Oscillatoria, Nostoc, Pleurosigma, Navicula, Nitzschia, Amphora, Amphipora, Coscinodiscus,*

Combylodiscus, Microcystis and *Merismopedium.* Miscellaneous items included roots of higher plants, decayed plants and animal matter, fish eggs and fish scales. Microfauna included copepods, amphipods, gastropods and polychaetes.

From the water sample and soil sample analyses, it has been observed that diatoms were dominant during the study period, except during June and July 1993 when filamentous algae like *Oscillatoria* and *Spirogyra* have dominated in Station I. In Station II and III diatoms were dominat throughout the study period.

Samples of *E. suratensis* were brought to the laboratory for gut content analysis and grouped into 4 size groups *viz.* 60 - 89 mm, 90 - 119 mm, 120 - 149 mm, 150 - 179 mm. After noting the total length, standard length and weight of individual fishes the specimens were disected out. The digestive tract of each specimen was then carefully removed to note the intensity of feeding based on the amount of food present in the stomach and fishes were grouped as 1/4 filled, 1/2 filled, 3/4 filled, full and gorged (Prasadam, 1971, *J. Inland Fish. Soc. India,* 3 : 72-78). The gut contents were made up to a known volume and 1 ml of it was taken and analysed; and the number of diatoms, filamentous algae, detritus, digested organic matter and fragments of higher plants were recorded.

The temperature (°C) recorded and their range at three different stations are 27.0 - 30.5 in Station I, 27.5 - 31.5 in Station II and 27.5 - 31.0 in Station III.

The pH values have fluctuated from 6.40 to 8.03 in Station I, 6.93 to 8.4 in Station II and 7.13 to 8.5 in Station III.

Low salinity values were recorded during the monsoon season in all the three stations. These have ranged between 0.068 ‰ to 0.498 ‰ in Station I, 0.617 ‰ to 4.75 ‰ in Station II and 0.664 ‰ to 4.85‰ in Station III.

High dissolved oxygen values were recorded during the monsoon months in all the three stations. The values ranged between 2.73 ml/l and 4.835 ml/l in Station I, 2.361 ml/l and 4.578 ml/l in Station II and 2.65 ml/l and 4.8126 ml/l in Station III.

High values of nitrate-nitrogen were noticed during monsoon season in Station I and III, but no such trend was noticed in Station II. Nitrate value ranged between a maximum of 19.36 µg at/1 and a minimum of 5.6 µg at/1 in Satation I. In station II values ranged between a maximum of 9.6 µg at/1 and minimum of 4.8 µg at/1 and in Station III the values were 9.62 µg at/1 and a minimum of 5.98 µg at/1.

High nitrite-nitrogen concentration was noticed during the monsoon season in all the three stations. Values ranged between 0.46 µg at/1 and 1.64 µg at/1 in Station I, 0.15 µg at/1 and 2.2 µg at/1 in Station II, and 0.70 µg at/1 and 1.80 µg at/1 in Station III.

In Station I phosphate values ranged between a maximum of 14.2 µg at/1 and a minimum of 6.6 µg at/1. In Station II the values were 18.04 µg at/1 and 4.02 µg at/1 and in Station III the values were between 15.62 µg at/1 and 3.78 µg at/1.

Silicate concentration has fluctuated from 20.6 µg at/1 to 58.0 µg at/1 in Station I, 14.0 µg at/1 to 36.0 µg at/1 in Station II and 15.0 µg at/1 to 36.4 µg at/1 in Station III. The fluctuation was inconsistant in all the three stations.

Filamentous algae were observed to form the major food item in the guts of *E. suratensis* from Station I in all the size groups, and diatoms in Station II and III. In all the three stations gut contents were mostly made up of *Oscillatoria, Pleurosigma, Cyrosigma, Navicula, Nitzscia, Amphora, Amphipora, Diploneis, Rhizosolenia,* detritus, fragments of higher plants, sand grains, miscellaneous items including roots of higher plant, fish eggs, fish scales, copepods, amphipods and gastropods. In all the stations, size groups 60-89 mm and 90-119 mm were found to be omnivorous in habit while the larger size groups 120-149 mm and 150-179 mm were found to be more of a herbivorous in habit.

The feeding index was calculated using the formula :

$$\text{Feeding index} = \frac{\text{No. of fishes with filled stomach}}{\text{No. of fishes examined}} \times 100$$

The observations have shown a decrease in feeding indices with increase in size in all the three stations.

Food preference experiment

To find out the food preference of *E. suratensis* a feeding experiment was conducted in the laboratory using two weight groups of the fish (4 gm and 20 gm body weight) for a period of 5 days. The fishes were kept in plastic troughs containing 5% salinity brackishwater throughout the experiment and food materials like *Spirogyra, Salvinia,* clam meat and pelleted feed prepared from 25% *Spirogyra,* 25% ground nut oil-cake, 20% rice bran, 22% tapioca powder, 2% cod liver oil, 2.8% vitamin mix and 3.2 % mineral mix were given to them. The fishes were fed daily in the morning and evening hours at a rate of 10% of the body weight.

In the first weight group (20 g), the average food consumption of the individual fish from the evening feeding schedule were *Spirogyra* 14.75% of feed given, *Salvinia* 0.016%, pelleted feed 16.75% and clam meat 14.72%. In the same weight group, the average food consumption from the morning feeding schedule was *Spirogyra* 14.3%, *Salvinia* 0.0075%, pelleted feed 15.73% and clam meat 14.15%. In the second weight group (4 g), the average food consumption from the evening schedule were *Spirogyra* 15.8%, *Salvinia* 0.045%, pelleted feed 20.15% and clam meat 12.35%. In the same weight group, the average food consumption from morning feeding schedule was *Spirogyra* 18.55%, *Salvinia* 0.045%, pelleted feed 18.9% and clam meat 10.75%.

Experiments have indicated that among the four types of feed supplied the preference was for pelleted feed.

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THE EFFECT OF HEAVY METALS ON THE PHYSIOLOGICAL CHANGES OF MICROALGAE

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Introduction

With the large scale development of aquaculture practices around the world, there is an increasing trend to evolve noval techniques in hatchery operations and culture activities to maximise production. Mass culture of microalgae is one such innovative technique amenable to further development. In world aquaculture practices, the most important use of microalgae is as live food for rearing the larvae of bivalves, crustaceans and finfishes. Favoured species of microalgae for larval feeds include species of *Isochrysis, Chromulina, Dicrateria, Tetraselmis, Chlorella, Dunaliella* and *Chaetoceros.*

These phytoplankton play a vital role as the primary producers in the basic marine food chain. Living in the most dynamic of all ecosystems, these microalgae are constantly being exposed to several fluctuating environmental factors. These microalgae are more than often prone to the effects of pollutants both in the natural ecosystem and in culture systems through contamination of the medium, in which they thrive. To control the possible dangers that may be caused by transfer of pollutants through these primary producers, it is necessary to assess the potentiality of the pollutants in bringing about appreciable changes in the physiology of these microalgae.

With these view, this study has been undertaken to assess the effect of some heavy metals such as copper, Zinc and Lead which are usually present in the effluents of industries, on the marine microalgae *Tetraselmis gracilis, Chromulina Freiburgensis* and the diatom *Chaetoceros calcitrons* which are important live feeds in hatcheries for rearing the larvae of economically important cultivable organisms such as crustaceans, molluscs and finfishes.

Objectives

The objectives of this study are :

1. to find out the tolerance limit of a particular microalgae to a heavy metal or to find out the highest effective sublethal range of heavy metal concentrations on unialgal cultures,

2. to study the effect of heavy mentals on the natural phytoplankton population collected from mangrove and prawn culture ponds, and

3. to compare the toxicity of these heavy metals on both unialgal cultures and natural phytoplankton populations.

Material and methods

The three species of microalgae selected for the present study belong to phytoflagellates : *Tetraselmis gracilis* (chlorophyceae) and *Chromulina freiburgensis* (Haptophyceae), and the marine centric diatom *Chaetoceros calcitrans* (bascillariophyceae).

The seawater used for the experiment is filtered through Whatman filter paper and sterilized by boiling. The salinity of the seawater was maintained between 32-35 ppt.

The heavy metals selected for the present study are Copper (as Coppor sulphate), Zinc (as Zinc sulphate) and Lead (as Lead nitrate). Five different concentrations of each of these heavy metals were taken :

Copper - 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm.

Zinc - 30 ppm, 40 ppm, 50 ppm, 60 ppm and 70 ppm.

Lead - 25 ppm, 50 ppm, 100 ppm and 125 ppm.

Preliminary experiments to determine the tolerance limit of a microalgae to this heavy metals were conducted. Highest effective sublethal range of metal concentrations were the choice for present investigation.

Algal cells in exponential growth phase were used for inoculation. After determining the cell concentration of the stock culture of each species, 10-20 ml of the culture was inoculated to the duplicate flasks plugged with sterilized cotton

and kept in a wooden rack providing 1000 lux light. Known quantity of heavy metal stock solution was added to the medium just before inoculation. The inoculated flasks were kept under a photoperiod of 10 hrs light and 4 hrs of darkness. The period of study for each experiment was 16 days.

Obsevations on cell number, primary production and chlorophyll content were carried out. Determination of growth and productivity was done in every alternate days and the analysis of chlorophyll content once in 4 days.

For the quantitative estimation of the cells 1 ml of the culture were taken and fixed in Lugol's iodine. The cells were counted with a calibrated haemocytometer and represented as cells x 10^4/ml.

Productivity measurements were made in alternate days using Light and Dark Bottle Oxygen Technique. By using Winkler's method, the oxygen content of the sample were determined. The oxygen values then converted into their carbon equivalents applying a PQ of 1.25 and expressed as mg C/1/hr.

Estimation of the chlorophyll content were followed by the method of Parsons *et al.* (1984, *A manual of chemical and biological methods for sea water analysis*. Pergamon Press, Oxford, pp. 1-283). A known volume of the culture was filtered through a GFC filter paper. The pigments were extracted by adding 10 ml of 90% acetone to each filter. The extracts were contrifuged and decanted the supernatant into a 10 cm path length spectrophotometer cuvette and measured the extinction at the following wave lengths : 750, 604, 647, 630, 510 and 480 nm. Calculated the amount of pigments in the sample using the revised formula of Parsons *et al.* (1984, *loc. cit.*).

Two similar experiments were conducted with natural population of phytoplankton collected from a prawn culture pond and a mangrove ecosystem. All the above mentioned parameters are determined for comparative studies and cells were counted by using Sedge Wick Rafter chamber by the settling method.

A statistical analysis by 2 way Analysis of Variance also made, using the computer to verify the data obtained.

Results

Effect of heavy metals on growth rate, primary production and chlorophyll content

Inhibition of cell multiplication, photosynthetic rate and chlorophyll pigments were observed in the metal treated cultures of microalgae. The rate of inhibition was accelerating as the concentration of heavy metals increased. Maximum inhibition observed in highest concentrations of heavy metal treated cultures.

Copper inhibited the growth rate of *Tetraselmis gracilis* by about 90% in 25 ppm whereas primary production and chlorophyll content inhibited by about 94% and 95% respectively. From fourthday onwards culture in 25 ppm revealed declining tendency.

Chromulina freiburgensis exhibited 97% of growth retardation on 2nd day of experiment in 25 ppm copper concentration. Respiratory rate in this experiment was always higher than the total primary production of cells. 92% inhibition of photosynthetic rate observed in this level. Chlorophyll content, inhibited by about 80% on 4th day.

The marine diatom *Chaetoceros calcitrans* showed 90% of growth retardation, 85% inhibition of photosynthetic rate and 46% inhibition of chlorophyll content in 25 ppm level of copper.

The highest concentration of zinc selected for the present study was 70 ppm which expressed 94% inhibition of cell division in *Tetraselmis gracilis,* 90% in *Chromulina freiburgensis* and 92% in *Chaetoceros calcitrans. Tetraselmis* showed declining phase from 6th day onwards and *Chromulina* on second day onwards, but the marine diatom revealed declining tendency on 10th day onwards.

There was marked difference in growth of the metal treated cultures to that of control from 50 ppm of Lead. Maximum inhibition effect observed was in 125 ppm level. Growth of *Tetraselmis* inhibited by about 91% in 125 ppm level whereas *Chromulina* and *Chaetoceros* showed 93% and 58% retardation of cell multiplication.

Natural populations from prawn culture pond exhibited 100% retardation of growth in 15, 20 and 25 ppm of copper on 4th day. Almost 100% retardation of cell division happened in 60 and 70 ppm of zinc on 4th day of experiment. These phytoplankton populations expressed 100% of growth inhibition on 2nd day of experiment in 75, 100 and 125 ppm of Lead.

Phytoplankton populations collected from mangrove ecosystem expressed 100% retardation of cell number on 6th day in 25 ppm of copper. The observations revealed that 50% of cell division happened in 50, 60 and 70 ppm of zinc on 12th day. 100% of mortality determines in 100 ppm of lead on 12th day, but 75 ppm showed 84% growth inhibition at that day.

Toxicity of heavy metals to microalgae

It is evident from the present investigation that copper is the most toxic and lead is the least toxic heavy metal to these three species of microalgae. The order of toxicity of the three heavymetals to *Tetraselmis gracilis, Chromulina freiburgensis* and *Chaetoceros calcitrans* is Cu > Zn > Pb. The tolerance limit of this three species of microalgae to the heavy metals copper, zinc and lead was varying according to their physiological and morphological features. From the present investigation it is revealed that the phytoflagellate *Chromulina freiburgensis* is the least tolerant species to copper toxicity. However, the marine diatom *Chaetoceros calcitrans* was the most tolerant species towards zinc and lead toxicity. It is also revealed from the investigation that cell division and primary production were most inhibited than the chlorophyll contents in this three species of unialgal culture due to heavy metal toxicity. Respiration rate was enhanced in all the heavy metal treate cultures.

Toxicity of heavy metals to natural phytoplankton population

The present investigation proved that of the two ecosystems selected for the study, natural phytoplankton population collected from mangrove ecosystem was found to be more tolerant towards, copper, zinc and lead toxicity compared to the populations of prawn culture pond. Compared

to zinc and lead, copper was the most toxic heavy metal to these natural population of phytoplankton.

Heavy metal toxicity to microalgae

Metal	Algae
Copper > Zinc > Lead	Generalization
Copper	*Tetraselmis* > *Chaetoceros* > *Chromulina*
Zinc	*Chaetoceros* > *Tetraselmis* > *Chromulina*
Lead	*Chaetoceros* > *Tetraselmis* > *Chromulina*
Copper > Zinc > Lead	Natural phytoplankton population
Copper, Zink, Lead	Phytoplankton from mangrove ecosystem > Phytoplankton from prawn culture pond

Discussion

The adoption fo batch cultures allowed the simultaneous study of the effect of a wide range of metal concentrations upon cell population, all taken from the same stock culture and therefore initially in the same physiological conditions. The relationship between the supply of copper, zinc and lead and the growth of *Tetraselmis, Chromulina* and *Chaetoceros* have been considered from different aspects. The supply of these essential elements at higher concentrations limited the growth rate of this algae.

Of the three heavy metals, copper was found to be most toxic whereas zinc and lead were found to be least toxic to both unialgal cultures of microalgae and natural phytoplankton populations. Rao and Sivasubramanian (1985, In : R. C. Sharma [Ed.] *The oceans realities and prospects,* Rajesh Publishers, New Delhi, India, pp. 243 - 268) have reported that compared to copper, zinc and lead were less toxic to marine diatoms such as *Acanthes haukiana, Amphora coffeaeformis, Fragilaria pinnata, Synedra tabulata, Thalassiosira fluviatillis* and *Triceratium dubium.* The three species studied showed growth upto 20 ppm of copper, 60 ppm of zinc and 100 ppm of lead.

It is found that there is significant difference in between species to tolerate metal toxicity. The haptophycean flagellate *Chromulina freiburgensis* was found to be more sensitive to copper and zinc toxicity, while the chlorophycean flagellate *Tetraselmis gracilis* was found to be more tolerant to copper toxicity. But the marine centric diatom *Chaetoceros calcitrans* found to be least sensitive towards zinc and lead toxicity. This observation is supported by the fact that major trend among species in their resistence to heavy metal toxicity has been a phylogenetic one with the marine diatoms and chlorophycean members being the least sensitive, whereas haptophyceans and cyanophyceans being the most sensitive (Mandelli, 1969, *Contributions to Marine Science.* Univ. of Texas, **14** : 47 - 75; Erickson *et al.*, 1970, *J. Wat. Pollut. Cont. Fed.*, **42** : 270 - 270).

