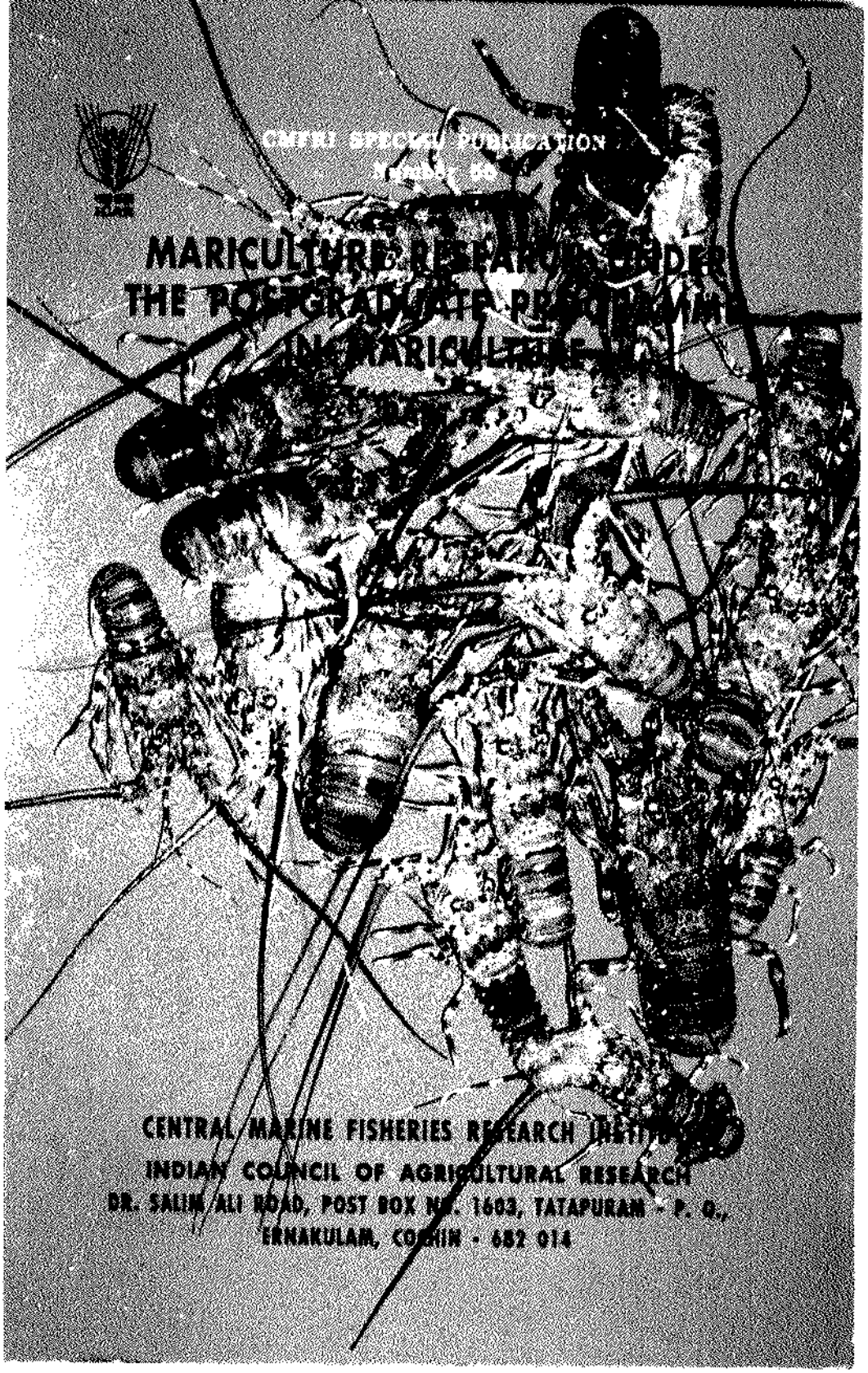

CMFRI SPECIAL PUBLICATION

Number 80

MARICULTURE RESEARCH UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
DR. SALIM ALI ROAD, POST BOX NO. 1603, TATAPURAM - P. O.,
ERNAKULAM, COCHIN - 682 014

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

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

— improve the quality of postgraduate education in mariculture;

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

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MARICULTURE RESEARCH UNDER THE POSTGRADUATE PROGRAMME IN MARICULTURE

PART 4

CMFRI SPECIAL PUBLICATION
Number 55

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
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NUTRITIONAL EVALUATION OF CHICKEN ENTRAILS AS PRAWN FEED FOR *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Aquaculture as an economic means for low-cost production of protein food is important. Culture of organisms in an aquatic system needs attention because of its capability of counteracting against unemployment, malnutrition, undernourishment and poverty in rural areas. Nutrition of prawns is a major impediment in culture operations, either due to non-availability of nutritious feeds or due to its high cost.

With the increase in meat production and consumption in India, slaughter-house and poultry wastes particularly cast-off chicken entrails, rich in protein is recommended as a potential supplement in prawn feeds (Mohapatra *et al.* 1991, *Natl. Semi. Shrimp Seed Prod. & Farm.*, Abst., p. 54). The present study was aimed at evaluating chicken entrails as prawn feed in the fresh and compounded forms.

Material and methods

Fresh and pelleted chicken entrails were given as experimental diets to the prawns. Fresh clam meat was used as control for comparison. Proximate analyses of the compounded feed and the individual feed ingredients were carried out to estimate dry matter (DM), ether extract (EE), crude protein (CP), ash, crude fibre (CF) and nitrogen free extract (NFE) following AOAC (1984, *Official methods of Assn. of Agri. Chem.*, Arlington, 7.025 - 7.032) methods.

The three feed treatments were :

Treatment I - fresh entrails

Treatment II - compounded diet with entrails

Treatment III - control (fresh clam meat)

Five replicates were maintained for 36 days. The stocking size of prawns were 16.0 mm in length and 30.0 mg in weight. Acclimatisation period was 5 days. Feeding frequency was twice a day at 20% of biomass. Unconsumed food and faeces were removed separately, dried and weighed. Length and weight of the prawns were recorded initially and subsequently on every sixth day on last day of the experiment. Hydrobiological parameters were maintained at optimum levels. Pearson's square method was used to compute a nutritionally balanced feed incorporating chicken centrails to achieve 40% protein in the final mix. Other ingredients *viz.* prawn meal, groundnut oil cake, wheat bran, tapioca powder, cod liver oil, vitamins and minerals were added on percentage basis. Indicator (chromic oxide) was added in the pellets to study its' digestibility. Water stability of pellets was also studied.

To study food conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency, K_1%, K_2%, weight gain, protein gain, growth and apparent digestibility; close monitoring of length, weight, moults, mortalities, amount of unconsumed food and faeces were done.

Results were subjected to statistical analysis by ANOVA and tested for significance.

Results

The weights in grams of fresh meat, raw entrails and cleaned entrails were 1241.44 (± 50.391), 93.4 (± 9.107) and 48.498 (± 3.043) respectively. Moisture content of chicken entrails was 80.307% (± 2.960); crude protein was 51.477% (± 0.031) in the dry condition and 42% (± 0.520) on boiling.

Proximate chemical composition of the three feed treatments I, II and III are given below :

	DM	CP	EE	CF	Ash	NFE	GE
Treatment I	19.693 ±0.025	51.477 ±0.031	26.05 ±0.227	0.0022 ±0.003	5.25 ±0.25	17.221	607.624
Treatment II	—	40.00	13.682 ±0.217	7.732 ±0.210	15.839 ±0.220	22.247	547.453
Treatment III	18.00	60.605 ±0.002	18.50 ±0.681	0.728 ±0.681	6.800 ±0.005	13.367	572.048

Pellets showed maximum leaching in the first hour itself *i. e.* 66.541% (± 0.02). But negligible loss occurred over the next four hours.

Build-up ammonia was fastest in the case of fresh entrails from 0.01 NH_3-N mg/l at 0.00 hrs to 0.1 NH_3-N mg/l at 1000 hrs. In the control the increase was from 0.01 to 0.08 NH_3-N mg/l and with componded diet it was from 0.01 to 0.065 NH_3-N mg/l in the same time period.

Length, wet weight and dry weight variations during the period of experiment are given below :

Particular	Treatment I	Treatment II	Treatment III
Absolute growth in length (mm)	10.131 ±0.765	22.222 ±1.031	15.438 ±0.337
Absolute growth in wet weight (mg)	63.179 ±0.982	117.204 ±2.297	99.646 ±1.685
Relative growth	2.077 ±0.120	3.852 ±0.126	3.346 ±0.047

Survival rate was highest with compounded diet 94.333% (± 4.633) and the least with fresh entrails 79.333% (± 11.148). Control had a survival rate of 88% (±10.431). Compounded diet gave the best overall moulting rate, followed by control.

FCR value was the least efficient for fresh entrails 4.815 (± 0.228). FCR value of compounded diet was the best, 2.463 (± 0.149) and control gave an FCR of 3.899 (± 0.147). PER values of Treatment I, II and III were 0.065 (± 0.002), 0.116 (± 0.003) and 0.060 (± 0.002) respectively. Conversion efficiency of fresh entrails, compounded diet and control were 20.8%, 40.6% and 25.7% respectively. The cost per kilogram of the 3 feeds were Re 1/- for fresh entrails, Rs. 7/- for compounded diet and Rs. 10/- for clam.