The high tolerance of the marine centric diatom *Chaetoceros calcitrons* to heavy metal toxicity may be due to the rigid silicious cell walls, while the other two species have cellulose cell wall. The chlorophycean member *Tetraselmis gracils* is found to be more tolerant to copper toxicity than *Chromulina* and *Chaetoceros.* Greater tolerance to *Tetraselmis gracilis* to copper can be accounted by the organisms capacity to block the entrance of cations to the cell.

Copper is known to affect cell separation in *Tetraselmis gracilis,* resulting in multicellular aggregates. Similar effect has also been indicated by Foster (1977, *Nature,* **269** : 322 & 323) in *Chlorella vulgaris.*

Inhibition of growth rate, primary production and chlorophyll pigments observed in all the metal treated, natural phytoplankton populations as symptoms of copper, zinc and lead toxicity. Patin *et al.* (1974, *Oceanology, Moscow,* **14** : 72 - 74) and Ibragin and Patin (1976, *Oceanology, Moscow,* **15** : 589 - 591) measured decrease in primary production rates caused by copper zinc and lead toxicity. Another important fact derived from the present study is that natural phytoplankton populations are more sensitive to copper, zinc and lead toxicity than the unialgal cultures. The main reason for this is the species diversity in the natural populations.

It is revealed that, if some heavy metals are required in trace amounts for growth and metabolism of microalgae, higher concentration of them cause inhibition of growth rate and primary production which will lead to the complete disappearance of that particular species. Moreover, the inhibition and stimulation of the growth of the micro-algae due to these heavy metals is controlled by many factors and the toxic threshold of these heavy metals is primary function of species composition of the microalgae.

Conclusion

From the present study, it is concluded that for the successful operation of hatchery system the seawater should have very low concentration of these heavy metals, *i.e.* below 5 ppm of copper, 30 ppm of zinc and 50 ppm of lead for rearing the larvae of economically important cultivable organisms.

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10

IMMUNOLOGICAL STUDIES IN *ETROPLUS SURATENSIS* (BLOCH)

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Introduction

In spite of all round developments in various aspects of aquaculture, the occurrence of disease still remains as a major problem to be solved. Hence development of preventive measures against diseases is of vital importance for the success of aquaculture programmes. Control of diseases using antibiotics and chemotherapeutic agents has many disadvantages. The relevance of immunization of fish bcomes more clear in this context. Vaccination is the main forte of immunology applied to fisheries. Since the immunological aspects of *Etroplus suratensis* (Bloch) have not yet been investigated, the present study was taken up in this species.

Objectives

This investigation is to :

1. evaluate the humoral immune response to different antigens,

2. assess the differential response to different doses of antigen,

3. evaluate the variations if any, in immunological response dependent on the route of antigen administration,

4. study and compare the immunological response in different size groups,

5. assess the immuno-suppressive effect if any, of the heavy metal copper (Cu),

6. test the effectiveness of immunization in protecting the fish against the bacterial infection *viz. Aeromonas hydrophila,* and

7. evaluate the cellular response along with humoral immune response.

Material and methods

The Pearl-spot *Etroplus suratensis* was collected from brackishwater ponds of Matsyafed, Narakkal and brought to the laboratory and acclimated to the laboratory conditions. Soluble protein antigens such as ovalbumin and Bovine Serum Albumin(BSA) and bacterial antigen *Aeromonas hydrophila* EMU 20 were used as antigens in the present study. Ovalbumin and Bovine serum Albumin were prepared by dissolving 0.5 g ovalbumin flakes and BSA powder in 100 ml of physiological saline and filtered through whatman No 1 filter paper. *Aeromonas hydrophila* was cultured in Zobell's agar and harvested. The cells were killed by adding 0.5 ml of 1% formalin and maintaining at 37°C for 12 hrs. The cells were washed in saline and adjusted to contain 1×10^8 cells in 0.2 ml.

The antigens were inoculated into fishes after homogenizing with the adjuvant which was prepared by homogenizing 1% Tween 80 in paraffin oil phase at 30 : 70 ratio.

Antigens were administered through either intramuscular or intraperitoneal or oral routes. Blood was collected from the inoculated fishes by heart puncture. The blood samples from different fishes were kept separately in sterile glass bottles and allowed to clot and the serum was separated and centrifuged for 10 minutes. The supernatant serum was collected and heated for 15 minutes at 45°C, and was used for antibody detection.

Antibody was detected and quantified by precipitation and agglutination tests. Precipitation test was carried out in liquid medium and in agarose gel (single diffusion in one dimension, double diffusion in one dimension and double diffusion in two dimensions). Agglutination test was carried out on glass slides and used for the detection of antibodies against the bacterial antigen *Aeromonas hydrophila*. The cellular immune response was studied by RBC count, leucocyte count and differential count. Counting of blood cells was done by improved Neubaurer haemocytometer. Blood smears were prepared and different blood cell types were identified.

Experimental designs and results

Preliminary trials were carried out to ascertain the ability of *Etroplus suratensis* to respond immunologically to ovalbumin, Bovine serum Albumin and to *Aeromonas hydrophila*. These trials indicated humoral immune response to all these antigens. Considering the thicker precipitation band formation with ovalbumin, it was selected as the antigen of choice for detailed investigations.

Dose determination

Ovalbumin was inoculated to three groups of fishes at doses of 12.5, 25 and 50 µg/g body weight. Control group was maintained without antigen injection. Primary response was noted on 9th day. A booster dose of the same dose was given on 10th day. Secondary response was measured on 15th day.

The highest antibody titre was from the inoculation of 25 µg ovalbumin/g body weight. One way ANOVA indicated highly significant difference among the primary response to different doses of ovalbumin inoculation. The response from both 12.5 µg and 25 µg inoculations were higher than that of 50 µg inoculation and this differences were highly significant. The secondary response was higher than the primary response in all cases. The mean precipitation titre values were 8.506 ± 0.15, 8.574 ± 0.16 and 8.814 ± 0.09 (expressed in log 2) in the low, medium and high doses respectively. One way ANOVA showed that the antibody titre values on 15th day also differed significantly. The dishes which received 50 µg ovalbumin g body weight developed necrotic lesion and growth of wood like fungus at the inoculation site. Considering the high primary response and absence of tissue damage, 25 µg/g body weight was selected as an appropriate dose for inoculation of ovalbumin for further studies.

Comparison of the routes of antigen administration

The appearance of antibodies in the sera of the two groups of fishes which were inoculated with ovalbumin at the rate of 25 µg/g body weight intramuscularly or intraperitoneally were monitored from the next day onwards

along with the control group which received no antigen. Antibody was detected on 3rd day in the sera of fish which received i.m. inoculation whereas in i.p. inoculation it was on 6th day. The mean titre values for 9th day sera were 8.058 ± 0.22 for i.m. inoculation and 6.531 ± 0.48 for i.p. inoculation. The sera collected on 5th day after booster dose gave mean precipitation titre value of 8.574 ± 0.16 and 7.588 ± 0.18 in the i.m. and i.p. routes respectively. Student's test indicated that i.m. inoculation of ovalubumin produced significantly higher primary and secondary response as compared to i.p. inoculation.

Immune response in different size groups

Immune response in different size groups (mean weight 5.074, 15.133, 32.49 and 100.21 gm) was tested using ovalbumin as antigen (dose 25 µg/g body wt). The mean precipitation titre values on 9th day and 15th day did not show any significant difference *Etroplus suratensis* is capable of mounting humoral immune response even at the weight of 5.074 gm. Even at this size, the immune system is well developed as in the adult fish to produce specific antibodies against an antigen.

Immunotoxicity of copper

To investigate the immunotoxic effect of copper in *Etroplus suratensis,* ovalbumin was administered intramuscularly to two groups of fishes of which one group had a prior exposure to the heavy metal copper at 5 ppm level in water. Though the titre value increased in both the groups over the days, the rate of increase in copper exposed animals was much lower than that in the unexposed controls. After the administration of booster dose, there was a sudden increase in the titre value in the control group whereas in the Cu treated group the increase was marginal. This result indicate a clear cut immuno-suppressive effect of copper in *Etroplus suratensis.*

Immune response to Bovine serum albumin

BSA at the rate of 25 µg/g body wt was administered to fishes. The mean titre value on 9th day was 3.17+0.46 and

5.04 + 0.25 on 15th day. Compared to BSA, the response was of high magnitude with ovalbumin.

Immune response to Aeromonas hydrophila

Three groups of 10 fishes each were given formalin killed *Aeromonas hydrophila* cells at a dose of 1 x 10^8 cells through intramuscular, intraperitoneal and oral routes. Control group was maintained without antigen injection. Primary response was evaluated on 12th day and secondary response on 24th day with booster dose administration on 19th day.

Agglutination test using 12th day sera from i.m. inoculation showed clumping of bacterial cells. The sera from control fishes did not show any clumping, suggesting the absence of antibody agaist *Aeromonas hydrophila* in the blood. Intraperitoneal inoculation resulted in the highest agglutination and precipitation titre values both in the primary and secondary response. The result showed that i.p. inoculation is the route of choice for *Aeromonas hydrophila.* Oral inoculation resulted in poor immunological response. In the case of i.p. and i.m. inoculations, the antigen directly comes in contact with the vascular system. This may help better antibody production whereas in the case of oral administration, antigen has to cross the barriers of the intestinal tract to enter into the blood system. Moreover, in oral inoculation stomach and foregut before they reach the immune sensitive areas of the lower gut.

All the above four groups of fishes (10 No. in each group) were challenged with live *Aeromonas hydrophila* at a dose of 1 x 10^4 cells intraperitoneally. The mortalities of fish in each group were recorded. In the control group, out of the 10 fishes challenged, all but one died. In the group which received oral immunization the mortality was 70% and in the intramuscular immunization 60%. In the case of intraperitoneal inoculation the mortality was only 20%. The Chi-square test showed that the i.p. immunization was the most effective where the mortality was only 20% compared to the 90% mortality in the control group. The Chi-square value was significant at 1% level. It is to be recalled here that it was the i.p. inoculation of *A. hydrophila* that has given rise to the highest titre value. The high titre value and lowest mortality

in this group points towards the presence of appreciable quantity of specific antibodies against the bacteria *A. hydrophila* in the blood and its effectiveness in protecting the fish against the *A. hydrophila* challenge.

Cellular immune response

The cellular response observed on the 15th day following the i.m. inoculation of ovalbumin at the rate of 25 µg/g body wt on first day as primary dose and a similar booster dose on 10th day are presented below.

The erythrocyte count ranged from 2.14 x $10^6 mm^{-3}$ to 3.2 x $10^6 mm^{-3}$ in control fish. The man value was 2.67 x $10^6 mm^{-3}$. There was no significant difference in the erythrocyte count before inoculation and on 15th day after the inoculation. The leucocyte count ranged from 5,200 mm^{-3} to 11,500 mm^{-3} before inoculation. The mean value was 8,350 mm^{-3}. There was a marked increase in the number of leucocytes after the antigen administration. On the 15th day of inoculation of ovalbumin, the mean leucocyte count reached 40,310 mm^{-3} whcih ranged from 35, 640 mm^{-3} to 44, 980 mm^{-3}.

The differential count of leucocytes showed that the lymphocytes constituted 27% of the leucocytes before the antigen administration. Increase in the lymphocyte count was observed following i.m. injection of ovalbumin.

An effort was made to identify and characterise the various blood cells of *Etroplus suratensis*. The blood cells identified were lymphocytes (small and large), monocytes, neutrophils, macrophages, three types of thrombocytes (oval, 'tear-drop' shaped and spindle shaped) and Red Blood cells (oval and round).

Conclusion

Etroplus suratensis immunologically responded to all the antigens tested *viz.* ovalbumin, Bovine Serum Albumin and *Aeromonas hydrophila*. The specific antibodies produced against these antigens could be detected by precipitation and/or agglutination tests. Clear precipitation bands were produced in liquid medium as well as in agarose gel by single diffusion

in one dimension and double diffusion in one dimension. Agglutination test was demonstrated on glass slides. Ovalbumin at the rate of 25 µg/g body weight was found to be the appropriate dose for evoking good primary and secondary responses. Intramuscular inoculation of ovalbumin produced higher antibody titres than the intraperitoneal route both in the primary and the secondary responses. There was no significant difference in the antibody titre values among different size groups of *Etroplus suratensis* with ovalbumin as the antigen. Booster dose stimulated antibody production resulting in the sudden increase of titre values.

Copper was shown to have immuno-suppressive effect in *Etroplus suratensis*. Bovine Serum Albumin had a weaker immunological response when compared to that of ovalbumin. *Aeromonas hydrophila* produced precipitating and agglutinating antibodies in *Etroplus suratensis*. Intraperitoneal inoculation of the bacteria produced the highest antibody titres compared to intramuscular and oral inoculations. On challenge experiment with live bacteria, intraperitoneal immunization gave 80% protection. The result pointed to the possibility of immunization of *Etroplus suratensis* against *A. hydrophila* through intraperitoneal route. Leucocyte count had increased significantly following ovalbumin inoculation. There was a marked increase in the number of lymphocytes, Red Blood Cells, lymphocytes, monocytes, macrophages, neutrophils, thrombocytes, etc. were identified in the blood smears of *Etroplus suratensis*.

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MARKETING CHANNEL AND PRICE SPREAD OF AQUACULTURE PRODUCTS

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Introduction

Liberalisation of the Indian Economic policies has given maximum thrust to Exports. Fish and fishery products are the main item having immense potential and good scope to increase our exports. India is exporting a variety of marine products comprising shrimps, lobsters, squid, cuttlefish and a number of commercial finfishes valuing about Rs. 1767 crores during 1992-'93. Among the various fishery products, shrimps command a leading position in the global market by virtue of its ever increasing demand and competitive international prices which contributes to about 70% of our fishery export earnings. However, there is not much scope for increasing our shrimp export by depending on capture fisheries, hence prime importance has been given for the development of aquaculture.

Production and marketing should go hand in hand for the fast development of aquaculture in India. In fact the entire development of prawn culture depends on the export market as 90% of shrimp production through aquaculture are exported to Japan, USA and EEC. Shrimps are exported either in IQF, HL, HO Block frozen, PUD, PD, etc. depending on the size, freshness and quality of the raw material. An efficient distribution net work with sufficient infrastructural facilities in the marketing system has to play a vital role for further development of aquaculture in India.

Objectives

Eventhough many aquatic organisms such as edible molluscs, seaweeds, lobsters, crabs and finfishes are suitable for aqaculture, at present production restricts mainly to shrimp farming, because of its high demand in the export market.

All these factors made it imperative to conduct a detailed study on marketing channel and price spread of aquaculture products to assess the marketing efficiency with the following specific objectives.

1. To review the overall production and marketing trend of aquaculture products.

2. To identify the different marketing channels and to analyse the comparative marketing expenses and margins of aquaculture products.

3. To carry out a comprehensive study on price spread for commercially important varieties of cultured shrimps and to estimate the share of producers and intermediaries in consumer rupee.

4. To find the interrelationship between prices of different levels of the marketing system.

Materials and methods

Fifty prawn farms representing 20 Seasonal, 10 Perennial and 10 Extensive methods were randomly selected from various representative regions like Vypeen Island, Kannamally and Chellanam in Ernakulam District of Kerala State. Data were collected from 10 commercial semi-intensive farms from Andra Pradesh and Tamil Nadu. Information available from the Prawn Farming and Marketing section of MPEDA were also made use of for collecting data regarding export marketing. Data were also collected from peeling sheds, 10 intermediaries, 10 processing centres and 10 exporting units in Cochin region. 10 local markets were also included in the present study to analyse the domestic marketing channels. Based on this investigation, 3 types of schedules were prepared, pre-tested and used for data collection.

Schedule I : It is common for all type of prawn farming practices to gather information from the producers. This provided information about the technology of farming, profitability, production details, marketing channel and the price received for each specific count of the raw material.

Schedule II : It is specific for the peeling shed owners, collecting agents and other intermediaries who play an important role

in the marketing of products from seasonal and perennial fields. It dealt with the grading methods, peeling charges, transportation and marketing expenses and marketing channel of the semi-processed raw material.

Schedule III : It is specific for the exporting units and it provided information regarding the price of the raw material, processing methods, marketing of export products, Foreign market trend and the export of value added products.