Apparent protein percentage of Treatments I, II and III were 13.103 (± 0.338), 73.199 (± 4.114) and 22.988 (± 1.049) respectively. K 1% of the same were 0.648 (± 0.016), 1.191 (± 0.181) and 0.795 (± 0.033). Gain in protein was highest in compounded diet fed group, 660.267 mg (± 35.217). Minimum gain was obtained with fresh entrails 103.227 mg (± 1.526). Control group gained 311.158 mg (± 7.349).

Dry matter digestibility of the Treatments I, II and III were 68.431 (± 0.355), 74.076 (± 0.093) and 73.204 (± 0.662). By the direct method, protein and lipid digestibilities respectively were 80.609 (± 0.218) and 50.354 (± 7.691) for fresh entrails, 89.015 (± 0.037) and 71.342 (± 0.097) for compounded diet and 82.209 (± 0.432) and 77.128 (± 0.567) for control. By the indirect method, protein and lipid digestibilities of the pelleted diet were 91.52% (± 0.014) and 77.88% (± 0.124) respectively.

Statistically, significant difference existed between the effect of the three feeds, with compounded diet being highly significant at 1%.

Discussion

The crude protein content of 51.477% for chicken entrails is in accordance with 52.9% for chicken viscera as reported by New (1987, *FAO, ADCP/REP/87/26*). The low cost - high protein content combination of chicken entrails forms a major attractant for its choice as shrimp feed supplement.

Colvin (1976, *Aquacult.*, **16** : 199-209) had reported a growth rate of 44 mg/day with initial weight of 0.95 g for a diet with 43% protein in *P. indicus*. Ali (1982, *Proc. Symp. Coastal Aquacult.*, **1** : 321-328) reported 23 mg/day from a stocking size of 0.2 g for the same species fed with 50% crude protein content. With fresh entrails, compounded diet and control in the present study, growth rates were 1.757 mg/day, 3.313 mg/day and 2.768 mg/day from an initial weight of 0.03 g for the same species fed on a compounded diet containing 40% protein. The results are comparable, taking into consideration the protein contents and initial weights. Results show compounded diet as most growth promoting, underlining the fact that one feed material alone may not be self-contained and nutritionally balanced.

Kanazawa *et al.* (1970, *Bull, Jap. Soc. Sci. Fish.*, **36** (9) : 949-954) had reported the growth rates with 4 diets containing components of silk-worm, chinook salmon, brine shrimp and short-necked clam each at 20%, 25%, 63% and 72% on *P. japonicus*. In comparison growth rates for fresh entrails, compounded diet and control were respectively 175.7%, 331.3% and 276.8% and were several times higher.

Sick *et al.* (1972, *Fish. Bull.*, **70** (1) : 101-108) had shown survival rates of 80-90% when the stocking density was 10 animals/m^2. The survival rates for fresh entrails, compounded diet and control at the density of 10 animals/0.8 m^2 were 79.333%, 94.33% and 88.0% respectively, showing all of them comparable to aforesaid findings. Among the diets, fresh entrails showed a greater incidence of mortality. This may be due to higher levels of ammonia build-up in water for the treatment.

Sick *et al.* (1972, *loc. cit.*) had reported an FCR of 5.5 with semipurified pelleted diets for penaeid shrimp. FCR values of fresh entrails, compounded diet and control in the present study were 4.815, 2.463 and 3.899 respectively. In this superiority of compounded feed is obvious. An FCR of 4.185 and high levels of NH_3-N concentration in water under controlled experimental

conditions in the present investigation need not rule out the applicability of chicken entrails in shrimp culture. On the contrary, in extensive culture systems where stocking density is low and supplementary feeding augments natural productivity, an empirical input of oil cakes and bran if possible blended with entrails should prove effective in terms of yield per unit area, though detailed investigations are needed in this direction to draw any definite conclusion.

Sambasivam *et al.* (1982, *Proc. Symp. Coastal Aquacult.*, **1** : 406-408) working on *P. indicus* observed high conversion efficiency and growth rates in diets having 60% protein and highest survival with 50% protein. Highest survival of 94.33% and conversion efficiency of 40.6% were obtained with compounded diet having 40% protein content.

Among the diets, compounded feed had the best values for PER, protein gain, apparent protein percentage and apparent productive protein value during the study. This indicates better efficiency of the protein fraction of the particular treatment.

Raman *et al.* (1982, *Proc. Symp. Coastal Aquacult.*, **1** : 337-343) had observed in juvenile penaeids that diet containing prawn waste gave in FCR of 12.94 and cost of production for 1 kg of prawns amounted to Rs. 11.35. Similarly, FCR for bajra was 37.08 and production cost worked out to Rs. 38.56/kg of prawn. In comparison the cost efficiency of fresh entrails, compounded diet and control were Rs. 4.82, Rs. 17.24 and Rs. 39.00 respectively per kg of prawn. Costwise fresh entrails is economical.

Tapioca was the binder chosen for the study, due to its availability, binding capacity, cheap cost and the added advantage of being a carbohydrate source. It gave about 65% water stability, comparable to that reported by Pascal and Tabbu (1979, *Quart. Res. Rep.*, **3** (1) : 1-6).

The apparent dry matter digestibility (ADMD) of fresh entrails, compounded diet and control were 68.43%, 74.076%

and 73.004% respectively. Akiyama *et al.* (1988, *Bull. Jap. Soc. Sci., Fish.*, 55 (1) : 91) however, obtained an ADMD of only 56.8% for shrimp meal with penaeid shrimps.

Chicken entrails, therefore provide a good source of protein for prawn feeds. It is a cheap source of protein and has no seasonality in its availability. It is better utilised in the pellet form, compounded with shrimp meal which serves as the attractant. It gives good conversion and protein digestibility (91.52%). Utilisation of entrails as prawn feed provide effective means of constructive waste disposal.

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A STUDY ON FLUCTUATION OF ZOOPLANKTON IN THE ESTUARINE WATERS AT COCHIN DURING MAY-SEPTEMBER, 1991

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Introduction

In a broader sense, plankton is considered as an index of fertility, as the fishery resources of any aquatic system mainly depend on the magnitude of phytoplankton and zooplankton production. These, in turn, are influenced by various physical, chemical and biological factors. Scrutiny of literature indicates that since 1976, little work has been done in the Cochin Backwater on the zooplankton fluctuation and abundance in relation to the environmental characteristics. The present investigation has been carried out in the Ernakulam Channel of the Cochin Backwater extending between the railway-cum-road bridge (in the south) and Fairway buoy, on the fluctuation and abundance of the dominant zooplankton groups in relation to the environmental parameters such as rainfall, temperature, salinity, dissolved oxygen, tide and freshwater flow during the southwest monsoon season commencing from May to September 1991.

Material and methods

The study area covered three zones - (1) the lesser saline zone (opp. shipyard), (2) the middle zone (between Fort Cochin and Vypeen Island) and (3) the higher saline zone (near Fairway buoy) in the Ernakulam Channel of the Cochin Backwater having a depth range of 6-10 m. Based on the preliminary survey conducted in May, three stations were fixed, one each in the three zones with a distance of about 5 km in between the stations. Weekly sampling was made regularly from the three stations from the surface, midwater and near-bottom during

0830-1030 hrs. Apart from weekly collections, monthly diurnal observations were conducted from 0700 to 1900 hrs to obtain bihourly data on hydrographic parameters and zooplankton and hourly data on tidal amplitude.

Hydrographic parameters were estimated by the standard methods. As poor sampling design is one of the serious defects in the studies of secondary production, the sampling method of zooplankton was standardised and the programme discussed in detail in the original Dissertation.

Bongo net and 0.5 m diameter nets were used. The samples collected were preserved in 5% formalin in separate bottles.

As the plankton samples from the backwater stations contained detritus, phytoplankton and decaying leaves, twigs, etc., the numerical estimation alone was considered in the present investigation.

Zooplankton were analysed qualitatively and quantitatively applying suitable formulae for both surface water and bottom water of the study area.

Considering their frequency of occurrence and numerical abundance (excluding unusual swarms) in the samples for June-September, groups such as copepods, chaetognaths, cladocerans, lucifers, medusae, decapod larvae and fish eggs and larvae were treated separately as individual groups. While the groups constituting less than 0.5% in total (excluding the blooming/ swarming samples) were included under 'others' in the present investigation.

Since the measurements were subjected to diurnal, micro-distributional and experimental sources of variability, as far as possible, individual values were not considered for the results and discussion. From the weekly data collected, fortnightly, monthly and season's average were estimated. Depending on the intensity of rainfall, the period of study was divided into

onset of monsoon (May), peak monsoon (June-August) and closure of monsoon (September) in the present investigation. The values obtained for the three stations were pooled together to get the average picture of surface and bottom waters in the Ernakulam Channel for different parameters. Linear correlation coefficients were worked out to examine the influence of hydrographic parameters on total zooplankton and the different zooplankton groups.

Observations

Rainfall : The Cochin Backwater had the local rainfall of 2600 mm during May-September 1991. The monthly rainfall were 80, 1492, 541, 433 and 54 mm from May to September showing the peak in June.

Water temperature : Ranged between 26.83 - 32.05°C in the surface and 24.87 - 95°C in the bottom. The mean values were 28.84 and 27.32°C respectively.

Salinity : The surface and bottom waters ranged between 1.18 - 30.83‰ and 9.66 - 34.51‰ respectively with a mean of 14.79‰ at surface and 26.32‰ at the bottom. There was generally a decrease in the surface salinity during June - August while during June - September there was occasional unusual increase in bottom salinity (>23.7‰). The overall average for the entire water column was 20.56‰.

Dissolved oxygen : At surface and bottom ranged between 3.32 - 4.82 and 1.32 - 3.93 ml/l with the mean of 4.04 and 2.76 ml/l respectively. The overall average for the entire water column was 3.40 ml/l.

Zooplankton : During May-September, marine, estuarine and freshwater members of different zooplankton groups were noticed. The groups of zooplankton encountered were copepods, cladocerans, chaetognaths, lucifers, medusae, decapod larvae, fish eggs and larvae, appendicularians, ctenophores, doliolids, dinoflagellates, isopods, ostracods, planktonic poly-

chaetes and siphonophores. Their abundance and fluctuation are discussed in detail elsewhere.

The total zooplankton number ranged from 720 - 4269/m^3 at the surface and 1264-4214 nos/m^3 at the bottom layer with the mean of 2242 and 2520 nos/m^3 respectively in the entire study area during May-September. The overall average for the entire study area was estimated as 2381 nos/m^3.

Plankton blooms/swarms : Blooms of diatoms and dinoflagellates, and swarms of decapod larvae and pelagic polychaetes were recorded in the study area. A dense swarm of decapod larvae (brachyuran zoea) was observed on 1-6-1991 amounting to 44140 and 57006 nos/m^3 in the surface and bottom layers at station 1. The other two stations did not show high values.

On 20.7.1991 a bloom of dinoflagellate *Noctiluca* was observed in the study area with the maximum number recorded in the surface at staion 1. The estimated values were 21765 and 5541 nos/m^3, 8344 and 1592 nos/m^3, and 10955 and 2802 nos/m^3 in the surface and bottom layers at stations 1, 2 and 3 respectively.

Abundance of cladocerans was noticed in the samples with highest number recorded at station-1. The estimated values were 2468 and 3333 nos/m^3, 1783 and 2962 nos/m^3 and 1035 and 604 nos/m^3 in the surface and bottom layers of stations 1, 2 and 3 respectively. Along with this, abundance of pelagic polychaetes to the extent of 462 and 844/m^3 was recorded at station 2 in the surface and bottom respectively. Along with these two, dense bloom of *Fragilaria* and *Ulothrix* were also observed especially in the middle zone (station 2).

Results and discussion

The total rainfall recorded for this season was more than 80% of the annual mean indicating good rainfall during the southwest monsoon season in 1991. The intermittant high and low values seemed to have direct or indirect influence on the hydrographic parameters and zooplankton fluctuation.

Reduction in water temperature was noticed in the surface layer during the peak monsoon months due to the influence of monsoon rainfall. During the peak monsoon months, the salinity values at the surface and bottom layers indicated vertical gradients especially at stations 2 and 3 with low value at surface, indicating the influence of freshwater flow at surface and tidal flow in the bottom layer. The relatively high values of dissolved oxygen recorded during peak monsoon months at the surface layer is attributed to the freshwater discharge and primary productivity.

The results indicated that the bottom waters occasionally registered unusually low values of temperature and dissolved oxygen and high salinity values during June-September which are characteristic features of the offshore bottom waters. The unuaual increase in the bottom salinity during the third week of June, first and third week of July, third week of August and first week of September with low values observed in the intermittant weeks at the middle zone in relation to the relatively low dissolved oxygen level and low temperature (in general); and the occurrence of the same trend in lesser magnitude at station 1 indicated the incursion of upwelled water into the backwater.

To check whether these high values of salinity in the backwater were due to tide, the bihourly diurnal data on salinity obtained at station 2 on a 'normal day' in June and July were examined. The diurnal data revealed that the salinity at the highest tide never exceeded 16.75‰ in June and 24.0‰ in July, whereas the bihourly diurnal data obtained on an 'upwelling day' (first week of September) showed the highest salinity value of 34.64‰ during high tide. It was clear from the values of the previous and succeeding weeks that the incursion of the upwelled water into the backwater was not a continuous process and the intensity varied from time to time and it might become weak or absent even within the fortnight itself and might start again during this season. Ramamirtham and Jayaramn (1963, *J. mar. biol. Ass. India*, 5 (2) : 170-177), Shynamma and

Balakrishnan (1976, *J. mar. biol. Ass. India*, **15** (1) : 391-398) and others have also reported the incursion of upwelled water into the Cochin Backwater during Southwest monsoon season.

The relatively low density of zooplankton number observed in the surface and bottom waters at station 2 (middle zone) than at the other two stations indicated high mortality rate at the middle zone by the relatively high mixing up of the low and high saline waters from the other two zones.

Considering the fortnightly mean of total zooplankton number, the fluctuation was considerably more at stations 1 and 2 in the surface and bottom water columns.

Copepods play a vital role among the zooplankton communities in the freshwater, estuarine and marine ecosystems. Their peak at station 1 during first fortnight of May, first and second fortnight of July and first fortnight of September in the surface and bottom waters indicated the succession of species of different salinity tolerences. Silas and Pillai (1975, *Bull. Dept. Mar. Sci. Univ. Cochin*, 7 (2) : 329-355) also have recorded this phenomenon. Station 2 also reflected almost the same trend. The bottom layer of the backwater stations seemed to show some relation in the copepod abundance corresponding to the increase in salinity during July and September by the incursion of upwelled water. But, in the higher saline zone, the surface waters showed an increasing trend from second fortnight of May to first fortnight of July with decrease in salinity. Menon *et al.* (1975, *J. mar. biol. Ass. India*, 13 (2) : 220-225) reported a similar increasing trend when salinity decreased. In the bottom waters, the increasing trend was noticed during the same period when the salinity was steady. These observations confirm that salinity is not the only factor to limit the copepod population in the estuaries during the monsoon season. Temperature and dissolved oxygen also did not seem to have any direct relationship with the fluctuation and abundance of copepods in the present investigation. These results indicated that the biological factors such as food availability, species composition, their life span and reproductive potential in space and time

might limit the population. This is in view with George (1958, *Indian J. Fish.*, 5 : 375-401). The copepod population has also been reported to be limited by the occurrence of other zooplankton groups (Menon *et al.*, 1971 *loc. cit.*, Madhupratap, 1987, *Bulletin of plankton Society of Japan*, 34 (1) : 66-81).

The distribution of chaetognaths was very much restricted in the backwater zone of the study area (stations 1 and 2) while it was evenly distributed in the surface of the higher saline zone. It was also seen that they were more during the onset and closure of monsoon indicating their affinity to higher salinity. This is in accordance with the findings of Srinivasan (1971, *J. mar. biol. Ass. India*, 13 (2) : 173-181). The remarkable increase in the bottom water could be related to the incursion of colder upwelled water into this region. A similar increase in chaetognath number has been reported by Menon *et al.* (1971, *loc. cit.*) and Vijayalakshmi (1971, *J. mar. biol. Ass. India*, 13 (2) : 226-233).

The relatively less number of cladocerans during May and June in the bottom at all stations, its reduction from station 1 to station 3 in the surface with increase in salinity (24-32‰) and progressive increase in number observed during August-September indicated their preference to the estuarine habitat. A similar trend was noticed by Menon *et al.* (1971, *loc. cit.*), but the density of cladocerans was high during the closure of monsoon.

Lucifers were present in all the fortnightly samples of surface and bottom layers during the southwest monsoon period with their maximum recorded during the onset of monsoon. George (1958, *Indian J. Fish.*, 5 : 375-401) and Madhupratap and Haridas (1975, *Indian J. mar. Sci.*, 4 : 77-85) have recorded the absence of lucifer from July onwards in the southwest monsoon season. Their unusual occurrence at intervals during June-September might be attributed to the incursion of upwelled water into the backwater at intervals.

The distribution and abundance of medusae during the beginning and closure of monsoon when the salinity was

relatively high revealed their affinity to saline condition. This is in confirmity with the observations of Santhakumari and Vannucci (1971, *J. mar. biol. Ass. India*, **13** (2) : 211-219). In general, medusae were relatively more at the surface layer.

The results revealed that the decapod larvae were generally abundant during the onset of monsoon especially at station 1. From the record of dense swarms of brachyuran larvae in the lesser saline zone, it appeared that the initial monsoon rainfall occurred in May resulting in the sudden changes in salinity and other water characteristics might have triggered the spawning of brachyuran decapod in the backwater environment. Their absence at stations 2 and 3 as well as in the next weekly observation at station 1 indicated heavy mortality of these larvae as the result of heavy rainfall in June. Menon *et al.* (1971, *loc. cit.*) reported a similar trend. George (1958, *Indian J. Fish.*, 5 : 375-401) recorded the minimum number in June which could be attributed to the fluctuation in the onset of monsoon during the respective years of investigation.

The distribution of fish eggs and larvae showed wider fluctuations between surface and bottom layers and among stations. The results revealed that their abundance is more related to the habitat of the breeder. This is in accordance with the findings of George (1958, *loc. cit.*). The relatively high number at station 1 and less number at station 3 during the onset of monsoon revealed that these eggs and larvae might belong to the estuarine species or group. The abundance of fish eggs and larvae at station 3 during August at surface and bottom waters and their occurrence at the bottom waters at station 1 and 2 in considerable number indicated that they might belong to a demersal species or group and their transport from the sea to the backwater could be due to influence of coastal upwelling currents as evidenced from the hydrographic features and by tidal flow.

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PRODUCTIVITY AND PROFITABILITY OF PRAWN FARMING PRACTICES - AN ECONOMIC ANALYSIS

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Introduction

Among various existing aquaculture systems, prawn culture is the most developed and is the dynamic field receiving widespread attention. Most of the current prawn culture practices are at an elementary level and husbandry methods are only recently evolved. The technology followed by most of the prawn farms are evolved by trial and error rather than scientific research.