In the international markets, traders use various trade names instead of the scientific and local names. Shrimps are graded based on their size and weight. As culture shrimps belong to a uniform size grade of 10-20 gm (white) and 20-30 gm (Tiger), only 6 grades of white, 6 grades of tiger and 3 grades of brown were selected. For convenience and uniformity of the present study the US dollar price for each grade were worked into rupees per kg, based on the dollar exchange rate (1 US $ = Rs. 31.25/-). At various stages of the marketing system unit weight of shrimp undergoes considerable weight loss and corresponding price increase. To maintain uniformity the actual price of the raw material was worked out. An interrelation between the number of culture days, weight of shrimp, their corresporling price and grade were prepared. The grading system of number of prawns per kilogramme were maintained through out the study.

Domestic market products were graded into 2 - 3 groups based on their size. The gross marketing margin and producers' share in consumer rupee and share of intermediaries were worked out using suitable economic formulae.

Results and discussion

Production and marketing of aquaculture products : During the last ten years our shrimp export has increased only less than half times in quantity whereas the shrimp production has doubled. At present about 38% of total shrimp production is reaching the export market. Our shrimp export in the processed form (74,393 tonnes in 1992-93) is only 23% of our total shrimp production. About 66% of the shrimp export is contributed from marine sector and rest of the quantity is supplied from aquaculture sector.

A comparison between the production and marketing among the traditional ponds of Vypeen, extensive farms of Chellanam and Kannamaly and semi-intensive farms of Nellore, Guntur and Tuticorin shows that the average production per hectare from the 20 traditional farms were only 500 kg/ha/crop, extensive farms 1,000 kg/ha/crop and semi-intensive farms 4,000 kg/ha/crop. The traditional farm production is a mixture of prawns, finfishes and crabs. The production from the traditional ponds shows a declining trend over the years. (George, 1974, *Indian J. Fish.*, **21**(1) : 1 - 19; George, 1980, *Proc. First. natl. Symp. on shrimp farming*, pp. 131 -137; Purushan, 1987, *Seafood Export Journal*, **19**(4) : 15-19; Sathiadhas *et al.*, 1989, *Seafood Export Journal*, **21**(11) : 9-21; Nasser and Noble, 1992, *Proc. Workshop on Aquaculture Economics, Spl. publ., 7, Assian Fishing Society Indian Branch, Mangalore*, pp. 1-94). This may be due to the indiscriminate fishing in Cochin Brackwater and the increased natural seed collection methods. The present study shows that out of the 500 kg of total production, about 80 kg are finfishes and 20 kg are crabs (20%) which are moving into the domestic market. Finfishes received only about Rs. 15/kg and small crabs *(Scylla serrata)* fetches Rs. 30/kg. *Metapenaes dobsoni* (Thelly) and *M. monoceros* (Chooden) are the dominant species (67%) among prawns and these are less preferred in the International market. This fetches hardly Rs. 32-40/kg, whereas the white and Tiger prawns which constitute about 31% of the total production fetch about Rs. 150-200 per kg. So the average unit value of the total production from a traditional pond is only about Rs. 45/kg.

Production and marketing from the extensive farms of Chellanam and Kannamaly involves only the export marketing system. The average production from these farms were about 1000 kg/ha, comprising of either White or Tiger prawns. This is a mixture of almost uniform size grades which mainly belong to four different grades of Tiger prawns such as 25-35 g (60%), 20-25 g (20%), 16-20 g (10%) and 12-20 g (10%). White prawns belong to 16-20 g (10%) and 12-16 g (10%). White prawns belong to 16-20 g (60%), 12-16 g (10%) and 8-10 g (10%). The average price per kg of each grade of

prawns can be calculated. Marketing channels of production from these farms move directly to the processing centre. The producers are getting about Rs. 150/kg for White prawns and Rs. 200/kg for Tiger prawn.

Products from the commercial semi-intensive farms are directly reaching the processing centres. The marketing system of commercial semi-intensive farms are the simplest and correspondingly the producers are getting the maximum price for the raw materials.

The marketing system of traditional farms are more complicated than the scientific farms. Many middlemen and money lenders are involved in the marketing system of products from traditional ponds.

Marketing channels : Aquaculture products are moving both in export and domestic marketing channels. Even though, shrimp is the major item of aquaculture, finfishes and crabs are also contributing a minor share. Production from a typical traditional pond is a mixture of prawns (80%) finfishes and crabs (20%) whereas production from a scientific shrimp farm is exclusively of either White or Tiger prawns. Finfishes and crabs move to the domestic marketing channels as they have no demand in export market. High unit value prawns move directly or indirectly to the exporting units. Based on the marketing channel studies and price behaviour, the exact difference in earnings and profitability of different shrimp culture systems can be highlighted.

The export marketing system has 5 main marketing channels where the products passed from one to four intermediaries. The domestic marketing system can also be classified into five channels. There is considerable variation in the unit price of products moving through export and domestic marketing channels. Export marketing channels are having about five times higher unit price than the products moving through the domestic channels. There is interrelationship between the different intermediaries involved in the marketing flow.

Export marketing channels

The marketing channels for traditional systems are much complicated than the marketing system of scientific farms. The main export marketing channels prevalent are as follows:

1. Producer → Financier cum Agent → Preprocessing Centre → Processing Centre → Exporter → Foreign Buyer → Consumer.

2. Producer → Pre-harvest contractor → Preprocessing Centre → Processing Centre → Exporter → Foreign Buyer → Consumer.

3. Producer → Collection Depot → Processing Centre → Exporter → Foreign Buyer → Consumer.

4. Producer → Processing centre → Exporter → Foreign Buyer → Consumer.

5. Producer → Processor cum Exporter → Foreign Buyer → Consumer.

Domestic marketing channels

The Domestic marketing items of aquaculture products are mullets, pearlspot, tilapia, milkfish and crabs. Domestic marketing pattern is applicable only for the traditional ponds, because only these farms products are a mixture of different organisms. Among the shrimp, the small size prawn varieties rarely reach the domestic market. The present study identified five types of channels in the domestic marketing.

1. Producer → Preharvest Contractor → Wholesaler → Retailer → Consumer.

2. Producer → Wholesaler → Retail Commission Agents → Retailer → Consumers.

3. Producer → Wholesale Commission Agents → Local Market Agent → Retailer → Consumer.

4. Producer → Worker cum Vendor → Fisherwomen Vendor/Retail Agents → Consumer.

5. Producer → Consumer.

In general, the aquaculture products in the domestic marketing system do not move long distance. The present study reveals that about 40% of the products are sold to consumers within 10 km of the production centres, 20% reached the nearby city or town markets, 15% handled by door-to-door fisher women vendors, 20% consumed by the producer and labourers and about 5% reaches the nearby hotels and cold storages. The domestic fish marketing chain consists 1 to 3 intermediaries before it reaches the ultimate consumer.

Product movement and price behaviour

Price spread of aquaculture products can be defined as the difference between the prices received by the producer and those paid by the consumer. It includes the payment received by all intermediaries performing services in moving the products from production site to exporter, ensuring the quality of the products. Price spread is composed of margins at various levels. Unlike agriculture products, aquaculture products are highly perishable and the price of a particular grade of shrimp is determined by the buyer in the foreign country and accordingly price is offered to the preprocessor and finally to the producer. So the producer has the least role of deciding the price of the material produced by him. Gross marketing margin is the difference between the retail 'price paid by the consumer and the price received by the producer. The gross marketing margin consists of various sub-marketing margins like Pre-processor's Margin, Processor's Margin, Exporter's Margin, and Buyer's Margin. The gross marketing margin for White Prawn ranged from 27 - 48%, the average being 36%. Gross marketing margin for Tiger prawn ranged from 29 to 54% with an average of 39%. For Brown prawn the gross marketing margin ranged from 28 to 42% with an average of 33%. This shows that the marketing margin for Tiger prawn is the highest followed by White and Brown prawns. So the gross marketing margin is directly related to the economic importance of the species. Since production from the traditional prawn farmers and intermediaries are getting a lessor profit for the products as they are handling mostly Brown and White prawns.

The producer's share in export marketing channel 1 was only 58% and this increases upto 70% in channel 5. At present many of the traditional farmers are loosing about 12% of the consumer rupee mainly bcause of the inefficient and complex marketing system. Pre-processor's margin ranges between 2.77% and 7.08%. The processor's margin is only 2.83% of the consumer rupee. The exporter's margin and buyer's margin remains same for all the channels. The average exporter's margin is only 6.9% of the consumer rupee. The foreign buyer's margin is about 7.6% and these products are used as raw material for reprocessing and repacking. Corresponding to their value addition the retailers and super markets are geting 12.16% of the consumer rupee. Exportor and foreign buyer are the two essential intermediaries applicable for all the channels.

Live crab marketing from crab fattening ponds

The live crab marketing flourished only during the last five years. A district level commission agent collects these live crabs from various local commission agents and transport in bambo baskets. Each basket contains 50-60 crabs weighing about 25 kg. Each individual crab is tied securely by attaching the chelate legs to the abdomon, to reduce the cannibalistic tendancy. From the district level commission agents these bamboo baskets are sent to Madras Airport and transported to Singapore, Malaysia, Japan and Hong Kong. The state level commission agents (Exporter) check the quality of the product and discard the dead and weak specimens. Healthy quality crabs can withstand the strain of transportation upto three to four days.

Crabs fattening is profitable than the crab culture technique as it involves short duration. It is a holding operation during which immature crabs and newly moulted are kept in enclosures and fed until their gonads develop or their shell hardens. This best quality product is known as *mud*. The poor qualities are named *water, local, small* and *dead*. Crabs fattening is profitable because of the fast turn over, low operating cost, high survival rate, controlled cannibalism, migration and good market demand. The newly moulted crabs (*water*) are discarded in the market and this can be converted into top quality

(*mud*) just by holding in small pits or cannals for 4-5 weeks. Live crabs are marketed through the following marketing system such as Producer → Local Commission Agents → District level Commission Agents → State level Commission Agent (Exportor) → Buyer → Consumer.

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INFLUENCE OF DIFFERENT TYPES OF SOIL ON THE WATER QUALITY IN CULTURE PONDS

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Introduction

Sustainable development in aquaculture is possible by adopting environmental friendly technologies where soil and water play important role. An ecologically non-degrading, technically appropriate, economically viable and socially acceptable aquaculture practices can be developed by proper management of soil and water.

The interaction of soil and water in culture ponds is a subject of unusual scientific and ecological interest. It's scientific interest springs from its application in aquaculture, limnology, pollution control and geochemistry. It's ecological importance needs no emphasis, since 71% of earth surface is covered by submerged soil or sediments. Pond soil is an integral part of every pond and play important role in the production cycle *viz.* in the storage and release of nutrients to water, in the mineralisation of organic bottom deposits, in regulation of pH, in oxygen balance of water and in sulphur, phosphorus, nitrogen and carbon cycles.

While relatively more comprehensive data are available on water quality of culture ponds in India, there has been practically very little study of the soil of brackishwater culture ponds. Although result of such studies in inshore water, backwater are invaluable in understanding exchange between soil and water it is realised that the conditions in culture ponds are far more complex because of diversity of culture practices, differential stocking density and different feeding patterns.

Kerala is a State with notable physical features of backwater areas adjoining the sea. Out of the different types of soil in Kerala such as red loam, laterite and brown

hydromorphic association, coastal alluvium, riverine alluvium, greyish onattukara, hydromorphic saline, acid saline, black soil and forest loam, etc., brackishwater/coastal areas are mainly of hydromorphic saline, riverine alluvium, coastal alluvium and acid saline type of soil. The fish production is badly affected by low pH, unfavourable ionic composition of pond water, the frequent presence of finely dispensed ferric hydroxide scum and poor supply of algal feed.

Objectives

The present work was carried out with the objective of elucidating the behaviour of phosphorus, nitrate, carbon, sulphur, anions and cations in different types of soil subjected to different salinities in different seasons and their influence on water quality in culture ponds.

Attempts were made :

i. to establish the normal range of selected parameters in different types of soil which have important bearing on the productivity of water,

ii. to determine the inter-relationship between selected parameters of soil condition and water quality, and

iii. to define the inherent fertility status of these culture ponds so that an efficient fertilization scheme can be developed.

With this view, an attempt had also been made in experimental work to study the relative response of three common fertilizers (urea, Ammonium sulphate and Superphosphate) in three types of soil differing in their reaction.

Materials and methods

The study area : Representative soil and water samples were collected from thirtyseven perennial brackishwater ponds in four centres comprising eleven stations. Centre I having hydromorphic saline soil located at Vyttila with three stations Station 1 Vennala, Stn. 2 : Vyttila, Stn. 3 : Kundannoor. Centre II is situated on the bank of branch of River Periyar. It has two stations *viz.* Stn. 4 : N. Paravur - 1 and N. Paravur 2. Centre III located in coastal alluvium soil is having 3 stations *viz.* Stn. 6 : Cherai, Stn. 7 : Narakkal, Stn. 8 : Puthuvypeen.

Centre IV is of acid saline types having 3 stations *viz.* Stn. 9 : Vechoor. Stn. 10 : Karumady, Stn. 11 : Poothotta. In each station a group of minimum three to maximum six ponds were selected depending upon the availability of ponds with culture practices.

The method of collection in shallow ponds was by a soil sampler which consists of a metal cylinder whereas in deep ponds by vanveen grabs. Water samples were collected 15-20 cm below the surface level. Regular monthly sampling was done from all these stations. To make the sample representative a number of samples were collected from different places of pond and mixed to have a composite sample.

In laboratory experiment the soil layer of 7.5 cm was spread in the rectangular perspex tank of 200 1t capacity. Slaked lime $Ca(OH)_2$ is applied to increase the pH from acidic (4.5-5.5) to neutral (7-7.5) in tank - 2 and from acidic to Alkaline (9.5-10.5) in tank - 1. A water column of 30 cm having salinity of 15 ‰ was maintained. Urea and Ammonium sulphate are two nitrogenous fertilizer applied at the rate of 250 kg/ha individually to each soil type. Samples of water and soil were collected from these tanks once before fertilization and subsequently after each six days.

Soil samples were analysed for hydrogen ion concentration and redox potential (using digital pH meter), texture (International pipette method), conductivity (conductivity bridge), cation exchange capacity (Iswaran, 1980, *A Laboratory hand book for agricultural analysis.* Today & Tomorrow Printers & Publishers), total exchangeable metallic cations (Firman, 1964, *Chemistry of soil.* Oxford and IBH Publishing Co., New Delhi), exchangeable cations K^+, Na^+ and Ca^{++} (using flame photometer), alkalinity and nitrate nitrogen (Adoni, 1985, *Work book on limnology.* Pratibha Publishers, New Delhi, pp. 1-60), organic carbon (Walkly and Black wet oxidation method) and available sulphur (Ganguly, 1982, *Laboratory practical manual for postgraduate studies in soil chemistry and plan nutrition*).

Water samples were analysed for pH (using digital pH meter), salinity (Mohr's titration method), dissolved oxygen

(Winkler's method), total hardness and alkalinity (Adoni, 1995, *loc cit.*), nutrient (Strickland and Parsons, (1968, *Bull. Fish. Res. Bd. Canada,* **167** : 1-311), chlorophyll and carotenoid (Parsons *et al.*, 1984, *A manual of chemical and biological methods for sea water analysis.* Pergamon Press, pp. 177), primary production (Gaarden and Gran, 1927, *Rapp. Proc. Verb. Cons. Intr. Explor. Mar.*, **42** : 1-148) and growth estimation (using haemocytometer).

Results and discussion

Morphological features of these soils indicate that soild of different centres are differing in their characteristic colours, consistency, plasticity, permeability and texture. Majority of the ponds studied are sandy loam, sandy clay loam and clay loam in their texture. It was found that available nutrients of soil increases with decreasing grain size.

Water pH ranged between 6.67-8.78, while soil pH (dry) betwenn 3.55-7.28. Acid and hydromorphic saline soils are highly acidic (3-5), while alluvial soils are moderately acidic to neutral (pH 5.5-7). However due to submergence, pH is stablised at neutral pH in all types of soil. On the basis of dry soil pH, around 84-89% of the ponds studied are found to have acidic soil and 11-16% of ponds are having neutral to weakly alkaline soil. A highly significant correlation exists between soil and water pH. Overall decease of pH during monsoon was observed. Soil pH had positive significant correlation with cations, soil alkalinity, but inverse correlation with available phosphorus in soil. Redox potential ranged between +228 and +549 mv thus establishing the oxidised state of all these ponds. Dissolved oxygen of water ranged between 0.7 ml/1 to 7.65 ml/1 and is found to be correlated with pigments.