In the last two decades considerable progress has been achieved in the field of prawn farming, but there is a dearth of information regarding economics of various culture practices. The existence of various culture practices and the reluctance of traditional prawn farmers to adopt the semi-intensive culture methods has made it imperative to conduct a comparative economic analysis. The present investigation was carried out with the following specific objectives : (i) to assess the productivity and profitability of different types of prawn culture practices, (ii) to analyse the comparative economic efficiency of prawn farms based on their location, size and technique, and (iii) to estimate the input-output relationship for prawn production in the semi-intensive culture system.

Material and methods

Prawn farms representing different culture systems were randomly selected from various representive regions such as Narakkal, Kadamakkudy, Maradu and Kannamaly in Ernakulam District of Kerala and Tuticorin in Tamilnadu. A total of 82 farms with a coverage of 375.6 hectares were observed.

Seasonal farms (195 ha), perennial farms (132.5 ha) and semi-intensive farms (91.8 ha) were selected for the study.

Based on the preliminary investigations, carried out on prawn farming in Ernakulam and Tuticorin regions, 3 types of schedules were prapared, pre-tested and used for the collection of data. Schedule I gathered basic information regarding the prawn operations whereas schedule II and III were used to get the information regarding the costs and earnings of the traditional and semi-intensive culture systems respectively.

Prawn farms were classified based on their location, size and technique. Based on their size, farms were divided into marginal (less than 2 ha), small (more than 2 ha and less than 10 ha) and large farms (more than 10 ha).

An input-output relationship was also worked out using Cobb-Douglas production function to find out the efficiency of individual inputs, their influence on the total production and to estimate the maximum profitable level of input application.

Marginal Physical Product (MPP) was calculated to find out whether the use of each variable input were optimal in terms of maximising this profits.

$$MPP = b.\frac{\bar{y}}{\bar{x}}$$

Where MPP is the marginal physical product of the input, b is the partial elasticity of production of the input, $\bar{y}$ is the estimated production at geometric mean of all inputs $\bar{x}$ is the geometric mean of the input.

By comparing the MPP with the input-output ratio and solving for the variable, input, the maximum profitable level per hectare of area, stocking density, feed and labour worked out.

Results and discussion

Seasonal prawn farming in paddy fields : The practice of growing prawns in rice fields on a commercial scale is an age old practice in Kerala. Paddy is raised from June to September

and prawn is cultured during November to April. Once the paddy is harvested, smaller holdings of the paddy fields are pooled together and leased out to contractor for prawn filtration for a period of 5 months. The lease value of the farms vary from place to place, year to year and highly influenced by the market demand and price of prawns.

The average operating cost per hectare was found to be Rs. 7021 and about 64% of the operating expense was labour cost and the maximum number of man-days were required for the preparation and maintenance of the pond followed by harvesting and watch and ward.

The average catch of the seasonal farm was found to be 1947 kg. The catch was comprised of *M. dobsoni* (51%), *P. indicus* (20%), *M. monoceros* (6%), *P. monodon* (0.2%), fishes (22%) and crabs 0.5% of the catch. The initial phase of the culture period is dominated by *M. dobsoni* and the later half by *P. indicus*.

About 50% of the total revenue was realised from *P. indicus* followed by *M. dobsoni* (32%), *M. monoceros* (6%), *P. monodon* (1%) and fishes (10%), and profit was Rs. 11,658 per hectare. The production cost per kilogram of the product was Rs. 17.5 whereas the profit margin of the product was Rs. 11.1.

The production and profit were found to vary from place to place. Among the three centres selected for the study, seasonal farms at Narakkal (1232.5 kg) has given the maximum production followed by those at Kadamakkudy (1155 kg) and Maradu (538.8 kg). This decreasing production is attributed mainly to the increasing distance from the barmouth. However the lower production at Maradu may also be due to the release of industrial effluents from the fertilizer plants and paper mills.

The profit per hectare was Rs. 11412 at Narakkal, Rs. 12935 at Kadamakkudy mainly due to the lower lease value and Rs. 9807 at Maradu.

Marginal farms (1108 kg/ha) was found to be more productive than small (1051 kg/ha) and large farms

(1031 kg/ha). The average profit per hectare was found to be maximum in marginal farms (Rs. 13834) followed by small (Rs. 13604) and large farms (Rs. 12260). More production in the marginal could be due to better management procedures like additional stocking of seeds collected from the wild and periodic feeding in small quantities.

About 60% of the farms in the surveyed area were found to be stocking seeds of *P. indicus*. The economic efficiency of seasonal farms which were stocking additional prawn seeds (A) and those depending only on the natural entry of seed (B) were analysed. The average production per hectare in the former was 1163 kgs and 952 kgs respectively. The percentage prawn production in farm A was 80% and that of farm B was 74% of the total catch. The contribution of *P. indicus* to prawn catch was more in farm A (28.8%) than in farm B (13.8%). The average net profit per hectare was worked out to be around Rs. 18448 in farm A and Rs. 7330 in farm B.

From the above result, it is evident that the current production and profit of the seasonal pokkali fields can be increased by the additional stocking of prawn seeds. The increment to the total production by the additional stocking at Narakkal and Maradu were 4.9 kg and 5 kg respectively, whereas at Kadamakkudy it was 10.2. It can be assumed that prawn farms nearer to the barmouth were getting enough prawn seeds from the wild by the traditional method and hence the additional stocking of seeds were not causing much difference to the total prawn production. Hence a substantial increase in production can be achieved in the seasonal farms away from the barmouth by additional stocking.

Perennial prawn farming : Perennial ponds are water impoundments which will have water throughout the year and the traditional trapping and holding method is practised throughout the year with periodic harvesting during the new moon and full moon days. These farms are leased out to contractors for a period of 12 months. The lease value ranges from Rs. 10,000 to 20,000 per hectare per annum between different farms and the

average lease value was worked out to be Rs. 12,424. The annual expenses including the lease value was worked out at Rs. 21,548 per hectare and 40% of it was incurred on labour.

The annual production per hectare was 887.7 kg in the perennial farms. About 46% and 28% of it was contributed by *M. dobsoni* and *P. indicus* respectively. *M. monoceros* constituted about 8%, and fishes and crabs 28% was of the total each. Maximum production of *M. dobsoni* was observed during February and March. The peak periods of *P. indicus* was during the February-March and during May-June.

About 57% of the gross revenue was realised from *P. indicus*. The annual profit per hectare was Rs. 9,368. Break even cost per kilogram was Rs. 24.3 whereas the profit margin per kilogram was Rs. 10.5.

The perennial farms were divided into small and large farms based on their size. The average lease value, labour charges and material cost per hectare were more in small farms. The labour cost was found to be declining sharply with the increase in culture area. The average annual production per hectare in small farms (1070 kg) was more than that of large farms (812 kg). Lesser production in large farm was mainly due to inefficient management and the inability to retreive the entire stock. The annual profit per hectare in small and large ponds was Rs. 11,832 and Rs. 8,363 respectively.

About 90% of the perennial farms surveyed, were stocking additional prawn seeds collected from the wild or hatchery. The average additional seeds per hectare was found to be 15,250 in the perennial fields. George (1974, *Indian J. Fish.*, 21 (1) : 1- 19) has reported a production of 838.6 kg/ha/yr during 1969-72, without any kind of additional stocking. But, inspite of the additional stocking of seeds practiced at present, the production has not increased considerably.

Semi-intensive culture system : The semi-intensive farms at Kannamaly were modified pokkali fields. The species used for

the culture practice was *P. monodon* with a stocking density of 30,000 - 60,000 per hectare per crop and generally two crops were cultured in an year.

The average capital investment involved in the construction of the pond was Rs. 34,108. About 41% of it was incurred for the pond preparation and 33% and 26% for the sluice gate and diesel pumps respectively.

Feed and seed were the major variable costs covering 47.3% and 16.7% respectively, wages constitute about 29% of the variable costs whereas 3% used for the purchase of diesel. Electricity was not used for the culture operation. Prawn feed was clam meat with a conversion ratio of 3.8. In certain farms a better FCR was obtained, when the clam meat was mixed with other feed ingredients and like ground nut oil cake, rice bran, cooked cattle liver, sardine oil, poultry mineral mix. Most of the farm labourers were employed on a permanent basis and additional labourers were also employed now and then on daily wage basis.

The average production per hectare per crop was 788 kg. The size of the harvested prawn ranges from 15-30 gm and this size was attained in a period of 100-120 days. The average size of the prawn was 21 gm whereas the survival rate was 79%.

The harvested product was sold to the exporting companies which offered the maximum price for the product. Price realised was based on the number of prawns in one kilogram. The average price realised for the cultured product was Rs. 105 per kilogram.

The average profit per hectare per crop was Rs. 30,166. The average capital investment involved in the marginal farms was greater than that of small farms. Number of man-days required per crop for the marginal farms (220) were more than that of small farms (190). The cost of eradication is less in marginal farm. The stocking density of marginal farm (48,000/ha/crop) was more than that of small farm (41,476/ha/crop).

The feed application/ha/crop in marginal farms (2,934 kg) was more than that of small farms (2,773 kg) and a better FCR of 8.5 was observed in the marginal farms compared to 4.1 in the larger farms.

The production/ha/crop was 839 kg in marginal and 664.5 kg in small farms respectively. The profit/hectare realised from the marginal farms (Rs. 34,174) was more than that of small farms (Rs. 27,333).