Overall salinity fluctuation was found between 0.08% and 27% whereas soil conductivity (S.EC) ranged between 0.5 and 18 mmho/cm and a distinct pattern of decreasing trend in monsoon was observed in both of these property. Soil EC and water salinity are significantly correlated. Water hardness ranged between 27.03 and 48.38 mg/1 as $CaCO_3$ and had significant correlation with soil EC and salinity. Soil EC had significant correlation with soil clay and soil pH. However,

it is clear from the present study that decomposition of organic matter is lower under high EC.

Water alkalinity ranged between 12 to 185 mg/1 as $CaCO_3$, while that of soil ranged between 0.29 and 17.05 mg/gm and both these properly are showing high degree of correlation ($r = 0.783$, $P < 0.01$). Out of the total ponds studied, 0-8% of ponds showed low productivity, 14-51% low to medium, 41-75% medium to high and 0 to 22% highly productive as far as water alkalinity is concerned. Alkalinity of these brackishwater ponds are caused by carbonate and bicarbonate fractions.

Cation exchange capacity varied from 6 to 28 me/ 100 gm whereas TEC ranged from 0.88 to 26.2 me/100 gm. Seasonal fluctuation in CEC and TEC were inconsistant. ANOVA showed that there was no significant variation in CEC and TEC between the centres.

Exchangeable K^+, Na^+ and Ca^{++} in soil was found to range from 55 to 2819 ppm, 113 to 4575 ppm and 320 to 6870 ppm respectively with highest during premonsoon months and lowest during monsoon months.

Soil organic carbon varied between 0.15% and 3.0% and had no significant seasonal variation. ANOVA revealed that centres were not differing significantly as far as organic carbon is concerned. The ponds studied can be classified as 3-16% of ponds low, 48-64% average, 22-30% optimum and 3 to 11% highly productive. Organic carbon was found to have significant correlation with water phosphorus, water nitrate and chlorophylls ($P < 0.01$). Similarly with available phosphorus, alkalinity and clay content of soil, organic carbon showed significant positive correlation.

Available sulphur ranged from 113 to 4375 ppm and was significantly varying between different soil types. Apart from clay content in soil, other parameters such as soil EC and organic carbon were found to influence on available sulphur content of soil. Alkalinity was negatively correlated with available sulphur which emphasizes that in tropical estuarine condition, the *in situ* carbonate accumulation and high pyrite formation are mutually exclusive.

Water reactive phosphorus in water varied between 0.08 to 66 µg at/1, nitrate from 0.31 to 74 µg at/1, nitrite from 0.02 to 7.0 µg at/1 and silicate from 12.46 to 86 µg at/1. Available phosphorus in soil fluctuated between 4.48 to 414 µg/gm whereas nitrate nitrogen of soil ranged between 4.08 and 16.05 µg/gm.

Out of the total ponds studied, taking all seasons into account 17-48% of ponds were low, 8-11% average and 33 to 46% productive as far as soil available phosphorus is concerned. Similarly with respect to water phosphorus 73-78% of ponds were low, 14-19% fair and 5-11% good in productivity.

Soil pH has a negative correlaion with available phosphorus. This is in well agreement with previous reports that available phosphorus is a function of pH. In acidic pH, phosphate tends to be bound to solid phase by Fe^{3+} & Al^{3+} either by precipitation or by adsorption while in alkaline pH it tends to be bound to calcium and precipitated as calcium phosphate. In freshwater conditions, transformation of native phosphorus depends on soil reaction, but in brackishwater condition salinity also influenced available phosphorus. It may be due to large amount of cations present in these environment.

Soil phosphorus and water reactive phosphorus are significantly correlated, while there exists no correlation between soil nitrate and water nitrate. Organic carbon is correlated with available phosphorus, but not with soil nitrate. This indicates that while there is a close relationship exists between cycle of phosphorus and organic production, the nitrogen cycle is completely unconnected with organic productivity rhythm.

From the significant correlations obtained in the present study, it can be inferred that nutrient status of water may be influenced very much by nutrients in soil.

The pigment contents varied in different location and showed appreciable increase during monsoon.

Significant correlation was found between these pigments and organic carbon, available phosphorus and exchangeable potassium in soil. This confirms that apart from light and

dissolved oxygen, photosynthetic pigments and thus primary production are influenced by soil types and water quality. Carbon to phosphorus ratio in soil ranged from 48 to 864, the lowest being at centre I and the highest at centre IV. This may be because the conversion of organic matter into inorganic forms are instant and effective in centre I, but reverse in centre IV.

Inorganic nitrate to phosphate ratio in water ranged from 0.18 to 11.74. This low ratio indicates that nitrogen is the limiting factor in all these brackishwater culture ponds, and emphasizes the need of nitrogen fertilization which is also supported in the laboratory experiment.

In the laboratory experiment also highly significant correlations were established between soil pH and water pH, soil alkalinity and water alkalinity, available phosphorus in soil and water reactive phosphorus which highlights the result obtained in the field condition.

Despite fertilization, acidic and alkaline systems did not show good production. So it can be concluded that without proper reclamation of acidity/alkalinity fertilizer application will not have much effect.

Nitrogenous fertilizers especially ammonium bases were found to be better in influencing gross production under neutral pH range of soil. In acidic and alkaline conditions, relatively higher phosphate fertilizer may give better production.

On the basis of the overview of this investigation, it is concluded that culture ponds in different types of soil are independent with regard to physicochemical parameters of soil and water and the contributing factor varies from one to other types of soil.

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13

GENOTOXIC STUDIES IN *ETROPLUS SURATENSIS* USING MICRONUCLEUS TEST

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Introduction

In fish, genetical changes could be an useful indicator of the effects of pollution and provide a quantitative and qualitative measure of the biological damage being produced by pollutants and hence genetic bio-markers for environmental monitoring and chemical hazard evaluation are now being recognised by aquatic toxicologic researchers. Genetic toxicity assays have been enriched by the large number of methods for characterising the genotoxicity of chemicals and other environmental pollutants. However, micronucleus test is considered to be the best short-term screening test over the other chromosome aberration (CA) and sister chromatid exchange analysis for many reasons.

Micronucleus is a small membrane bound mass of chromatin material present in the cytoplasm of dividing cell populations resulting from chromosome aberrations in the cell cycle following clastogenic exposure, either occurring fused with the principal nucleus or remaining as a separate, small secondary nucleus in the cells, scorable in telophase stage. Micronucleus may arise from different types of chromosomal aberrations and hence appears of different size and shape which may arise either from acentric or centric fragments of chromosome of chromatin materials due to chromosome breakage (structural chromosome damage - acentric micronucleus) or whole chromosome leading to the production of numerical chromosome damage (centric micronucleus). Scoring of micronuclei may indicates chromosome aberrations and genotoxic effects through clastogenic and spindle apparatus damage. Micronucleus test which utilises fish erythrocytes, was termed as "Piscine erythrocytic micronucleus test".

Objectives

In the present investigation the study was conducted in *Etroplus suratensis* with the following objectives : (i) to detect various types of nuclear anomalies of fish erythrocytes, (ii) to detect artifacts seen in Piscine erythrocytic micronucleus test, (iii) to generate base-line data to screen known chemicals for genotoxic potentials and (iv) to assess genotoxic effects in fish from suspected polluted sites with a standardised protocol for Piscine Micronucleus Test.

Material and methods

For the laboratory and base-line studies, specimens of size ranging from 70 - 90 gm with a mean weight 83.4 (S.D. = 7.1) were collected by cast net from fish ponds of Narakkal Research Centre of Central Institute of Brackishwater Aquaculture (CIBA) and Matsyafed fish farm, Narakkal.

The genotoxic effects of selected chemicals *viz.* Colchicine, Cyclophosphamide-Endoxan Asta, Mitomycin - C and Cadmium were studied in *E. suratensis* in the form of nuclear lesions comprising micronucleus and nuclear anomaly. In the laboratory the animals were exposed to three sublethal doses (96 hrs tolerance limt was highest dose and subsequent doses were the fractions of the highest dose) of the aforesaid chemicals and the blood samples were collected from these animals after 24, 48, 72 and 96 hrs and were analysed for nuclear lesions. All the test solutions were prepared in physiological normal saline (0.9% NaCl). A dose of 0.5 - 1.0 ml of these mutagenic solution was injected intraperitoneally using 1 ml tuberculin syringe. The effect of dose and time of exposure on micronucleus and nuclear anomaly incidences were analysed by two-way ANOVA and by critical difference analysis.

Live specimens of *E. suratensis* were collected from Narakkal Matsyafed fish pond (control station), Narakkal waste water canal and Eloor industrial area (of Periyar River), Kochi and were analysed for genotoxicity. Small amount of blood (0.06 - 0.125 ml) was collected from test animals by cardiac puncture using heparinized (4 mg/ml) 1 ml syringe.

Blood smear was made on an acid washed clean slide by the standard smearing method. After air drying period the slides were kept for overnight maturation. These slides were fixed in absolute methnol for 10 - 15 min, stained with freshly prepared and cloth filtered 20% in sorenson's buffer (pH 6.8) for 15 - 20 min. After staining the slides were washed gently with tap water and then air dried after distilled water wash. Two slides per individual were prepared. Unmounted slides were screened for estimating the rate of micronucleus and nuclear anomalies under 1000 x magnification with oil immersion objectives through Nikon optiphot No.1 (Japan) microscope equipped with a built-in automatic camara for microphotography. Six to ten thousand erythrocytes per individual were observed. The rate of micronucleus and nuclear anomaly formation was expressed as micronuclei and nuclear anomalies per thousand cells. The total count of micronucleus and nuclear anomaly was denoted as nuclear lesion.

Results and discussion

Although both sodium citrate (2%) and heparin prevented clotting effectively, the latter was found to be better anticoagulant for the erythrocytic micronucleus test. Sodium citrate badly damaged the cells through hypotonic effect and led to more artifacts. Different procedures were tried to get an optimum method for blood smear preparation and staining. Giemsa stain (20%) made in sorenson's buffer (pH 6.8) yielded good contrast between cytoplasm and nucleus of erythrocytes and was found to be the best for piscine erythrocytic micronucleus test.

Consistent variations in the shape (smooth and elliptical) of the mature erythrocyte nucleus were noted in *E. suratensis*. These variations constituted the lesions of the nucleus in the form of micronucleus and nuclear anomaly.

Micronucleus consideration was given to the cells with clear and membrane bound nuclear mass, smaller than the main nucleus (1/10th to 1/2.75 of main nucleus), but with similar colour intensity. However most of the micronuclei ranged between 1/4th to 1/3rd of main nucleus, not more than one micronucleus per cell was noted. Mostly micronucleus was attached with main nucleus and rarely seperated

micronuclei were noted. Micronuclei shape varied between round, almond and avoid.

Erythrocytic anomaly includes mainly changes in nucleus structure in erythrocytes. Different types of nuclear anomalies are noted in pearlspot erythrocytes and they constituted blebbed nucleus, lobed nucleus and bilobed nucleus.

The bilobed nucleus was relatively small evagination of the nuclear envelope which ranged in shape from a slight protrusion to a stalked structure. The lobed nucleus was considered on the basis of the nuclear evagination, longer than the blebbed ranging from simple swellings of the nuclear surface to the presence of multiple lobes, etc. The bilobed nucleus was a nuclear strcuture constricted at centre giving the nucleus a dumbbell shape. It was differentiated from micronucleus by the depth of invagination at centre. One half invagination of the nucleus represented micronucleus whereas lesser than half represented bilobed nucleus.

Low counts of micronuclei (MN) and nuclear anomalies (NA) were found in the animals collected form Matsyafed and CIBA fish ponds, Narakkal. The counts ranged from 0.0-0.3 per thousand cells for micronuclei and 0.2 - 0.6 for nuclear anomalies. Which provided the base-line frequencies (background counts) for the studies conducted in the laboratory animals treated with known chemical mutagens and collected from the suspected polluted areas.

Many artifacts in the form of overlapping cells and ruptured cells were observed alongwith micronuclei and nuclear anomalies. Stain particles and acidic materials other than chromosomal fragments were the main intervening artifacts and were excluded on focussing due to their refractile nature while comparing with the main nucleus.

The mutagens well proved in mammals, induced genotoxic effects in *E. Suratensis,* because of the universality of the DNA molecule. Further it is reported that fish species are having increased level of similar mixed function oxidases with that of mammals and reduced activity of DNA polymerase, an enzyme important in DNA repair also would have resulted in the toxic effects.

Cyclophosphamide

Animals exposed to different doses (0, 100, 150 and 200 ppm) of cyclophosphamide had significant effect of dose and time ($P < 0.01$) on micronucleus and nuclear lesion incidence. Maximum frequency of micronucleus and nuclear anomaly was observed in the animals exposed to highest dose (200 ppm) for 96 hrs. In other animals exposed to lower level of doses and for lesser time, the frequencies of micronucleus and nuclear anomaly production were higher than that of control animals.

The effective response of cyclophosphamide noted in the present study might be due to the bio-activation of the promutagen (CP) by microsomal enzyme system.

Mitomycin - C

An increasing trend in nuclear lesions, with the increase in dose (0, 0.5, 1.0 and 2.0 ppm) and time of exposure was observed in animals exposed to mitomycin - C. Dose and time had significant effect on micronucleus ($P < 0.01$); nuclear anomaly ($P < 0.01$) and nuclear lesion ($P < 0.05$). Total nuclear lesions ranged from 5.4 to 8.6‰. The significant increase in micronucleus frequency with increase in exposure time might be due to the high number of damaged cells undergoing mitosis enabling production of micronucleus and its accumulation over a period of time.

Colchicine

Increased incidences of micronucleus, nuclear anomaly and nuclear lesion observed in the animals exposed to different dose-levels of colchicine (0, 0.1, 0.5 and 1.0 ppm) than that of control animals. Two-way ANOVA showed that the levels of doses ($P < 0.01$) had significant effect on micronucleus, nuclear anomaly and nuclear lesion incidence. Whereas time ($P < 0.05$) had significant effect on nuclear anomaly and nuclear lesion incidences, but not micronucleus.

Dose dependent micronuclei and nuclear anomalies were produced by colchicine. However, a marginal increase noted between first sampling (24 hrs) and last sampling (96 hrs) times for 0.1 and 0.5 ppm doses were not significant. This

finding of no significant effect of time on micronucleus production may be due to the direct action of colchicine on microtubules causing metaphase arrest after administration.

Colchicine which is representative of clastogenic agents, caused damage to the erythrocyte nucleus of *E. suratensis* giving rise to micronucleus and nuclear anomaly. This may be due to the spindle disturbing nature of colchicine and its effect on microtubules and thus resulting in failure of chromosome segregation.

Cadmium

Like other chemical mutagens, cadmium also induced the elevated micronucleus and nuclear anomaly in treated animals against a low incidence of control. An increasing trend in micronuclei, nuclear anomalies and nuclear lesions was observed with the increase in dose and time. Significant variation ($P < 0.01$) between doses was observed in micronucleus, but not nuclear anomaly induction. Significant variation between time of exposure was observed for nuclear anomaly ($P < 0.01$) and nuclear lesion ($P < 0.05$), but not micronuclei.

Increased micronucleus and nuclear anomaly incidences after cadmium exposure might be due to the inability of the fish to detoxity the poison and the existing unexcreated toxic metabolites effect leading to the loss in integrity of nuclear membrane and membrane bound enzymes by enhancing lipid peroxidation. The time dependent nuclear anomalies might be due to the effect of cadmium on erythropoietic cells rather than on the circulating erythrocytes. According to critical difference analysis, in all the mutagen treatments highest significant variation was observed between lowest and highest dose and first and last sampling times. The results of this study indicate that the three chemical mutagens and one heavy metal selected are genotoxic to *E. suratensis*.

Genotoxicity of all these chemicals might be a result of bio- activation resulting in reactive metabolites and free radicals generation, reaction with bio-molecules to yield increased DNA damage through alkylation, cross-linking of DNA change and interaction with membrane, induction and persistence of micronucleus and nuclear anomaly.

Field study

Field study was conducted by analysing the natural incidence of micronucleus, nuclear anomaly and nuclear lesion in the animals occurring in suspected polluted areas (Eloor Industrial area of River Periyar and Narakkal waste water canal, Kochi). The micronuclei and nuclear anomalies varied between 6.8 - 8.2 and 11.4 -14.4‰ to respectively in animals from Eloor Industrial area. Whereas in animals from Narakkal waste water canal micronuclei and nuclear anomalies ranged between 2.0 - 2.6 and 5.4 - 6.2‰ to respectively. The nuclear lesion in Eloor Industrial area and Narakkal waste water canal respectively showed a 52 and 11 times increase than the control incidence. This result coincides with the earlier reports of contamination in the sites.