Production function : An input-output relationship was estimated by Cobb-Douglas production function. The coefficients of all variables except the cost of eradication have the expected positive sign. The coefficients of four variables X_1, X_3, X_4 and X_5 were significant at 5% level; whereas the coefficient of cost of eradication and capital investment were not significant. The production elasticities of the variables X_1 X_2 X_3 X_4 X_5 and X_6 were 0.1466, -0.1282, 0.4166, 0.3892, 0.204 and 0.0846 respectively. The production elasticity of X_3 (0.4166) indicates that a 10% increase in the use of an input X_3 could produce a 4% increase in the total production. Seed and feed were the most important factors that influence the production followed by labour, size of the pond and the capital investment. The cost of eradication was showing a negative relationship with the production. This could be due to the fact that the eradicators were necessary only for those ponds which cannot be drained fully.

The sum of the production elasticities was 1.113, which indicates that the production function exhibits an increasing return to scale.

The marginal physical product of the inputs X_1 X_2 X_3 X_4 X_5 X_6 were 77.8, -0.07, 443.7, 0.074, 0.757 and 0.0023 respectively. The price ratio of the inputs X_1 (50) X_2 (0.17) X_3 (142.8) X_4 (0.057) X_5 (0.38) were more than their respective marginal product and hence the use of area, seed, feed and labour should be increased to maximise profit.

The maximum profitable level of water area was worked out to be 2.16 hectare whereas the maximum profitable level of

the feed application was 5042.5 kg. The input of seed can be increased to the biologically possible level eventhough the maximum profitable level of stocking rate is 2.16 lakhs. The profitable level of labour input was worked out to be 396 man days.

Semi-intensive culture of P. indicus *in salt pan areas* : The scientific culture of *P. indicus* in the salt pan areas is fast growing in Tuticorin region. Most of the prawn farms in this area are operated by the salt producing companies as part of their salt industry for an additional source of income. The prawn farms are constructed above the mean sea level and can be easily drained completely. The size of each pond ranges from 0.5 to 1.0 hectare. Two crops are cultured in an year and the culture period of each crop is 120-125 days. *P. indicus* seeds collected from the creeks are stocked at the rate of 60,000 to 1,20,000 per hectare crop.

The semi-intensive culture of *P. indicus* at Tuticorin was highly capital intensive with an average initial investment of Rs. 1,04,421 per hectare. About 42% of the initial investment was used for the construction of the pond followed by the purchase of pumps (14%), aerators (15%) and the machinaries required for the feed mill (18%).

About 63% and 11.2% of the total variable cost were used for the purchase of feed and seed respectively. The ponds were fully drainable and hence eradicators were not used for killing the predators. The energy requirement for pump operations was mostly met with the use of diesel. The average diesel required per hectare per crop was 1015 litres constituting 12.2% of the total costs. The feed was prepared in the feed mill of the farm itself. The major constituents of the feed were groundnut oil cake, rice bran, squid meal, fish meal, etc. The average FCR of the locally prepared food was 3.3.

The average production per hectare was 955.7 kg/ha/ crop. The average size of the prawn was 15 gm and the survival

rate was 70- 80%. Regression analysis revealed that seed, feed, labour and capital investment were the major factors influencing the prawn production in the semi-intensive farms at Tuticorin. The profit realised per hectare per crop was Rs. 21,638.

The capital investment of small ponds were more than that of marginal farms. Small farms were well established and each farm was self-sufficient with the entire infrastructure facilities required for the prawn farming operations, whereas most of the marginal farms were recently established with less infrastructure facilities. The average stocking density per hectare in small farms was 1,00,000 seeds whereas in marginal farms it was 70,000. More quantity of feed with a better conversion ratio was provided in the small farms.

The average prawn production per hectare per crop in small farms were 1000 kg whereas in marginal farms it was 824 kg. The average profit per hectare per crop in the marginal farms was Rs. 15,998/- and in small farms Rs. 23,535.

Comparative economic efficiency

Production : The average annual production per hectare was 1912 kg for the semi-intensive culture at Tuticorin, 1476 kg for the semi-intensive culture at Kannamaly, 1046.6 kg for the seasonal pokkali farms and 887.7 kg for the perennial farms of Ernakulam District respectively.

Profit : Although maximum production was observed for the semi-intensive culture of *P. indicus* at Tuticorin, maximum profit was for the semi-intensive culture of *P. monodon* at Kannamaly. This was mainly due to the disparity in the price realised for *P. indicus* (Rs. 72) and *P. monodon* (Rs. 105).

The average annul profit was Rs. 60,344 for the semi-intensive culture of *P. monodon*, Rs. 43,294 for the semi-intensive culture of *P. indicus*, Rs. 11,658.8 for seasonal farms and Rs. 9,368 for the perennial farms.

Conclusion

The present study has proved beyond doubt that the semi-intensive culture system is more productive, profitable and generate more employment opportunity than the traditional culture system. Hence, the traditional pokkali fields can be converted into semi-intensive farms wherever it is possible. Two crops of *P. indicus* or *P. monodon* can be cultured during the high saline periods and during the monsoon period. Euryhaline fishes such as mullets, pearlspot, milkfish can be cultured along with the freshwater prawn.

In some regions, due to social, economic and political reasons, cultivation of paddy is unavoidable and hence in such region, the existing integrated culture of prawn and paddy should be continued with certain modifications. Annually, one crop of prawn during the summer season and a crop of paddy during the monsoon season can be carried out. The preparation of the field and the farm management can be followed as recommended by Unnithan (1985, *CMFRI Spl. Publ.*, **21** : 1-92). Prawn production can be increased considerably by this method, but the availability of sufficient hatchery reared prawn seeds and quality prawn feed should be ensured by government and private agencies before advocating the scientific culture practices.

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OBSERVATIONS ON "LAB-LAB" CONSTITUENTS IN SOME CULTURE SYSTEMS IN AND AROUND KOCHI, KERALA STATE

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Introduction

"Lab-Lab" is the biological plant/animal complex. This is composed of several microscopic organisms dominated chiefly by the blue-green algae *Oscillatoria, Phormidium, Lyngbya, Spirulina, Anabaena, Microcoleus, Chroococcus* and *Gomphosphaeria;* the diatoms *Navicula, Pleurosigma, Amphora, Nitzschia, Gyrosigma, Nostogloia, Stauroneis,* etc. and other organisms like protozoa, bacteria, the microfauna represented by copepods, amphipods, ostracods, nematod worms, polychaete worms, molluscs, cladocerans, isopods, tanaids, etc. It forms the natural food and is the main food item of milkfish, prawns and mullets. From a review of literature it is evident that the sustained growth and maintenance of "Lab-Lab" in culture ponds is the key factor for high production of the milkfish. The production of herbivorous fishes and their food organisam depend on soil fertility and the supply of nutrients from tidal water.

Various authors have studied in detail the phytoplankton production (primary productivity) and zooplankton distribution of the Cochin Backwater system. But the prawn culture fields in this area were not studied extensively until recently. In view of the importance of prawn culture in these areas a general ecological study was taken up. Qualitative and quantitative aspects of "Lab-Lab" in the seasonal and perennial prawn culture fields of the Cochin Backwater in relation to the environmental parameters were taken up and studied.

Material and methods

Four representative cuture systems were selected and the samples were collected fortnightly from each station. The first station is the seasonal prawn culture field at Cherai, the second station is coconut groves at Narakkal, third station is an experimental perennial culture pond belonging to Central Institute of Brackishwater Aquaculture (CIBA) at Narakkal and the fourth one is the supply canal to CIBA.

Environmental parameters such as water depth, temperature, dissolved oxygen, pH, salinity, free carbondioxide, phosphate and Nitrate were determined fortnightly. Sedimentological parameters such as soil organic carbon, organic matter, total nitrogen, available phosphorus and texture of sediments were determined. Algal and animal ones were observed.

Bottom water samples were collected by a bottom water sampler. Soil samples were collected using a Van-veen grab. "Lab-Lab" is scrapped off from the bottom with a "Lab-Lab" sampler consisting of a basal plate of 15 x 15 cm of Galvanised Iron (GI) sheet and from this an area of 10 x 10 cm is scrapped off and the samples were made up into a known volume and preserved in 5% formalin.

For the estimation of dissolved oxygen, salinity, nitrate and phosphate standared methods were followed (Strickland and parsons 1968, *Bull. Fish. Res. Bd. Canada,* **167** : 1-311). The Free CO_2 is estimated by the method cited by Adoni (1985, *Workbook in Limnology*). Available phosphorus was determined colorimetrically using spectrophotometer (Olsen, 1954, *Circ. US Dept. Agricult.*, pp. 19-39). Total nitrogen and Organic matter were calculated following Jackson (1973, *Soil Chem. Analy.* Prentice Hall (India) Pvt. Ltd., New Delhi). Organic carbon is determined by "Wet oxidation method". Texture of soil was determined by the Pipettee method.

"Lab-Lab" : The samples from an area of 10 x 10 cm^2 were made into a known volume with water and the number of algae and other constituents of "Lab-Lab" from an aliquot

were counted under a microscope. The length of the algal filaments were recorded and mean length of each sample was calculated. The total length of the filaments per 100 cm^2 is computed by multiplying the estimated number of filaments by their mean length in each case. For the estimation of aquatic fauna like copepods, amphipods, cladocerans, polychaete, etc. the organisms were separated from an area of 10 x 10 cm^2 by seiving it through a tub of water, to avoid any damage to the animals while sieving. After seiving, the organisms were preserved in 5% formalin with rose bengal to provide colour contast between the animals and sediment fraction. The rose bengal was added at a rate of 1gm/l of formalin. The number of organisms was counted and the total number of organisms is expressed as per 100 cm^2 area.

The respective mean values of surface and bottom water parameters were computed. The values of each parameter for twelve collections for each station were fed to the computer for one way analysis and the test of significance between stations was made. In order to find the influence of environmental parameters on the production of "Lab-Lab", linear regression analysis was also done.

Results

In the four stations, the depth of water varied from 18 to 45 cm at Cherai Pokkali fields, 52 to 72 cm at Narakkal coconut groves, 47 to 57 cm at CIBA experimental pond and 48 to 85 cm at CIBA supply canal.

The monthly rainfall data recorded from April to October 1991 were 71, 80, 1492, 541, 433, 54 and 49 mm for the respective months with the peak in June.

The variations in temperature was 26.5°C - 34.63°C and pH 5.64 - 8.25 in these stations. Salinity showed drastic changes of 0.63 - 27.0 ppt in all the systems. The free carbondioxide content was between 4 mg/l and 17 mg/l and the dissolved oxygen was between 1.01 ml/l and 6.74 ml/l. The nitrate values ranged from 0.10 µg at/l to 2.79 µg at/l. The phosphates showed

fluctuations between 1.1 µg at/l and 14.75 µg at/l. In May, the salinity and temperature attained their peaks and during June after the onest of Southwest monsoon, their values have decreased considerably. Dissolved oxygen, water nutrients, phosphate and nitrate values showed and increasing trend during the monsoon period.

The organic carbon varied between 0.9% and 3.48% and the organic matter between 1.55% and 5.99%. Higher values of organic carbon and organic matter were observed in Cherai pokkali fields throughout the study than in other stations. The percentage of total nitrogen ranged between 0.09 and 0.29% in all stations. The concentration of available phosphorus was from 38.0 µg/gm to 134.44 µg/gm. The texture of the sediment in Cherai pokkali field was clayey, but in Narakkal coconut groves, the CIBA experimental pond and the CIBA supply canal, this was sandy in nature.

The "Lab-Lab" present in the substrata in these stations was composed mainly of blue green algae - *Oscillatoria* spp., *Phormidium* spp., *Lyngbya* spp., *Spirulina* sp.; the diatoms - *Pleurosigma* spp., *Navicula* spp., *Amphora* spp., *Nitzschia* spp. and *Coscinodiscus* spp. and the microfauna - copepods, amphipods, polychaete worms and Lamellibranch spat. All these were studied for biomass calculations.

The "Lab-Lab" production in Narakkal coconut grove was relatively higher, compared to other systems followed by Cherai Pokkali fields and the CIBA experimental pond. The production was poor in the CIBA supply canal. The total production of "Lab-Lab" was very low in all the four stations during June and July. A good production was observed in May and September in all the stations.

Oscillatoria spp. showed a positive relationship with dissolved oxygen in station 1. *Spirulina* spp. and *Oscillatoria* are postively co-related to dissolved oxygen at Station 2. *Spirulina* is postively related to dissolved oxygen in the CIBA experimental pond and polychaete worm is directly related to soil phosphate.

Copepods showed a positive relationship with organic matter, dissolved oxygen and water phosphate. A similar relationship has also been obtained between polychaete worms and soil organic matter at Cherai pokkali fields. Copepods showed positive relationship with water phosphate in station 2. In CIBA supply canal, copepods showed a positive relationship with organic matter.

Rain fall and dissolved oxygen showed a negative relationship with total "Lab-Lab" production, whereas temperature, salinity, pH, free CO_2 nitrate, phosphate, organic carbon, organic matter, total nitrogen and available phosphorus showed positive relationship in all the four stations.

Discussion

The culture ponds, the pokkali fields and the canal systems studied, are mostly extensions of the estuarine and backwater masses and therefore are subjected to wide variations in their environmental conditions. The temperature regime of Cochin Backwater system generally within a narrow rage and has been found to be influenced by various factors such as rain fall and freshwater influx.

The fluctuation in temperature and salinity in all the stations were mainly due to the freshwater influx thus agrees with the earlier observation. According to Tang and Chen (1967, *FAO Fish. Rep.*, 44 (3) : 198-209) temperature from 25°C to 33°C and salinity between 10 and 25 ppt were suitable for "Lab-Lab" growth. This agrees with the present observation and showed a positive relationship with "Lab-Lab" production. According to Tang and Chen (1967, *loc. cit*) the pH value ranging from 8.0 to 9.5 was considered to be optimum for growth of "Lab-Lab" and this agrees with the present observation.

Since "Lab-Lab" is growing on the pond bottom, the nutrients of the soil are very important. Tang and Chen (1967, *loc. cit*), stated that higher organic matter, organic carbon, phosphorus and nitrogen were essential for better growth of "Lab-Lab". In the present study the soil nutrients showed a

positive relationship with "Lab-Lab" production and they also observed that the production of "Lab-Lab" during monsoon period was low. In the present study also, the production of it has been less in the monsoon period.

From the foregoing discussion it may be seen that the distribution and abundance of "Lab-Lab" constituents are influenced by various hydrological and sedimentological parameters which enable us to have some insight into the available "Lab-Lab" constituents in the culture system in and around Kochi and influence of ecological parameters on their production.

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QUALITY EVALUATION OF *PENAEUS INDICUS* FROM DIFFERENT PRODUCTION CENTRES

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Introduction

For estimating the freshness of seafood products, subjective and objective methods can be used for assessment. Subjective methods are universally applied to estimate quality and freshness of fish. This method requires the services of different types of panels of judges. Since the capacity for sensory discrimination in different individuals is different, we have to depend on the opinion of group of judges rather than on a single judge, in order to evaluate the product varieties. The methods by which the judges can be selected are the duo tests, duo-trio tests and the triangular tests. In the present study a primary taste test was conducted for selecting a panel for evaluation of quality of fishery products.

Among the subjective methods of assessment of sensory characteristic, the method of paired comparison is very important. Most of the studies using paired comparison was done in wheat varieties, bread and other backery products. The method of paired comparison is now employed in fishery products. An attempt is being made here to evaluate the quality of *P. indicus* on consumer point of view.

Material and methods

The primary aim of the experiment was to select the best treatment and a sub-set containing the best treatment with a certain probability of correct selection, using paired comparison with the help of a panel of judges. The judges were formed based on their sensitivity to primary tastes like salty, sweet, sour, alkalinity and bitter. The concentration of solutions used to represent the basic tastes were sodium chloride 0.5%, Sucrose

1%, hydrochloric acid 0.25%; Sodium carbonate 0.25% and quinine sulphate 0.05%. Each of these solutions were diluted to give five samples of varying concentrations as 0%, 25%, 50%, 75% and 100%. These solutions were arranged in random order and supplied to 128 staff members of the institute. A score sheet was provided to them for recording the concentration of solutions in increasing order as per their judgement. Tasters whose score was 80% or more were considered successful and formed the subject for experiments in the selection of panelists.

A pilot study has undertaken to see whether there was any taste difference in different size classes of *P. indicus*. For this samples were collected from a small trawl net operated on board R. V. *Cadalmin* off Cochin. For detailed study, samples of *P. indicus* were collected from the following centres : Prawn farms of Matsyafed, Perennial prawn farm, Edavanakkad, Shakthikulangara landing centre and Fort Cochin Beach landing centre. Collections from all these centres were made on the same day.

The samples collected from each production centre were transferred to polythene bags and kept in an ice box containing crushed ice. Sexes were separated and the total length were taken. The prawn samples were grouped into following size classes *viz.* 70-100 mm, 100-130 mm and 130-190 mm for *Cadalmin* samples, and 100-130 mm, 130-160 mm and 160-190 mm for other centres. These prawn samples of the different size classes were then beheaded and stored separately in polythene bags in the freezer for a day. On the following day the prawn samples were thawed to normal room temperature, deveined and washed thoroughly with tap water. Each of the size group was further separated into equal portions and shell of the prawns of one portion was removed. All the different samples were then steam cooked under similar temperature and pressure conditions for seven minutes and were served hot to judges.

The method of paired comparison was used for evaluating the quality of prawns. The basic goal in this method is to

discriminate between t > 2 treatments on the basis of preference obtained by presenting the treatments in pair in a given order to a set of judges, according to a specified paired comparison design. The designs used in this present study were full paired (FP) and the Symmetrical paired (SP) comparison design in presence and absence of ties. An SP design is a type of fractional pair design designated by (T_1, T_2), (T_2, T_3), ... (T_t, T_1) which is one among the several SP.

Selection of the best treatment

In any experiments designed to compare 't' objects the primary interest lies in the detection of the best object. The treatment T (t) is better than T (t-1) and if the number 'n' of the replications is large enough, then T (t) should emerge with the highest score with a probability (P) as close to 1 as desired. 'n' can be determined by the following rules (R) :

(a) Find 'n' corresponding to given values of t, p and the configuration of preference probabilities $C(\pi_{ij})$.

(b) Perform the experiment and declare best, the object with the highest score; if m scores tie for the first place declare best one the corresponding objects at random.

It was assumed that there are no ties, no order effect, no replication effect and preference probabilities satisfy a linear model. The probability π_{ij} is called the preference probability of treatment T_i over T_j.