Conclusion

The present study indicated the rapidity, simplicity and accuracy of piscine erythrocytic micronucleus test over any other test system to monitor the aquatic pollution and to evaluate the genotoxic potency of commercial chemicals. Due to the nucleated erythrocytes in fish, the observation and characterisation of micronucleus/nuclear anomaly in such cells was easy and had the advantage of reducing the artifacts by direct comparison of micronucleus with main nucleus, which is not possible in mammalian micronucleus test. From the study, it is concluded that *E. suratensis* can be used as a good model to study the genotoxic effects of aquatic pollutants in fish. The high incidence of micronuclei, nuclear anomalies and nuclear lesions in animals from polluted areas showed that the aquatic animals in these areas are under serious threat of pollution. Since fish is an important part of our food it is recommended to undertake a large scale genotoxicity screening of chemicals, effluents and environmental residuces, etc. to check the level of contamination in fish of economic importance, where *E. suratensis* can be used as a cytogenetic model and piscine erythrocytic micronucleus test as a cytogenetic tool and thereby formulate the pollution control programmes accordingly.

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OBSERVATIONS ON THE POPULATION CHARACTERISTICS OF THE CORBICULID CLAM *VILLORITA CYPRINOIDES* (GRAY) IN THE CHITRAPUZHA PORTION OF THE VEMBANAD LAKE

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Introduction

Among the exploited bivalve resources of India, clams are the most abundant and widely distributed molluscs, supporting sustenance fisheries in the estuaries and backwaters of Kerala, Karnataka, Goa, Tamil Nadu and Andhra Pradesh. The black clam *Villorita cyprinoides* (Gray) contributes to about 67% of the clam fishery. Its meat is locally consumed and is also used as an animal protein feed supplement in various feeds, while the shell is largely used in lime-based industries. The near sedentary habits of the clam makes it particularly vulnerable to over-exploitation. This clam is admirably suited for on-bottom culture. Keeping in view the importance of *V. cyprinoides* in the rural economy of Kerala, a study involving ecological observations of clam beds, population characteristics, distribution patterns and some related biological and physiological aspects was undertaken during a seven month period (April-October) in a 12 km stretch of the Chitrapuzha Lake extending downstream to the Vembanad Lake, so that the information collected might prove useful in the management of this valuable resource.

Materials and methods

Stratified transect sampling was carried out once in every month at six stations along the study zone by diving and hand-picking. A 0.25 sq.m steel quadrat was used for density observations. Water samples were collected using a Casella water sampler. Depth was estimated with an aid of a lead sinker attached to a rope. Temperature was measured with a mercury thermometer. A Secchi disc was used to measure transparency of the water column.

The water sample was estimated for dissolved oxygen by Winkler's method, salinity by silver nitrate titration method and pH using a digital pH meter. Soil samples were subjected to texture anlysis following the international Pipette analysis. The soil organic carbon content was estimated following the Walkley and Black method.

The biological samples were subjected to morphometric and weight measurements. Gonadal smears were observed following the guidelines given by Narasimham [1988, *J. mar. biol. Ass. India*, **30** (1&2) : 137-150]. Salinity tolerance test and pH tolerance tests were conducted in the laboratory using live clams collected for the sampling site.

Analysis of data was done by the following methods :

1. Modal Progression Method for length-frequency data.

2. ELEFAN Program applied to fit von Bertalanffy Growth equation.

3. Beverton and Holt instantaneous mortality rates.

4. Estimation of Condition Index (CI) $= \frac{\text{Wet flesh weight}}{\text{Shell-on weight}} \times 100$

5. ANOVA and correlation matrices for various ecological parameters.

6. Regression equation ($y = a + bx$) for morphometric data.

7. 't' test to test the significance of 'b' value in the regressed length-weight equations.

8. Arc-sine transformation to work out salinity tolerance and pH tolerance from the experimental data.

Results

Depth was found to vary between 0.6 and 4.2 m between the stations at the lowest low water level (LLWL), while transparency which did not vary significantly between months at given station, ranged from 0.15 to 0.95 m. The water temperature varied from 27- 29°C differing significantly between stations and between months at 1% level. Water

salinity ranged from 0 - 19.25 ppt during the study period differing significantly between stations and between months at 1% level. In the bottom water, dissolved oxygen ranged from 0.52 to 4.79 ml/l with an average of 3.39 ml/l. The peak values were recorded during monsoon months and the lowest values in May.

The sediments, based on particle size, was classified and brought under the following types, as per Brady's classification :

Clay Loam	-	Station I
Loamy Sand	-	Stations II, IV and VI
Sandy Loam	-	Station III
Sandy Soil	-	Station V

For the study area as a whole, the proportions of sand, silt and clay were 79.48, 7.02 and 13.5 respectively. The organic carbon values of this sediment were generally high and ranged from 0.009 to 4.98%. Soil organic matter and organic nitrogen were found to range between 0.06 to 8.58 and 0.0008 to 0.439% respectively. Soil salinity and Soil pH were found to vary significantly between months, but not between stations.

Observations on the density distribution of clams revealed that Station I exhibited a total absence of clams during the entire study period. Station II had the maximum abundance of large clams while Station III had greater small clam distribution. Spatfall was noticed to be highest in Station III. The average standing stock of *V. cyprinoides* in the area was estimated at 42 t/ha.

The parameters of the von Bertalanffy growth equation, as computed by ELEFAN program, are

Asymptotic Length (L_∞) = 58 mm

Growth co-efficient (K) = 0.56 (annual basis)

Theoretical origin of the growth curve (t_0) = 0 (assumption)

The von Bertalanffy Growth Equation obtained is

$$L_t = 58\ [1-e^{-0.56(t)}]$$

Where L_t is length at time 't' and 'e' is the base of the Neparian logarithm.

The relative lengths estimatedto be attained at different ages *i.e.* after 6 months, 1st year, 2nd year, 3rd year and 4th year respectively are 14.16 mm, 24.6 mm, 39.09 mm, 47.19 mm and 51.83 mm. The largest specimen obtained in the collection measured 54 mm.

Significant correlation was obtained between the morphometric features studied *viz.* length (APM), height (DVM) and depth. Length-weight relationships were found to be allometric.

Poor Condition Index was noticed in smaller clams during the postmonsoon months and in larger clams during the premonsoon months. In general, low Condition Index prevailed in peak monsoon in both size classes, when maximum clam mortality was also noticed. Protracted spawning with a peak in May-June was observed. Differential spawning of size classes in different salinity regimes was observed.

The estimated mean instantaneous mortality rate 'Z' was 1.76 (SD = 0.613) for the black clam population in the study area.

Salinity tolerance limits in general were found to lie in the 0 - 13 ppt regime with the large clams showing good survival in 1-5 ppt and smaller clams surviving better in 5-10 ppt. The clams were found to exhibit 100% survival in water of pH 6-7. While moderate survival was noticed in pH levels of 4.5 and less survival was hampered by pH levels of 8.5 and above.

Discussion

Based on the observations and findings made in the course of the present study, the abundance and distribution of the balck clam in the study area can be said to be influenced mainly by the salinity conditions prevailing in the area and by the nature of the sediment. Depth does not play a significant role and dissolved oxygen tends to remain more or less stable at the stations throughout the period of investigation. It has been noticed that the animals thrive well in turbid waters.

Water salinity seems to be a major limiting factor in determining clam abundance and distribution, the experimental results tallying with the observations made from nature. Maximum clam mortality has been noticed during peak monsoon when water salinity drops to practically zero. Salinity has also been found to influence spawning activity, with different size classes tending to spawn in different salinity regimes. Together with salinity, sediment characteristics also play a significant role in clam abundance and distribution. While a high abundance of clams has been recorded in sediments containing 1-3% organic carbon, sparsity of occurrence is seen in sediments containing lower or higher levels of the same. The animals show a preference for sediments containing 70% sand, with 0-6% silt and 0-12% clay. Under highly favourable conditions, clam densities as high as 16000-17000 no/sq.m have been recorded.

The experimental studies conducted on salinity tolerance reveals that mantle fluid osmotic concentrations can be used as an index of the animals response to varying environmental salinities. The results of the present study could possibly be used to promote better indentification and management of this valuable resource.

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STUDIES ON THE SALINITY TOLERANCE OF THE VENERID CLAM *PAPHIA MALABARICA* (CHEMNITZ)

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Introduction

There is a wide scope for the culture of clams in our country due to the availability of vast suitable areas as well as seed clams from the nature or by producing them in hatcheries. Knowledge of the physiological impact of environmental parameters on the selected species is very essential for the successful operation of its culture. Of these, salinity is of great importance as it varies with the changes in other environmental factors, especially in a tropical country like India.

The clam *Paphia malabarica* (Chemnitz) which supports a very good, regular clam fishery with its rich resource in Ashtamudi Lake (Kerala), has good culture prospects. Hence the present study of salinity tolerance was carried out in the clam *Paphia malabarica* under laboratory conditions.

Objectives

The objectives of the present study were :

1. determination of optimum tolerable salinity ranges for the clam,

2. determination of lethal levels of lower salinity ranges,

3. relative survival rates of the clams at different levels of salinity,

4. determination of conditioning time of the clams to different salinities, and

5. studies on mantle fluid.

Material and methods

Test medium : Sea water collected 8-10 km off Kochi was used. This water was allowed to settle in the laboratory, filtered and used. Salinity dilutions were made by using the formula

$$V = \frac{\text{Required salinity}}{\text{Known higher salinity of sea water}} \times 1000$$

Where V gives the volume of seawater of known higher salinity to be taken to prepare one litre of the particular diluted solution. Higher concentrations were made by dissolving required amount of common salt, which was filtered after settling, then used for the study. The solutions made were checked by titration following Mohr's method.

Test animals : Live clams *P. malabarica* of two size groups were selected for the study, small clams (15-20 mm APM) and large clams (30-35 mm APM). They were transported from the natural bed at Ashtamudi Lake and acclimated in the laboratory for 48 hr in the habitat salinity. After acclimation, the animals were directly transferred to the experimental salinities.

Test containers : 40 lt capacity plastic tubs were used for acclimation. Plastic tubs of 8 lt capacity were used as experimental containers. Volume of the prepared salinity solutions given in each tub was 4 lt.

Tests on salinity tolerance

The salinity treatment in which at least 50% of the animals survived at the end of 10 day exposure have been considered to be within the tolerable range and rest as lethal. The clams were considered dead if they does not close the valves or react when touched with a needle.

Three sets of salinity tolerance experiments were designed as follows.

Experiment I : This experiment was meant for determining the approximate highest and lowest levels of salinity tolerated by the clams and the survival rates in between. The test salinities in this experiment varied from 0 ppt to 40 ppt at

5 ppt intervals *i.e.* 0, 5, 10, 15, 20, 25, 30, 35 and 40 ppt. A control was also set up with the habitat salinity of 22 ppt. All the sets were carried out in triplicate. Each tub was stocked with 10 animals each from both the size groups.

No feeding was done. The treatments were given uniform aeration. Water in each tub was changed once in 40 hrs. This experiment lasted for 10 days. During this experiment, observations were made on the conditioning time when at least 50% of the present animals opened their valves and started activity. Dead animals were immediately removed and water was changed.

Experiment II : This experiment was conducted for determining the lowest lethal salinity of both size groups where the 50% mortality sets in. Same stocking was done in salinities from 11 ppt to 14 ppt at 1 ppt intervals *i.e.* 11, 12, 13 and 14 ppt in duplicate. Conditioning time and temperature was monitored. Water exchange was done as before. No feeding for the whole duration of 10 days.

Experiment III : To study the effect of medium salinity on mantle fluid salinity. The treatment were 0, 1, 3, 5, 8, 12, 17, 22, 28 and 34 ppt, run in duplicate. Only large-clams (30-35 mm) were used for the study. Each tub was stocked with 22 animals. Duration of this experiment was 10 days. Dead animals were recorded and immediately changed. No aeration or feed was provided. Two animals from each tub was taken on the first, second, fourth, sixth and tenth days of the experiment, wiped dry with tissue paper, wedged open to drain out the mantle fluid into labelled test tubes. The salinity of mantle fluid was estimated. The mantle fluid of the acclimated samples also taken initialy.

Data analysis : The data of Experiment I was subjected to obtain ED-50 value of salinity by probit analysis. Analysis of variance (ANOVA) was done with the data of Experiment III to test the significance of treatments.

Results and discussion

The maximum survival rates (100%) was recorded between 17 ppt and 30 ppt for the large clams; 20 ppt and 30 ppt for the small clams. Maximum mortality was recorded

(100%) from 0 ppt to 8 ppt. 10 ppt recorded 69.98% and 66.63% mortality for the small and large clams respectively. 50% survival was recorded for both the size groups in 12 ppt salinity. Probit analysis showed ED-50 valves of 11.6787 ppt and 11.3915 ppt for the small and large clams respectively. The treatments for the Experimentt III was found to be significant at 1% level.

The lowest conditioning time of 15 min. was recorded in 22 ppt by both size groups and the highest in 10 ppt by the small clams (172 hr). In 0 and 5 ppt the size group of animals did not open their valves. The large clams took 148 hr to get conditioned in 10 ppt. At higher salinities smaller clams got conditioned earlier than the large ones.

Mantle fluid salinity studies showed the reaction of the animal to lowering of salinity. It is proved that the clams cannot survive in 0 to 8 ppt due to the hypertonicity of the mentle fluid which was a result of the indefinite closure of the shell valves in the unfavourable salinity conditions. They adjusted to the 12 ppt medium with partial mortality (50%) and acclimated gradually without stress from 22 ppt to 34 ppt although a negligible mortality was recorded in the latter media.

The present results are in agreement with the similar works on salinity tolerance carried out in other clams like *Sunetta scripta* (Thampuram *et al.*, 1982, *Bull. Dept. Mar. Sci., Univ. Cochin*, **13** : 46-52), *Meretrix meretrix* (Sundaram and Shafee, 1989, *J. mar. bio. Ass. India*, **31** (1&2) : 299-302), *M. casta* (Salih, 1978, *J. mar. biol. Ass. India*, **15** (1) : 345-353), *Katelysia opima* (Ranade and Kulkarni, 1973, *J. Bombay. Nat. Hist. Soc.*, **69**(3) : 616-634).

Davenport and Fletcher (1979, *J. Mar. Biol. Ass. U.K.*, **58** : 671-681) says that the permenant closing of the shell value in low salinity media weakens the adductor muscle after a period, which initiates the diffusion of low saline water into the mantle cavity. This diffusion disrupts the osmotic pressure inside damaging the internal organs like gill filaments and frontal gill cilia, resulting in mortality. In 12 ppt, it was proved that the animals which can tide over the adjustment period of mantle fluid with the external salinity only can

survive. When the mantle fluid salinity reached just above the medium salinity (14.85 ppt) the mortality ceased (at 50%). From 22 ppt - 34 ppt, the adjustment between the two fluids was rapid, especially in 22 ppt.

So it can be concluded from the conditioning time data and mantle fluid study that 22 ppt is the best salinity for the optinum living conditions of the clam *Paphia malabarica*. But further studies are in need, relating the temperature, age and condition of the animal to salinity. These facts can really enlighten the culture of this particular species on a commercial scale.

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METABOLIC ADAPTATIONS OF THE YOUNG ONES OF THE SEA-BASS *LATES CALCARIFER* (BLOCH) WITH SPECIAL REFERENCE TO ENERGY UTILIZATION

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Introduction

Lates calcarifer popularly known as sea-bass, is one of the commercially important marine food fishes and thrives well in brackishwater and freshwater environments. This large centropomid species is a euryhaline, eurythermal catadromous, highly predaceous and protandrous hermaphrodite, which grows to a comparatively large size. To understand the physiological peculiarities of sea-bass which has the adaptability to different aquatic environments, the present work was undertaken by determining the metabolic rates and quotients at different ambient oxygen concentrations under random swimming activity.

Objectives

Studies on metabolism in relation to several factors have been extensively carried out in a few species of fishes, such as goldfish, sakeye salmon, rainbow trout, tilapia and mullet, but the information available on sea-bass is inadequate. The metabolic rate or rate of expending energy is of great importance, because it affects the speed of all metabolic processes. The rate of metabolism is directly or indirectly influenced by many biotic and abiotic factors and among them ambient oxygen, temperature and activity exert the greatest effect on metabolism. Though considerable information has been already accumulated on different aspects of this species, *viz.* distribution, biology, age and growth, reproduction, genetic variations, pigmentation, culture, production, larval development, availability of the seeds, etc. the present work on energy metabolism by young ones of sea-bass is the first of its kind in this country.