Selection of subject containing the best treatment

Consider the set of treatments $t = (T_1, T_2, \ldots\ldots T_t)$ and let S be a sub-set of T consisting of those treatments with the highest scores. In this section the aim is to select 'S' just large enough to ensure, with at least a preassigned probability Pcs that the best object T(t) is included in S. Following Gupta and Sobel (1960) the decision rule R' is as follows :

Retain in S only those objects T_i for which $a_i \geq a_{(t)} - V$ where $a_{(t)}$ is the highest score and V a non-negative integer, is a function of t, n and Pcs.

The studies have been undertaken for the samples collected from different centres see whether there is any taste differences from different production centre over sexes and same groups. The following are the treatments for size group 100-130 mm and 130-160 mm.

Size group 100-130 mm

Treatments	Centre	Sex	Peeled/Not peeled
1	Matsyafed	Male	Not peeled
2	Matsyafed	Male	Peeled
3	Matsyafed	Female	Peeled
4	Matsyafed	Female	Not peeled
5	Fort Cochin	Female	Not peeled
6	Fort Cochin	Female	Peeled
7	Fort Cochin	Male	Not peeled
8	Fort Cochin	Male	Peeled
9	Shakthikulangara	Male	Not peeled
10	Shakthikulangara	Female	Not peeled

Size group 130-160 mm

Treatments	Centre	Sex	Peeled/Not peeled
1	Matsyafed	Male	Not peeled
2	Matsyafed	Male	Peeled
3	Matsyafed	Female	Not Peeled
4	Matsyafed	Female	Peeled
5	Shakthikulangara	Male	Not peeled
6	Shakthikulangara	Female	Not peeled
7	Edavanakkad	Female	Not peeled
8	Edavanakkad	Female	Peeled
9	Edavanakkad	Male	Not peeled
10	Edavanakkad	Male	Not peeled

The number of replications needed for detecting the best treatment for the size group 100-130 mm and 130-160 mm was obtained by using the following set of values for different designs without ties.

Design	P*	π	'n'	Annexure
FP	.95	.75	5	1
SP	.90	.95	5	2

for the same designs with ties the following set of values were concerned

Design	P*	π	0'/ø'	0/ø	n	Annexure
FP	.99	.75	.05	.1	5	4
SP	.95	.90	.05	.1	5	3

The data for preference pooled over replications for FP without ties, SP without ties, SP with ties and FP with ties for size group 100-130 mm is given in Tables 5, 6, 7 and 8 respectively and Tables 9, 10, 11 and 12 for size group 130-160 mm.

For the size group 160-170 mm the following 4 treatments were considered.

Treatments	Centre	Sex	Peeled/not peeled
1	Shakthikulangara	Female	Peeled
2	Shakthikulangara	Female	Not peeled
3	Edavanakkad	Female	Not peeled
4	Edavanakkad	Female	Peeled

The number of replications needed for detecting the best treatment for size group 160-190 mm was obtained as 8 by using the following set of values for different design without ties.

Design	P*	π	n	Annexure
FP	.95	.8	8	1
SP	.90	.8	8	2

With ties the following set of values were concerned

Design	P*	π	0'/ø	0/ø	n	Annexure
FP	.99	.75	.05	.1	8	4
SP	.95	.65	.25	.3	8	3

The data pooled over the 8 judges by using FP and SP designs with and without ties are given in the original Dissertation.

Results and discussion

Panel selection

Out of the 128 persons who participated in the panel selection experiment, 16 persons got more than 80% scores. 11 selected members were in the age group 21-30 years and the remaining in the higher age groups, showing that persons in the lower age group have got very good taste perception.

Pilot study

a) *FP design with and without ties* : By using FP design with ties treatment 8 was found to have the maximum score of 33 and hence declaring treatment 8 as the best sample preferred by the judges with a probability of .95.

In the case when the judges were allowed to make a tie by using FP design it was found that treatment 8 was again having the maximum score of 32. Hence declaring treatment 8 as the best with a probability of $P^* = .99$ by using the selection rule (R). It may be noted that when the judges were allowed to make a tie, probability of correct selection P^* has improved from .95 to .99. Hence for sensory evaluation studies using paired comparison designs, it is better to allow the judges to exercise the option of tie instead of forcing them to make a preference one over the another.

b) *SP design with and without ties* : In this case by using the selction rule (R) for the best treatment, treatment 2 was found to have the maximum score and thus has to be declared as the best treatment with a minimum probability of $P^* = .9$. By using SP design the number of comparisons is reduced very much. Hence the judges can avoid fatigue by using this design. Moreover SP design can be used to detect an outlier among the treatments, if ever it exists.

The results obtained using SP design with and without ties are not in agreement with that of FP designs, thereby indicating that there is no outlier among the treatments.

c) *Selection of a sub-set containing the best treatment using FP design with and without ties - FP design without ties* : By using the selection rule R' with a probability of correct selection Pcs = .75 for 10 treatments and 5 replications the value of the selection constant ('V') is 8. The maximum score obtained by FP design without ties was 33. Hence treatments whose scores are more than 25 *(i. e.* 33 - 8) was included in the sub-set.

FP design with ties : The selection constant 'V' was obtained as 6.5 with a probability of correct selection Pcs = .75, ø = .3, n = 5 and t = 10. Treatments T_8, T_6 and T_5 are selected in the subset containing the best treatment as their scores are more than 25.5 (*i. e.* 32 - 6.5, 32 being the maximum score). It may be worth mentioning that by allowing ties the value of 'V' has reduced from 8 to 6.5.

Size group 100-130 mm

a) *FP Design with and without tides* : It is seen that treatment 5 was found to have the maximum score of 33. Hence treatment number 5 was declared as the best with a probability of P* = .95.

In the presence of ties also it was seen that treatment 5 was with the maximum score of 29.5 and hence declared it as the best treatment with a preassigned probability P* = .99. Treatment 5 was female prawns cooked with shell, collected from Fort Cochin Beach landing centre.

b) *SP Design with and without ties* : As per the selection rule (R) treatment 5 which was found to have the maximum score was selected as the best with P* = .9 and .95 for SP without and with ties respectively.

It was observed that treatment 5 was selected as the best by using all the designs. This indicates that T_5 is clearly an outlier.

c) *Selection of a sub-set containing the best treatment using FP Design with and without ties - FP Design without ties* : The selection constant 'V' was obtained as 8 with Pcs = .75, t = 10 and

n = 5. The maximum score obtained by this design was 33. Hence treatments whose scores are more than 25 (*i. e.* 33-8) are included in the sub-set. The treatments T_{10}, T_7 and T_5 are thus retained in the selected sub-set containing the best treatment.

FP Design with ties : The selection constant 'V' obtained by using this design was 6.5 with Pcs = .75, ø = .35, n = 5 and t = 10. By applying the selection rule R' treatments whose scores are 23 (*i. e.* 29.5 - 6.5) or more than 23 was retained in the sub-set.

Size group 130-160 mm

a) *FP design with and without ties*

FP Design without ties : Treatment 8 has the maximum score of 30 and hence declared it as the best treatment with a P* of .95 for n = 5 and t = 10.

FP Design with ties : In case of ties also treatment 8 was found to have the maximum score and hence selected as the best with a probability P* = .99.

b) *SP Design with and without ties* : Treatment 8 and 10 are found to have the maximum score of 8. Hence one of these two treatments was declared as the best with P* = .9. Here treatment 8 was declared as the best.

In case of ties also treatment 8 was found to have the highest score of 8 and hence selected as the best with a probability P* = .95 for t = 10 and n = 5. Treatment 8 was a peeled female obtained from Edavanakkad centre.

From the results obtained by using the different designs for size group 130-160 mm it is obvious that treatment 8 is the best sample and thus is an outlier, when compared to other treatments.

c) *Selection of a sub-set containing the best treatment using FP Design with and without ties* :

FP without ties : The value of 'V' was obtained as 8 with a Pcs of .75 for t = 10 and n = 5. Treatments whose scores are more than 22 (*i. e.* 30 - 8) are therefore retained in the selected sub-set.

FP Design with ties : The selection constant 'V' was obtained as 6.5 with Pcs = .75, ø = .35 for n = 5 and t = 10. The maximum score obtained by using FP design with ties was 28.5 and hence treatments whose scores are more than 22 (*i. e.* 28.5 - 6.5) were retained in the sub-set.

Size group 160-190 mm

a) *FP Design with and without ties* : By using FP design without ties it was found that treatment 3 was found to have the maximum score of 16 and hence selected as the best treatment with P* = .95.

In the case of ties also treatment 3 still had the maximum score and hence declared as the best based on the scores of the judges with a preassigned probability of .99 for t = 4. Treatment 3 was a shell on female from perennial prawn farm, Edavanakkad.

b) *SP Design with and without ties* : By using SP design without ties also treatment 3 still has the maximum score of 10 and thus declared on the best with a preassigned probability P* = .9.

In the case of ties treatment 3 and 4 were found to have the maximum score of 9. Then one of them was declared as the best at random. Here T_3 was declared as the best one.

From the results obtained from the *Cadalmin* sample it is evident that there is difference in consumer preference over the size group. Here in this case female prawns in the size range 100-130 mm cooked with shellon had higher acceptability among the consumers. This has been confirmed by the detailed study conducted during the course of this study.