Material and methods

Young ones of *Lates calcarifer* (Bloch) were collected from brackishwater in and around Cochin mainly from Puduvyppu, an island about 45 km from Cochin. 221 fishes ranging in total length from 7.0 to 17.0 cm and weighing from 5.0 to 80.7 g were captured from May to August and November to December 1989 - 90.

Test fishes were stocked in stocking tanks initially and were acclimatized to sea water (30‰), brackishwater (15‰) and freshwater (0‰) by "Drip method" (Perry *et al.*, 1984, *In* : W. S. Hoar and D. J. Randall [Ed.] *Fish physiology*. Academic Press, New York, 325 pp). They were acclimated in different media in an acclimation tank provided with air diffusers, a biological filtering unit and thermostatic relays. The fish to be experimented were kept within a partition and deprived of food for 24 hrs (Brett, 1964, *J. Fish. Res. Bd. Canada*, **21** : 1183 - 1226; Peer Mohamed *et al.*, 1978, *Indian J. Exp. Biol.*, **16** (3) : 385 - 386) before the experiment, in order to maintain the physiological conditions of the fish almost uniform. The fish to be experimented was left in the respirometer overnight with continuous flushing and recirculation of the experimental medium.

All the experiments were conducted with the help of a respirometer. In order to get an accurate estimate of total carbon dioxide, decarbonated experimental medium (Peer Mohamed and Kutty, 1980, *Proc. Indian Acad. Sci. (Anim. Sci.)*, **89** (6) : 537- 542; 1981, *Hydrobiologia*, **76** : 3-9) was used. Owing to the limited amount of decarbonated experimental medium the experimental system was so designed for circulation of the same water throughout the experiment. The water from the overhead reservoir flushed through the respirometer to a 50 lt capacity fibre glass tank kept at ground level, from which it was again pumped up to the overhead reservoir by a constant water-level-device provided in the overhead reservoir.

Metabolic rates (oxygen consumption, carbon-dioxide production, ammonia excretion); quotients (respiratory quotient - R.Q. and ammonia quotient - A. Q.) and random swimming activity of sea-bass subjected to routine metabolism

(at high ambient oxygen near air saturation), hypoxic metabolism (from air saturation down to asphyxial level) and recovery metabolism (recovery after hypoxia) were determined in three different media such as sea water (30‰), brackishwater (15‰) and freshwater (0‰) at two different temperatures - 30 and 35°C. Analysis included the estimations of dissolved oxygen, total carbon-dioxide and ammonia. Finally the data collected were analysed statistically.

Results and discussion

It was noticed that oxygen consumption rates were high in the media to which the experimental fish were transferred (brackishwater and freshwater) than in natural medium (sea water), and also the rates were high at 35°C as compared to 30°C in all the media. The mean routine rate of oxygen consumption increased from 78.26 mg/kg/hr to 86.55 mg/kg/hr in sea water; 100.57 mg/kg/hr to 111.46 mg/kg/hr in brackishwater and 72.59 mg/kg/hr to 126.36 mg/kg/hr in freshwater, when the temperature was raised. The interaction between the experiment and environment was significantly different at 5% level with oxygen consumption.

Carbon-dioxide production during hypoxia compares well with the same condition in *Tilapia mossambica* (Peer Mohamed, 1974, *Ph. D. Thesis. Madurai Univ., Madurai*) at 30° and 35°C in sea water. Carbon-dioxide production and ammonia excretion showed similar trends at both the temperatures in sea water during hypoxia. The relative increase in ammonia production during anaerobiosis helps to prevent acidosis (Prosser and Brown, 1961). The changes in the rates of oxygen consumption, carbon-dioxide output and ammonia excretion at different temperatures and salinities are reflected in R.Qs. and A.Qs.

The R.Q. values were significantly above unity in normoxia and hypoxia clearly suggesting that considerable amount of anaerobic metabolism has taken place, resulting in the release of excess carbon-dioxide as in *Rhinomugil corsula* reported by Kutty and Peer Mohamed (1975, *Aquaculture*, **5** : 253 - 270); in goldfish by Kutty (1968 a, *J. Fish. Res. Bd. Canada*, **25** (8) : 1689-1728); in *Tilapia mossambica* by Kutty (1972, *Mar. Biol.*, **16** (2) : 126-133), Sukumaran and Kutty (1987,

Proc. Indian Acad. Sci. (Ani. Scit., **96** (6) : 705-714); in crucian carp by Privolnev (1954, *Fish. Res. Bd. Canada., Transl. Ser.*, **4222**) and fry and fingerlings of *Mugil cephalus* and *Chanos chanos* as reported by Usha Devi (1987, *M. Sc. Dissertation. Cochin University of Science & Technology, Cochin*). The size of the R.Qs over unity is indicative of the intensity of anaerobic metabolism.

The rise in A.Q. in all the experiments, in all the media, at 30° and 35° C clearly indicates the extra release of ammonia anaerobically. A.Q. values increased along with R.Q. under low oxygen suggesting a coupling of increased ammonia excretion with increased carbon-dioxide output. The A.Q. values obtained were higher during hypoxia than during normoxic condition, in all the cases at both the temperatures.

The random activity of the fish increased when the temperature was raised from 30° to 35°C in all the media during routine metabolism. The mean rates which were 35.98 c/hr, 24.19 c/hr and 23.17 c/hr in sea water, brackishwater and freshwater respectively at 30° C, rose to 39.93 c/hr, 24.27 c/hr and 53.00 c/hr at 35° C. When the oxygen was gradually reduced in the respirometer during hypoxia in sea water and brackishwater, the activity of the fish first increased and then decreased apparently to escape from the medium as in the case of *Tilapia mossambica* and *Chanos chanos*. The increased activity induced by hypoxia (in hypoxia - recovery phase) might allow the fish to move out of the hypoxic environment to more oxygeneted waters as in the case of *Rhinomugil corsula, Puntius sarana* and *Carassius auratus*, reported by Peer Mohamed and Kutty (1980, *loc. cit.*; 1982, *Experentia*, 38 : 1-587) and four marine teleosts as reported by Hamsa and Kutty (1972, *Indian J. Fish.*, **19** : 76 - 85). Thus sea-bass cannot be grouped singularly under passive or active group, since it is showing the characters of both depending on the situation.

During hypoxia, the asphyxial oxygen concentration (concentration at which the fish begins loosing its equilibrium) observed in sea water was 1.17 ml/l at 30° C and 1.45 ml/l at 35° C, but when the salinity was decreased to 15‰ (brackishwater) the asphyxial oxygen concentration was found

to be 1.53 ml/l at 30° C and 1.60 ml/l 35° C, while when the salinity was again decreased to 0‰ (freshwater) the asphyxia was noted at 1.54 ml/l oxygen concentration at 30° C and 1.60 ml/l at 35° C. Hence the decreasing tolerance of hypoxia in the three media can be arranged in the following order : sea water, brackishwater, freshwater at 30° C. The fish also showed relatively more tolerance in sea water at 35° C.

In recovery metabolism, in all the three media, after aspyxiation, the fish recovered subsequently in air saturated water, thus indicating that the anaerobic ability to survive hypoxia exists in this species. During the recovery phase, sea-bass showed a post-hypoxic repayment of oxygen debt. It is noted that fish repays almost entirely all the oxygen debt accumulated. The trends at 30° and 35° C are similar in the hypoxic phase and subsequently the recovery phase.

The observations were made at 30° and 35° C; the higher temperatures were chosen because of the scarcity of information on fish energetics at these temperatures and also because of their relevance to tropical conditions. Temperature did not show any significant influence in all the three media. It is also statistically proved that the temperature range (30° C - 35°C) does not cause a marked metabolic difference in the species, either in the same medium or in different media.

Finally, the amount of energy utilized by the fish aerobically and anaerobically from different substrates by sea-bass in sea water, brackishwater and freshwater at 30° and 35° C were estimated based on the assumptions by Kutty and Peer Mohamed (1975, *loc. cit.*). In aerobic condition, the non-protein R.Q. was below 0.7 which is quite below the conventional R.Q. values for fat. Hence the fish has used only proteins and fat and glucose is not involved in oxidative energy release. In anaeroic metabolism, the non-protein R.Q. values were above 1 which showed that anaerobic energy has been derived and that it is mainly from protein and carbohydrate. Energy utilization by sea-bass under hypoxia suggests that the fish derives considerable amounts of energy anaerobically, much more efficiently by pathways other than

the glycolytic path, such as those involved in the simultaneous breakdown of carbohydrates and amino acids (Hochachka and Samero, 1973, *Strategies of biochemical adaptaion.* W. B. Saunders Co., Philadelphia, 358 pp.) resulting in increased carbon-dioxide and ammonia excretion, as in the case of *Rhinomugil corsula* reported by Kutty and Peer Mohamed (1975, *loc. cit.*).

The present study indicates that among the euryhaline fishes, sea-bass shows remarkable adaptability to wide variations in salinity. The results of energy utilization during the current study have shown that the energy expenditure of this species is minimal in sea water and brackishwater compared to freshwater. Hence it is expected that growth rates would be best in these media. It has also been established through the current study that activity of the fish is relatively least in brackishwater leading to less energy expenditure and hence better food conversion to stored energy. This should contribute significantly to high production rates. Eventhough the growth rate will not be as high as in the other two media, this species can also be cultured in freshwater impoundments of inland waters. The prospects of culturing this species on a commercial scale in sea water and brackishwater impoundments should prove very successful due to the remarkable versatility and adaptability of the species to changing salinities, its voracious feeding habits, its phenomenal growth rates and the commercial value that the fish commands in Indian and overseas markets.

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STUDIES ON SOME ASPECTS OF BIOLOGY AND ECOLOGY OF CORAL REEF FISHES OF LAKSHADWEEP WITH OBSERVATIONS ON OTHER CORAL REEF ECOSYSTEMS OF INDIA

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Introduction

Coral reef science now potrays itself through many specialised fields in the tropics. While the current concern on the world's coral reefs is to maintain ecological 'health', India faces the stupendous task of stepping up reef-related research in comparison to other developing countries. Despite important studies on the systematics of corals, associated fishes, invertebrates and hydrographic surveys, there is an overall paucity in reef-related studies that could make available basic data and create environmental awarness among the concerned authorities and users.

Fishes are the most conspicious organisms of a coral reef ecosystem. With a genesis in mid - and late - 1950's, the interest on coral reef fish has been ever increasing. Four significant reviews that appeared ever since were those of Ehrlich (1975, *Annu. Rev. Ecol. Syst.*, **6** : 211-247), Goldman and Talbot (1976, *In :* O. A. Jones and R. Endean [Ed.] *Biology and geology of coral reefs.* Academic press, New York, pp. 125 - 154), Sale (1980, *Oceanogr. Mar. Biol.*, **18** : 367 - 421) and Doherty and Williams (1988, *Oceanogr. Mar. Biol.*, **26** : 487 - 551). From the Indian region, the most comprehensive systematic account on reef fishes was that of Jones and Kumaran (1980, *Fishes of the Laccadive Archipelago.* The Nature consevation and Aquatic science, Trivandrum, pp. 760) in which 603 fish species of the Laccadive Archipelago have been described. A greater ichthyological activity is evidenced by several publications related to the ecology, biology and distribution of coral reef fishes from Lakshadweep, while

similar studies covering the reefs of Andaman and Nicobar Islands, the Gulf of Mannar and the Gulf of Kutch are wanting.

Coral reef fishes are among the major exploited food resources from reefs (Salm, 1988, *In* : R. A. Kenchington and Brydget E.T. Hudson [Ed.] *Coral Reef Management Handbook.* UNESCO, Jakarta, Indonesia, pp. 15 - 21) and are picking up excellent market value (Nicoll, 1993, *Australian Fisheries*, 52 (1) : 2- 23), but on a world - wide basis, they are underexploited (Munro, 1984, *ICLARM Newsletter*, 7 : 3-4). The major Indian reef systems (Lakshadweep, Andaman Islands, the Gulf of Mannar and the Gulf of Kutch) can not only support a lucrative fishing activity, but also sustain ideal fish species suitable for cage culture (*e.g.* groupers) and a majority of small species that are popular marine ornamental fishes. In the late eighties, the world trade in ornamental fish, associated plants and accessories stood at US $ 7.2 billion (Andrews, 1992, *INFOFISH International* (2/92) : 25-29). Coral reef fishes also serve as early - warning mechanisms of a deleterious environment (Reese, 1981, *Bull. Mar. Sci.*, 31 : 594-604).

Objectives

In view of the high fisheries potential of coral reef fishes and rather inadequate attention paid to their exploitation and utilisation in comparison to other fishery resource in the country, the present study was conducted at Lakshadweep with observations on other coral reef ecosystems of India (Andaman Islands, the Gilf of Mannar and the Gulf of Kutch). The study attempted a classification and description of sub-habitats, distribution and community organisation of reef fish, food and feeding habits and reproduction of some important and common species and fishing methods. Research results of practical importance for the management and development of coral reef fishery resources are highlighted.

Material and methods

General zonation patterns on the atolls of Lakshadweep are described from observations underwater (snorkeling), from the water surface (glass - bottomed boat) and aerially (helicopter). The sub-habitats from the four coral reef systems have been classified based on physiographic and biological

characters. For studying the fish species distribution, collections have been made from Lakshadweep, Andaman Islands, the Gulf of Manner and the Gulf of Kutch using various fishing gears. Collections preserved in the museums of different institutes were also examined for the purpose of identification and various publications have been consulted.

Fish species enumeration and their distribution among distinct physiographic zones (sub-habitats) were studied at Kavaratti Atoll using the visual census technique. Belt transects of 30 m length were used to record all visible species and numbers from 2 m on either side on a pre-laid line from five sub-habitats *viz.* live coral, massive coral, rubble, sand and seagrass zones. The approximate area covered by a single census unit is 120 sq.m and a total of 27 censuses were conducted during an 18 month period from January 1991 to June 1992. Fish species from the outer reef slope were recorded using a 100 m belt transect running parallel to the reef crest, the total width of the transect being approximately 6 m, visually estimated (Roberts and Ormond, 1987, *Mar. Ecol. Prog. ser.*, **41** : 1-8). Vagile species encountered in large schools were eliminated to avoid overemphasis of a particular group, while those species that were difficult to distinguish underwater were treated as single groups (Bouchon - Navaro and Harmelin - Vivien, 1981, *Mar. Biol.*, **63** : 79-86; Sale and Douglas, 1981, *Environm. Biol. Fishes*, **6** : 333-339). Data collected from each census were grouped under families and species and transformed onto a data matrix for respective sub-habitats and reef slope to study aspects of community organisation, namely the species diversity (H') (Shannon and Weiner, 1949, *Mathematical theory of communication*. Univ. of Illinois press, Urban, pp. 117), evenness (J') (Pielou, 1966, *J. Theoretical Biol.*, **13** : 131-144), frequency of occurrence, total abundance, percentage abundance, family/species composition, presence/absence, habitat distribution and community diversity.

A total of 143 commonly occurring fish species mainly from Lakshadweep were examined for their qualitative and quantitative prey preferences. The main prey were sorted by groups and their relative importance was determined by points or numbers, expressed as percentage. The Shannon - Weiner

diversity index (H') was used to determine feeding diversity of each species. Diets of the same fish species occurring in the Gulf of Mannar region were also compared.

Twentyfive commonly occurring species were selected mainly from Lakshadweep and few from the Gulf of Mannar to study aspects of maturity and spawning. The percentages obtained from the two broad grouping of immature and mature, against their respective sizes were used to determine the size at first maturity. Sex - ratio was computed based on total number of females and males recorded. Fecundity was estimated by counting mature ova from preserved ripe ovaries. The percentage occurrence of ova in respective ova - diameter groups was used to draw frequency polygons from which spawning frequencies were determined, while the percentage occurrence of ripe and spent specimens on a monthly time scale were used to determine the spawning seasons. Based on nocturnal collections of juvenile fishes on seagrass beds, monthly abundance, monthly fluctuations and juveniles of fish families typically associated with seagrass beds were studied. The important ecological processes concerning juveniles on seagrass beds and seagrass beds as fish nurseries were highlighted. Rank correlation was used to test juvenile abundances against salinity and temperature, while the same in relation to moon phases was tested using ANOVA. Various fishing methods used to capture coral reef fishes in the four reef systems are described based on actual operations/observations.

Results and discussion

Sub-habitats on the Indian coral reef ecosystems

In the classification of sub-habitats, the Gulf of Mannar recorded the highest number of sub-habitats (10 No). The sub-habitats are zones made up of deed coral, *Enhales acoroides* (seagrass), live coral, mangrove roots, massive coral, *Pemphis acidula* (intertidal shrub), rubble, sand, *Sargassum*. spp. (seaweed and seagrass. Eight sub-habitats in the Andaman Islands consisted of dead coral, live coral, mangrove roots, rock, rubble, sand-mud, seagrass and soft corals. The six sub-habitats classified on the coral atolls of Lakshadweep are zones consisting of live coral, massive coral, rubble sand, seagrass

and boulder zone. Reef slope was not considered as a sub-habitat as this physiographic zone on coral atolls is a combination of characters derived from many sub-habitats coupled with greater water depth. The least sub-habitat diversity (5 No) was found in the Gulf of Kutch region consisting of nontypical reef habitats, but with lesser individual expanse while atolls of Lakshadweep have fewer sub-habitats, but each of them with a wider expanse.

Distribution of fishes in the coral reef ecosystems of India

A total of 421 fishes belonging to 26 families were studied. 12 new records were indentified from Lakshadweep, 3 from Andaman Islands and 19 from the Gulf of Mannar reefs. The nine new records for the Indian region are *Neoniphon opercularis* (Valenciennes, 1831), *Sargocentron praslin* (Lacépède, 1802), *Plectorhinchus gaterinus* (Forsskal), *Chaetodon oxycephalus* Bleeker, 1853, *Forcipiger longirostris* (Broussonet, 1782), *Apolemichthys trimaculatus* (Lacépède, 1831), *Labroides bicolor* Fowler and Bean, 1928, *Scarus rubroviolaceus* Bleeker 1847 and *Siganus luridus* (Rüppell, 1828).

The most specious families were Labridae and Pomacentridae that recorded 55 and 53 species respectively. The available information on coral reef fishes of India shows that Lakshadweep received considerable attention. Similar attention was not paid to coral reef fishes inhabiting the reefs of Andamans and the Gulf of Mannar. Gulf of Kutch received the least inchthyological activity. However, intensification of efforts in the Gulf of Kutch region is not likely to yield good collections due to an almost dead environment.

Coral reef fish community organisation on Kavaratti Atoll

Among the fish families on the sub-habitats, Labridae emerged as a generalist family found on all sub-habitats except on live coral zone. Subsequent positions were occupied by other families characteristic of particular sub-habitats, reflection primarily the feeding mode, site - attachment and other habits. With an exception on sand flats where only Labridae and Acanthuridae were dominant, 3 to 5 families from a collective list consisting of Acanthuridae, Balistidae, Chaetodontidae, Labridae, Mullidae, Pomacanthidae, Pomacentridae and Scaridae were dominant on each sub-habitat.

Equal number of families (27) represented on the reef slope and seagrass beds is because the latter has juvenile and sub-adult representatives of most adult reef fish fauna which inhabit other habitats. This was followed by 26 families recorded on the massive coral zone. As the live coral zone is composed of a single species of ramose coral (*Acropora formosa*) very few families (14) made use of the zone, while a similar pattern was evident on rubble zone (15 families). Despite featurelessness, the sand flats supported 17 families. The community diversity (H') was highest on the reef slope (3.32) followed by massive coral, sand flats, rubble, seagrass beds and live coral (2.96, 2.69, 2.68, 2.49 and 1.84 respectively).

Family assemblages on the sub-habitats and the reef-slope were not stable between censuses and pre-monsoon, monsoon and post-monsoon seasons. Fish assemblages on coral reefs were observed to be loosely structured and lack a high degree of stability (Sale, 1982, *ICLARM Conf. Proc.*, **9** : 241-253). Inherent behaviour of fish connected to modes of feeding, reproduction, shelter requirement and local migrations determined the variation in the observed assemblages. Non-biological factors like habitat structure, proximity to other species - rich areas and the southwest monsoon also influenced family composition on sub-habitats. When the distribution on all sub-habitats was considered, it was found that only few families exhibited a wider niche while a majority of them had restricted distribution. A wider habitat distribution could be due to generalised diet that increases the apparent eveness of resource distribution and also due to diversity of food habits found within a single family (Hiatt and Strasburg, 1960, *Ecol. Monogr.*, **30** : 65 - 127; Hobson, 1974, *Fish Bull.*, **72** (4) : 915 - 1031).

The reef slope, massive coral and seagrass beds showed a marked variation in species richness though the family composition was almost constant (26 to 27 families). The increase or decrease in species composition was contributed either by greater number of genera or species supported by a single family or vice-versa. The possible cause for this pattern is attributed to topographical complexity that may harbour a greater number of users of a particular sub-habit. A high species diversity on massive coral zone is due to its

proximity to the species - rich adjacent reef slope, while the same in the seagrass beds is due to their role as nurseries and foraging grounds for many fish species. Fish assemblages could be roughly predicted with space.

The reef slop recorded the highest number of fish species (121) followed by massive coral (89). Seagrass beds and rubble zones recorded 65 and 46 species respectively. While live coral zones were frequented by 39 species; the least species composition of 28 species was found on sand flats. The community diversity (H') for species on sub-habitats was highest on the reef slope (4.45) followed by massive coral (3.93). Rubble and sand flats recorded H' values of 3.58 and 3.25 respectively, while the same for live coral and seagrass beds was comparable (3.16 and 3.14 respectively).

Species assemblages varied highly between censuses and pre-monsoon, monsoon and post-monsoon seasons of the sub-habitats. Many physical and biological factors within a single sub-habitat are possible determinants of the community structure of species. Fish assemblages did not follow a definite pattern of distribution and therefore cannot be predicted with time. When individual species were considered for their habitat width one or two species from each of the dominant family were found to be generalists. For example, *Acanthurus triostegus* (Acanthuridae), *Rhinecanthus aculeatus* (Balistidae), *Chaetodon auriga* (Chaetodontidae), *Halichoeres scapularis* (Labridae), *Abudefduf sexfasciatus* (Pomacentridae) were species exhibiting wider habitat ranges. Habitat selection in these diurnal fishes highly correlates with their requirements of food and shelter. A greater topographic complexity provides a greater diversity of shelter sites and surface area for algae and invertebrates (Luckhurst and Luckhurst, 1978, *Mar. Biol.*, **49** : 317 - 323) which form important prey for reef fish. It is therefore concluded that habitat complexity supported greater species richness.

Food and feeding habits

Feeding habit of fishes are diverse within any single family [Hiatt and Strassburg, 1960, *Ecol. Monogr.*, **30** : 65- 127; Hobson, 1974, *Fish. Bull.*, **72** (4) : 915-1031].

Strict herbivorous species belonged to Acanthuridae, Kyphosidae, Scaridae and Siganidae. These fishes fed on a wide variety of green, brown and red algae. Filamentous algae was a dominant prey item. Other feeding groups could not be categorised as zooplanktivores, omnivores, carnivores or benthic carnivores owing to a high overlap in food preferences. The zooplanktivorous species belonged to families Apogonidae, Caesionidae and some pomacentrids. Copepods, decapod larvae, fish larvae, eggs, shrimp and megalopae formed dominant prey groups. Pomacentridae had species that were zooplanktivores, omnivores and herbivores. Feeding diversity (H') was in general high in zooplanktivores as a higher number of prey items were involved. Among the dominant omnivores were, Balistidae, Chaetodontidae and Mugilidae that fed both on prey of plant and animal origin. It was observed that varying proportions of these prey items could stem a particular species more towards herbivore or carnivore while an equillibrium status retained their identity as omnivores. A majority of species belonging to Cirrhitidae, Holocentridae, Labridae, Lethrinidae, Lutjanidae, Mugiloididae, Mullidae, Scorpaenidae and Serranidae could not be distinguished as either strict carnivores or benthic carnivores as their diets consisted of benthic crustaceans, molluscs, echinoids and a wide variety of fishes. Polychaetes, sipunculids and algae were also occasional food items. In comparison to all families, mullids showed the greatest tendency to feed on benthic invertebrates. Benthic crustaceans (especially small xanthid crabs) emerged as an important link in the food web concerning most species. Species of chaetodontids regarded as strict corallivores fed on equal proportions of algae and coral polyps indicating a slow adaptation by change in food habits with change in environmental conditions.

In comparing the commonly occurring species between the two localities, *viz.* Lakshadweep and the Gulf of Mannar, it was found that prey items in general appeared to be few in the latter region. The possible reason attributed to this is the difference in the nature of reef formation and types of varying substrata offered for a variety of prey items. Low H' values indicated a narrow/specific food requirement of a particular species while high H' values correlate with higher feeding diversity.

Reproduction

Among the 25 coral reef fishes studied for aspects of reproduction, sizes at first maturity depended on the maximum size that a particular species could attain. In general, pomacentrids matured below 40 mm SL while some chaetodontids, cirrhitids and labrids matured at sizes ranging between 90 and 120 mm. While protogyny was prominent in labrids (*Halichoeres centiquadrus, Thalassoma hardwickii* and *T. lunare)*, other species studied either matured synchronously or differentially, with males maturing earlier or later than females. Protogyny is strongly developed in Labridae with the occurrence of two types of males, *viz.* the primary and secondary males among a harem of females (Robertson and Choat, 1974, *Proc. Int. Coral Symp.*, 2nd, **1** : 217 - 225; Warner and Robertson, 1978, *Smithson. Contrib. Zool.*, **254** : 1-27). Sex-ratios indicated that females were dominant in most species. Fecundity estimates varied highly due to the fact that most species were continuous spawners generally spawning at very short intervals. The total fecundity range was 700 to 225,850 mature eggs per ripe female. Coral reef fishes are known to be highly fecund with an annual egg production range from 10,000 to 10,00,000 per female (Sale, 1980, *loc. cit.*). Based on ova diameter studies most species were observed to be continuous spawners, some had small breaks in spawning, while very few indicated biannual spawning. Spawning appeared to be minimum during monsoon and this perhaps to tide over the adverse conditions during monsoon season which would perhaps transport eggs and larvae in an unfavourable direction. Robertson (1990, *J. Exp. Mar. Biol. Ecol.*, **144** : 49-62) stated that rough physical conditions evidently are stressful for adults and depress their reproductive output.

Juveniles of Acanthuridae and Labridae were most dominant on seagrass beds. Schooling species belonging to Acanthuridae, Carangidae, Holocentridae, Mullidae, Scaridae and Siganidae showed clumped and a variable recruitment. Non-schooling individuals were rare in occurrence. Absence of juveniles on the adjacent sand flats clearly indicated that shelter, apart from food, was an important resource that seagrass offered. Where coral reefs occur in conjunction with seagrass beds many recruits of a number of species will spend

the first portion of their post-larval life in the seagrass beds and invade coral reefs at a later age when they are capable of evading predation (Shulman, 1985, *Ecology*, **66** : 1056 - 1066). Characteristic avoidance of monsoon seasons by adult spawners was reflected in a low number of juveniles during monsoon. Peak settlement was observed during pre-monsoon and post-monsoon seasons. A continuous occurrence of juveniles in different size ranges confirms the continuous spawning habits.

While juveniles of labrids showed a clear abundance during summer months, acanthurids were dominant both during pre-monsoon and post-monsoon. Though chaetodontids and holocentrids were not abundant on seagrass beds, they occurred consistently round the year. Seasonality in occurrence was most pronounced in Carangidae and Siganidae, while individuals of Apogonidae, Lutjanidae and Mullidae were moderately seasonal in occurrence. Seasonal abundance of juveniles was influenced by summer peaks in settlement, adult spawning, timing of settlement, magnitude of settlement and local migrations. Abundance of larvae was not effected by temperature, but increased with salinity. Occurrence of juveniles was found to be greater on days of new moon phases (dark nights) rather than during full moon phases (bright nights). Lower settlement rates during weeks around the full moon (brighter nights) was reported by Robertson *et al.* (1988, *Ecology*, **69** : 370-381).

Fishing methods

Regional fishing methods varied with the type of reef system. Both indigenous and introduced/modern methods are being practiced and a majority of them make use of the behaviour of fish for capture. A greater variety of methods were observed in Lakshadweep than elsewhere. The atoll topography with extensive lagoon areas permitted the operation of many gears like seine nets, gill nets and encircling nets. Though traps are successful on many reef systems, their popularity in Lakshadweep has dwindled almost to extinction. Light fishing and harpooning are important traditional methods. Operation of large gill nets outside the reef is less

popular because of the damage caused to nets by reefs and sharks. Trolling is a popular fishing method.

Owing to the fringing reefs of Andamans, the scope of operating a wide variety of gear is reduced. Hooks-and-lines yield highest catches followed by gill nets. Seine nets are operated in shallow bays with sand flats. Traditional methods like harpoons, light-fishing and traps are practiced, but are not popular. In general, low fishing activity was observed in Andaman Islands.

Traps are very successful and form a major reef fishery in the Gulf of Mannar region. Indigenous traps are operated on a daily basis by local fisherman on fringing reefs and rocky areas bordering the islands or on plain sandy areas. Munro (1983, *ICLARM Stud. Rev.*, 7 : 1-9) found that the use of traps for capturing reef fishes and crustaceans is a predominant feature of the Caribbean reef fisheries. This method could probably become popular in the Indian region where traps are less popular. Gill nets, hook-and-lines and shore seines are also operated. No observations were made form the Gulf of Kutch region.

Research results relevant to development and management

Countries having adjacent coral reefs have sound developmental programmes aimed at exploiting resources and conservation of the ecosystems. Such measures can only be based on research results obtained from the particular area. The classification of sub-habitats provides necessary back-stop for taking measures to protect nursery areas of commercially important fishes, predict richest habitats based on habitat - complexity, adopt biotope suitable gear and avoid damage, identifying mariculture locations, declaration and establishment of marine parks, development of tourism and environmental awareness.

The information generated on the geographic distribution of certain coral reef fishes in India can identify candidate fish species that can be captured or cultured at a particular locality, ornamental fish species that Indian reef systems support, formulating projects on their trade and preparation of basic

market information. Finally, such check-list help in bridging gaps in biogeographic studies on reef fishes.

Community organisation studies identify habitats that are species rich based on fish species diversity, provides information on species associations with particular habitats which in turn helps to direct fishing operations to such areas with suitable gear. Knowledge on specific habitat requirements helps in simulating natural conditions for fish in captivity, procurement of natural food for fishes based on clues provided by their assemblages, assessing an under-exploited, optimally exploited and over-exploited stock of fishes on a particular area. This further forms a basis to implement conservational and management measures. Finally, patterns of community organisation creates employment opportunities for local people to help guide fish-watchers (tourists) to ideal species rich locations and for basic education on principles of ecology, conservation and environmental awarness.

knowledge on food preferences on 143 fishes can be used to formulate artificial feeds, provide desired natural food for cultured and ornamental fishes. Based on preferred food items, the chemical composition of the prey can be determined to create artificial attractants that can enhance feeding in captive fish and lure fish for capture. Monitoring of the prey of indicator species speaks indirectly on the status of the environment, thus finding uses for this study in conservation and management.

Data on aspects of spawning and maturity help in monitoring the sizes of mature fish and their sex-ratios for the purpose of implementing management measures if a particular stock is over-exploited or their selection for trials on breeding based on the frequency of spawning and spawning seasons, the recruitment of larvae and occurrence of juvenile fish can be predicted for seed collection of reef fish can be spotted and used for capture in the case of an under-exploited stock, whereas the same information can also be used to regulate an over-exploited stock. Knowledge on the occurrence of juveniles can not only help in seed collection for culture, but also for collecting sub-adults for ornamental fish trade and declaration of protected fish nursery zones.

As the status of Indian coral reef fishery can be stated as under-exploited, a knowledge of the fishing methods used can be utilized to develop efficient habitat-suitable gear to enhance fish production from such region. The success of certain fishing gear in some specific areas can prove to be successful in other areas with similar topography. As most of the traditional methods seem to be developed based on fish behaviour, it would be necessary for the developmental measures to give prime importance to such characteristics of fish catching methods.

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IMPACT ASSESSMENT OF BIOCIDES ON MICROALGAE : A STUDY *IN VITRO*

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Introduction

The impact of some commonly used biocides on three microalgal cultures was investigated. The two unicellular algae selected are of importance for mariculture as larval and post-larval food in hatchery systems. The fresh water algae collected from paddy field, formed a mixture representing a natural population.

Obejectives

In order to find out the effective concentration of biocides that would inhibit the growth of algae by 50%, 96 hour bioassay tests were conducted. The number of cells and rates of carbon production were determined after 24, 48, 72 and 96 hour, and they were compared with that of control. The variations in pigment contents were also estimated. The significance of using three parameters is that one parameter does not give accurate results.

Results and discussion

The results of 96 hour bioassay tests revealed that there was variation in growth responses of microalgal species with three biocides. In almost all cases the rate of carbon production was found to be more affected than other parameters. It was also revealed that EC_{50} values varied with the duration of experiment.

The 96 hour bioassay tests did not give a real picture of the impact of various biocides. Therefore, long term experiments were conducted to understand the effect of different biocides on physiological and biochemical characters of three microalgal cultures. Each experiment was of 20 days duration. A total 21 sets of experiments were conducted.

Among the physiological parameters, growth, rate of carbon production, pigments such as chlorophyll *a* and *b* in green algae and chlorophyll *a* and *c* in brown algae as well as carotenoids were estimated during the peiod of experiments lasting 20 days. Biochemical parameters such as protein and carbohydrate contents were also estimated.

The five different biocides selected were of common use in agricultural fields. Hence their residues may be ultimately reaching the aquatic ecosystem. In nature mixtures of biocides occur and so combinations of five different biocides were also tested.

Due to the low water solubility in the case of 'B.H.C' and 'Carbaryl sevin', acetone was used as the solvent. Results showed that acetone has no stimulatory or inhibitory effect on microalgal culture.

The growth of algae was found to be affected by the presence of biocide. The effect of different concentrations was specific for each biocide. The lower concentration of most of the biocides caused a stimulatory effect especially in *Tetraselmis gracilis*.

The percentage inhibition of algal growth was found to increase with increased concentration of biocide. It was also observed that almost all biocides showed maximum toxic effect in the culture at the commencement of application. With the ageing of culture the toxic effect decreases. So it can be concluded that most of the biocides show an immediate shock response followed by a habituation response.

The results of experiment with mixed culture of fresh water algae showed that species composition was affected. In the natural population of phytoplankton, the chlorophycean members were found dominating *Chlorella ovalis* was the dominant species. The other species especially *Nitszchia longissima* was only nominally represented and this species was found seriously affected by biocide treatment.

The rate of carbon production was found to be affected by biocide treatment. In this case also lower concentration of some of the biocides showed stimulation in carbon production.

But the percentage stimulation was less compared to that observed for growth of microalgae.

With the increase in the concentration of biocide, the carbon production was inhibited. The maximum inhibition was observed at the beginning of the experiment. Compared to gross carbon production, net carbon production was less. The difference in gross and net canbon production may be due to the fact that, at higher concentrations of biocide treatment, due to increased respiratory rate, the reserve food material is completely metabolized and that leads to decrease in net production. The percentage inhibition in carbon production was found decreased with the age of culture.

The pigment content was also affected by biocide treatment. At lower concentrations the chlorophyll pigment was stimulated especially in *T. gracilis* and mixed culture of fresh water algae. The higher concentrations showed very low values for pigments especially at the beginning of the culture.

In the case of control cultures, after an exponential phase, the chlorophyll pigments decreased, but carotenoid content increased. But a reverse condition was observed in treated cultures especilly at higher concentrations. In the case of *D. inornata* the pigment content was very much affected.

Another peculiarity observed was that even though the treated cultures show inhibitory response with respect to pigment, the percentage inhibition was less compared to photosynthetic rate. It shows that chlorophyll activity was inhibited. Among the estimated pigment, dead chlorophyll may also come.

The biochemical products such as proteins and carbohydrate of algae were found affected by biocide treatment. In the case of control cultures, the protein content was found to decrease with age of culture and carbohydrate content was found to increase. The lower concentration of some of the biocides tested showed stimulation of proteins and carbohydrates. But the percentage stimulation was high with respect to carbohydrate content. This showed that carbohydrate was resistant to biocides than protein. With the increase in the concentration of biocides, the protein and carbohydrate

content was found inhibited. One peculiarity observed with Nuvacron treatment is that in the case of *T. gracilis* the protein synthesis was not much affected even at 100 ppm concentration of Nuvacron. This may be because of the fact that, the Nuvacron undergoes degradation and these metabolites may act as nutrients in the medium. These increased nutrient uptake may ultimately leads to increased protein content. Thus it can be concluded that there was a relationship between the available nutrients and protein synthesis in microalgae.

Morphological deformities like cell wall breakage, chloroplast disarrangement, gigantism of some of the cells, and even clumping of cells were also observed as a result of biocide treatment.

The results of bioaccumulation studies revealed that the estuarine form of an alga *T. gracilis* had an ability to absorb the organochlorine insecticide from the medium and got accumulated in it. The degree of accumulation was found to increase with the concentration of toxicant in the medium and longer duration of contact. It was observed that nearly 86% of the toxicant present in the medium was concentrated by the alga at 4 ppm concentration.

Statistical analysis of data was done using analysis of variance. In most cases the variation in growth responses of algae as a result of biocide treatment were highly significant.

Out of the three microalgal cultures investigated the estuarine form *T. gracilis* was found to be resistant species. Because of this character and also due to the capacity to accumulate toxicant this alga can be recommended for waste water treatment as scavengers.

Among the different biocides, the fungicide - Cuman $L^{(R)}$ was found most toxic. The organophosphate insecticide Nuvacom was less toxic and because of its highly degradable nature it can be recommended for field use.

The combination of biocides showed more toxicity than individual biocides, except the fungicide Cuman $L^{(R)}$

It was observed that the lethal limit of five different biocides tested here was low as compared to the concentrations

of biocides that farmers are applying in the field. So caution is advised in applying different biocides in the field.

The above findings revealed that the interaction between different biocides and the microalgae could play a significant role in the alterations in aquatic ecosystem.

Further research is needed especially in the case of biodegradation and bioaccumulation characters of microalgae. This area should focus on the identification of kind of algae that take up different pollutants into their cells and how the pollutants are degraded or are passed on to consumer organisms.

With the liberalization of global trade and tariff it is possibe that several toxic pesticides might reach the country in the name of plant protection and increasing agricultural production. Some of this algae can remain as very useful sentinels for protecting the ecosystem from superfluous toxicity and bioaccumulation.

The protection of aquatic resources requires the quantities of biocides used to be held to the minimum possible. It is hoped that the use of natural biocides and also by adopting the biological control of pests would provide an answer for a safer environment in future years.

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MICROBIAL PRODUCTION OF ANTIBIOTICS FROM MANGROVE ECOSYSTEM

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Introduction

The mangrove ecosystem comprises a group of floristically diverse trees and shrubs which characterise the intertidal vegetation of many tropical and subtropical areas. Mangroves are one among the several specialised marine ecosystems in which the productivity at different trophic levels and energy flow assume unusual importance as it has direct influence in enriching the inshore environment (Heald and Odum, 1962, *Proc. Gulf. Caribb. Fish. Inst.*, **22**, *Ann. Sess*, pp. 130 - 135).

The mangrove ecosystem is one of the most productive in the world and plays an important role in the ecology of nearshore waters. Mangrove swamps comprising of foliage as a major organic material, support a detrital type of food chain in the tropical marine environment (Odum and Heald, 1975, *In* : L. E. Cronin (Ed.) *Estuarine Research.* Academic Press, New York, 1).

Mangrove swamp forests are complex ecosystems that occur along intertidal accretive shores in the tropics dominated by estuarine trees, they draw many of their physical, chemical and biological characteristics from the sea, inflowing freshwater and upland forests. Mangrove swamps serve as ecotones between land and sea and elements from each other are stratified both horizontally and vertically between the forest conopy and subsurface soil (Gerald and Walsh, 1974, *In* : R. Reimold and W. Queen (Ed.) *Ecology of halophytes.* Academic Press, New York, pp. 63).

In recent years there is steady increase in awareness of mangrove's ecological significance and benefits to mankind. Many aspects to this ecosystem are still unknown like the

distribution of antagonistic actinomycetes. Limited investigations are being made in India on ecology, phytogeography, microbiology, forestry, etc. of mangrove ecosystem.

Objectives

The principal interest of the present investigation was to determine seasonal variations of antagonistic actinomycetes in selected mangrove ecosystem. The microbial interrelationship in mangrove sediiments was found out by constructing the ratio between bacteria and actinomycetes, bacteria and fungi, and fungi and actinomycetes. In addition temperature, pH, salinity, dissolved oxygen and organic carbon were determined seasonally and their possible relationship was statistically analysed and the results are presented in this write-up. Isolated actinomycetes were subjected to cross streak assay to know their nature of antibiotic activity against test fish pathogens and crude antibiotics were extracted from selected isolates. Their inhibitory activity was studied and the results discussed.

Materials and methods

The present study was carried out for a period of one year from January to December 1991 in four selected stations *viz.* Managalavanam, Narakkal, Puthuvyppu and light house area of Puthuvyppu. Though much emphasis has been given to the occurrence and distribution of actinomycetes, an attempt was also made to understand the distribution patterns of other microflora in the sediments. Data on physico-chemical parameters were also collected to find out their relationship, if any with the microflora.

Results and discussion

Seasonal distribution of actinomycetes showed that monsoon recorded the highest number of actinomycetes. The actinomycetes ranged between 1.0 and 243 x 10^4/gm in monsoon, 1.0 - 111.0 x 10^4/gm in postmonsoon and 1.0 - 13.0 x 10^4/gm in premonsoon.

Whenever number of actinomycetes increased, number of genera of actinomycetes decreased. It was also found that whenever number decreased, type of genera increased.

Streptomycetes was always found predominant in all the selected stations sampled especially during pre-monsoon season.

The isolates usually had broad salinity and temperature spectra. Analysis of variance of the microflora studied (bacteria, fungi and actinomycetes) did not show any significant difference between the seasons or stations.

Distribution of bacteria showed a positive correlation with B/F and B/A ratio in all the four mangrove stations studied.

Distribution of fungi showed a positive relationship with F/A ratio at all the four stations and at the light house area of Puthuvyppu the fungal distribution had a negative correlation with B/F and B/A ratio.

Negative correlation was found between the distribution of actinomycetes and B/A, F/A ratio at Mangalavanam. Distribution of actinomycetes at Narakkal showed a positive correlation with pH of water, pH of sediment, organic carbon and a negative correlation with B/A. At Puthuvyppu distribution of actinomycetes did not show any relation with any of the parameters studied. Positive correlation existed between total actinomycete and total bacteria at the light house area of Puthuvyppu.

Positive correlation at 5% level was found between bacteria and actinomycete at Puthuvyppu and light house area of Puthuvyppu in the post-monsoon season. The pooled data for all the season showed positive relationship with 5% significant difference at light house area of Puthuvyppu for total counts of bacteria and actinomycetes.

Direct relationship was found between fungal and actinomycete counts at 5% level in the pre-monsoon season at Mangalavanam and Narakkal and during monsoon at Narakkal, Puthuvyppu and light house area of Puthuvyppu.

1591 actinomycetes were retrieved from the four fixed mangrove stations. Maximum number [427 (44.71%)] of actinomycetes were obtained from Narakkal during monsoon. In the post-monsoon season maximum number of

actinomycetes [183 (33.83%)] were recorded at Narakkal and 35 (36.84%) was the maximum number of actinomycetes recorded during pre-monsoon at Mangalavanam.

Among 1591 actinomycetes encountered, five colour series were recorded *viz.* white (42.50%), grey (52.80%), red (4.40%), orange (0.25%) and green (0.06%). Grey colour series dominated followed by white colour series in abundance. 62.62% of grey coloured actinomycetes were encountered during the period of monsoon. Only one green coloured actinomycete was observed throughout the period of study.

Five selective media were used to retrieve actinomycetes *viz.* oat meal agar, Grein and Meyer's agar, glucose asparagine agar, Kuster's agar and sea water agar. Out of these 5 media used sea water agar was found to be best for isolation and maintenance of actinomycetes.

Out of 1591 actinomycetes encountered 104 cultures were isolated and maintained in sea water agar. Among 104 actinomycetes isolated 26 were from Mangalavanam, 26 from Narakkal, 27 from Puthuvyppu and 25 from light house area of Puthuvyppu.

Maximum number of actinomycetes were isolated during pre-monsoon 55 (52.89%) followed by 27 (25.96%) during monsoon and 22 (21.15%) during postmonsoon.

White colour series dominated among the isolates 60 (75.69%) followed by grey coloured series 29 (27.89%), red colour 10 (9.62%), orange colour series 4 (3.85%) and only one (0.96%) green coloured actinomycete, 50% of the isolated actinomycetes were subjected to characterization according to the methods recommended by ISP and identified upto species level. Among 18 *Actinomycetes* identified 10 species were recorded and 24 species were identified out of 35 *Streptomyces* cultures.

In order to characterize the actinomycetes, aerial mass colour, spore and sporophore morphology, pigment production and carbon utilisation were carried out and the species description of 52 actinomycetes were outlined.

White, grey and red were the predominant colour series observed in the isolated actinomycetes. Rectiflexible (RF); Retinaculiaperti Spiral (RAS); Spiral (S) and RARF were the four types of sporophore morphology recorded, among which (57.96%) RF type dominated. Spore-morphology of actinomycetes can be grouped into smooth, spiny, warty, hairy and rough type, however only smooth and spiny type were recorded during the present study.

D-glucose, L-arabinose, D-xylose, i-inositol, D-mannose, D-fructose, rhamnose, sucrose and raffinose were selected to study the carbon utilisation of the actinomycetes in order to identify them upto species level. Only glucose was utilised by all the actinomycetes identified. Most of the isolates were not able to utilise either L-arabinose, D-xylose or D-fructose. Only few were not able to utilise D-mannose. About 30% of the isolates were not able to utilise either i-inositol, rhamnose or raffinose. Only 50% of the isolates were able to utilise sucrose.

Sodium chloride tolerance test was carried out to know the origin of the isolates. It was found that most of the isolates (46.15%) were able to tolerate sodium chloride upto 3% level. It was also noted that, most of the actinomycetes studied exhibited (53.85%) good growth even when sodium chloride and sea water was omitted in the medium.

The antagonistic activity was tested using 14 test pathogens *viz. Vibrio anguillarum, V. cholerae, V. alginoliticus, V. parahaemolyticus, Aeromonas, Pseudomonas, Salmonella-I, Salmonella-II, E. coli, Bacillus, Staphylococcus, Rhodotorula rubra, R. marina* and *Cladosporium.* All the cultures tested showed antagonistic activity towards one or more of the test pathogens.

Out of 104 actinomycetes tested for their antimicrobial activity, about 56% exhibited antagonistic effect towards Gram-negative bacteria, 35.6% towards Gram-positive bacteria. 100% of the isolates were able to inhibit the growth of the filamentous fungi *(Cladosporium)* and 90% of the isolates were antagonistic towards non-filamentous fungi *(R. marina* and *R. rubra).*

Antibiogram of the actinomycetes against test pathogens showed that two extreme zones (Category I and Category X) of inhibition was exhibited by most of the antagonistic actinomycetes.

Six isolates exhibiting different antimicrobial activity were selected and mass cultured for the extraction of crude antibiotics.

It was found that a few test pathogens which were resistant to antibiotics were sensitive when tested with their culture extracts.

pH 4.0 was found to be suitable when ethyl acetate was used as a solvent for the extraction of antibiotics and pH 7.0 was found to be optimum when ethyl ether and chloroform were used.

From the present study it was found out that most of the isolates (56%) were able to inhibit Gram-negative bacteria and all the isolates were able to inhibit the filamentous fungi *Cladosporium.*

Extracted antagonistic compounds were able to inhibit most of the pathogens tested exhibiting the broad-spectrum antibiotic activity. These studies considerably enhances our knowledge of distribution of antagonistic actinomycetes in mangrove environment of Cochin which plays a significant role in the economy of the sea. Further studies in the isolated antagonistic compounds are needed in order to produce new antibiotics effective against fish pathogens.

MANUALS OF RESEARCH METHODS AND SPECIAL PUBLICATIONS ISSUED UNDER THE POSTGRADUATE PROGRAMME IM 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 considerations of shellfish sanitation. *CMFRI Spl. Publ.*, **16**, 1984, 100 pp.

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

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

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

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

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

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

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

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

17. Mariculture research under the Postgraduate Programme in Mariculture, Part 2. *CMFRI Spl. Publ.*, **53**, 1993, 176 pp.

18. Mariculture research under the Postgraduate Programme in Mariculture, Part 3. *CMFRI Spl. Publ.*, **54**, 1993, 155 pp.

19. Mariculture research under the Postgraduate Programme in Mariculture, Part 4. *CMFRI Spl. Publ.*, **55**, 1993, 134 pp.

20. Mariculture research under the Postgraduate Programme in Mariculture, Part 5. *CMFRI Spl. Publ.*, **56**, 1993, 154 pp.

21. Mariculture research under the Postgraduate Programme in Mariculture, Part 6 *CMFRI Spl. Publ.*, **61**, 1995, 122 pp.