In all the samples collected, from Matsyafed, Fort Cochin and Shakthikulangara, the preferred size range was 100-130 mm or 130- 160 mm and that too female prawns cooked with shell. Whereas samples from Edavanakkad showed that peeled prawns in the size range of 130-160 mm was preferred. *P. indicus* are known to mature in the size range 130-150 mm and it can be assumed safely that the prawns which are falling in the size

range of 100-130 mm and 130-150 mm are maturing prawns. Hence maturing prawns were preferred. We can say that prawns have got more consumer acceptability when served after cooking cooked with shell. Prawns from the Edavanakkad perennial prawn farm was preferred by most of the panelists. None of the treatments from Shakthikulangara landing centre was preferred when compared to samples collected from in and around Cochin.

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ECO-BIOLOGICAL STUDIES OF MANGROVE *RHIZOPHORA* SPECIES

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Introduction

The mangrove ecosystem in the Cochin estuarine system found to be formative and the dominant vegetation constituted by the species of *Acanthus, Avicennia, Clerodendrom* and *Rhizophora.* Mangrove areas contribute significantly to fish production by (1) sustaining a fishery (2) as a suitable site for culture and (3) by providing breeding and nursery ground for many finfishes and shellfishes. With this view, the present work attempts to understand the various factors both ecological and biological which control a *Rhizophora* dominated community in the Cochin area.

Material and methods

Salinity, dissolved oxygen, nutrients and chlorophyll were studied following standard methods. Grain size analysis, pH, organic carbon, organic matter, total nitrogen, phosphate and cation exchange capacity of the sediments were analysed following the methods of Jackson (1973, *Soil Chem. Analysis.* Prentice Hall Pvt. Ltd., New Delhi). The biological study of the plant carried out as follows : *phenology* by observing the percentage of the buds, flowers and fruits in different months; litter fall estimated by collecting the mangrove leaves, twigs, fruits, etc. from a fixed quadrant of 1 sq. m which was collected fortnightly and dry weight was taken. Decomposition of leaves was done followed by "litter bag" method.

Results

Water

Temperature : The fortnightly variation was more or less similar in all the stations with low values during second half of June which increased and reached a peak in the second half of August. The values at Murukkumpadam was low during most of the period and recorded values lower than the other two stations. The change was significant ($P < 0.05$) between stations and between months ($P < 0.01$).

pH : pH values showed a maximum concentrations from June second week to the last week of September. A sharp decline was observed in June first week at all stations. There was no significant variation among the stations. However significant ($P < 0.01$) variation was observed between months.

Salinity : With the onset of monsoon in the latter half of May, salinity declined in all the stations and remained at less than 10 ppt throughout. The change was significant between the months ($P < 0.01$).

Dissolved oxygen : Narakkal showed a maximum dissolved oxygen content than other stations during the initial months and a decline was observed during the second week of July. The variations at the other two stations were almost similar. Significant differences between the months were observed. However the variations among the stations were not significant.

Nitrate : A gradual increase was observed at Narakkal during the first two months. But thereafter it reduced and remained low for rest of the period. On the other hand at Murukkumpadam a sharp decline was observed in the initial period and here also it continued to remain at a low concentration. At Puduveypu the concentration was low with a peak in August second week. The change was significant between months ($P < 0.05$).

Nitrite : From all the 3 stations the nitrite concentration was from June 3rd week to July 3rd week; with a peak at August 1st week. There was not significant variation among the station or months during the study period.

Ammonia : At Narakkal the concentration of Ammonia showed a gradual decrease from May 1st week to July second week. Thereafter it slightly increased upto September second week. At Murukkumpadam and Puduveypu station a peak was observed in June 3rd week and August 1st week. No significant variation among the station or month was observed.

Phosphate : Fortnightly variations were more or less similar (low values) at Murukkumpadam and Puduveypu. There was a sharp decline in July second week at Murukkumpadam. At Narakkal the concentration was low during most of the months and recorded lower values than the other two stations. The change was significant ($P < 0.01$) between station and between the months ($P < 0.05$).

Silicate : A decline was observed at 3 stations due to the onsent of monsoon which remained in same range throughout the period. A significant difference between the months for all the 3 stations was observed ($P < 0.01$). However the variation among the months also significant ($P < 0.01$).

Chlorophyll a : The productivity of Chlorophyll *a* was high in all the stations in September. However at Murukkumpadam the values of Chl. *b* were marginally greater than Chl. *a* during September.

Soil

Grain size analysis : The grain size of sediment showed sand as the major constituents; followed by clay and silt. The percentage of sand, clay and silt was as follows.

	Sand	Clay	Silt
Murukkumpadam	78.4%	19.8%	8.8%
Puduveypu	84.2%	13.8%	2.0%
Narakkal	78.2%	10.8%	8.0%

pH : Except for a sharp decline in the pH during the first week of June, the pH in all stations remaind above 8.

Organic carbon : At Narakkal concentration of organic carbon showed much lower values than the other two stations. At Puduveypu and Murukkumpadam a peak value was observed during June 3rd week and a decline occurred in August 1st week. The significant variation among the station ($P < 0.01$) and months ($P < 0.01$) was observed during the period.

Organic matter and total nitrogen : As the values were computed from the estimation of organic carbon, total Nitrogen showed a similar pattern to that of organic carbon.

Available phosphorus : Murukkumpadam station showed more concentration than the other 2 stations. Narakkal and Puduveypu had a similar range during the observation period. But a peak value was recorded in July 1st week at all 3 stations. The change was significant between stations and months ($P < 0.01$).

Cation exchange capacity : At Narakkal the CEC of soil indicated a steady level during the study period. At Puduveypu station, an increasing trend was observed from June 1st week to August 1st week. A peak value recorded in June 1st week at Murukkumpadam, thereafter it was almost same range as Narakkal. The change was significant ($P < 0.01$) between stations and between the months ($P < 0.05$).

Biological parameters

Floral phenology : The flower buds were observed in August-December and maximum number of flowers were observed during November-December and the propagule grow to a size of 24 cm to 36 cm and maximum number of propagule drop off during July-August, though falling starts in June itself.

Litter : A maximum litter fall was observed during July. With the contribution from fruits accounting for 90% of total litter. During the 5 month period the percentage contribution by twigs, fruits and leaves were 12.9, 63.6, 23.5 respectively. Fruits had a maximum fall during July. The total litter production during the study period was 357 $gm/m^2/5$ month.

Decomposition experiment of leaves : The experiments were conducted in the laboratory. The rate of decomposition of *Rhizophora* leaves was low during the first 28 days. Thereafter, the decomposition rate has become rapid. 39% of total weight was reduced during the period of study (49 days). A linear relationship between days and loss of weight gave an equation : $y = 10.295-0.088 x$ and $r = -0.98$. Expected days for 50% decomposition is calculated to be 60 days.

Germination experiment : For the experimental purpose 20 matured propagules (seed) were planted in 3x1 m area at Puduveypu station. Out of these, 16 were survived, 4 of them decayed due to the influence of flood and predators such as crabs and other micro-organisms. After plantation a continuous observation were made and noticed the appearence of the 1st leaf and its growth rate were measured (leaf length and breadth). The present observation indicates that about 4 months time it took to reach a 4 leaves stage. Through this experiment the growth rate were found very slow, it may be due to the effect of several factors such as tidal variation and monsoonal fluctuation in temperature and salinity and other biological factors.

Discussion

Organic carbon and hence organic matter and total nitrogen were found to be low at Narakkal when compared to the other stations. This may be due to a greater water exchange, because of its proximity to the main canal. Twilley (1985, *Estuar. Coast. Shelf Sci.*, **20** : 543-557) found that the export of organic carbon is associated with increased tidal amplitude. The values obtained for the present study compare well with the observations of Alongi (1990, *Estuar. Coast. Shelf Sci.*, **31** : 581-598).

The soil pH varried from 6.53 to 8.21 in all the stations. This however, is within the normal range of coastal soil pH. The estimate of organic matter varied from 0.7 to 7.48%. These had close relation with the monthly values for the organic carbon content.

The available phosphorus in the three station ranged from 11.6 to 76.0 µg/gm and showed wide variation during different months. Mortimer (1971, *Limnol. Oceanogr.*, **16** : 387-404) reported that progressive decline in the dissolved oxygen at the water sediment interface was accompanied by transfer of phosphorus to the overlying water. In the present study also the inverse relationship between high dissolved oxygen and low phosphorus content was observed in August and September. Sterling and Wormald (1977, *Estuar. Coast. Mar. Sci.*, **5** : 631-642) reported that reductions in salinity enhance adsorption. In general salinity drops drastically in the mangrove waters during June to August.

The monthly variations of hydrological parameters in the mangrove waters showed general fluctuation typical of an estuarine system which is influenced by rainfall during mansoon, river run off and recovery of essential parameters during postmonsoon months.

Uncles *et al.* (1990, *Estuar. Coast. Shelf Sci.*, **31** (5) : 651-665) observed that the ecological conditions of mangrove system and its associated coastal waters and fisheries is influenced by stratification caused by slow currents and weak tidal mixing.

In mangrove swamps, primary productivity can be attributed to several sources, the mangrove trees themselves, from their associated attached macrophytic vegetation and algae, from free floating macrophytic vegetation and from phytoplankton or benthic micro algae (John and Lawson 1990, *Estuar. Coast. Shelf Sci.*, **31** (5) : 505-518). Productivity calculated from phytoplankton without taking into account the respiration and other losses were 335, 490 and 500 mg $C/m^3/day$ at Murukkumpadam, Puduveypu and Narakkal respectively. This is comparable with the values for phytoplankton production at Ghana by Kwei (1981, *Hydrobiologia*, **56** : 157-174).

The total litter production at Puduveypu is estimated to be 8.568 tonnes/ha/year of which 12.7, 23.5 and 63.6% is contributed by twig, leaves and fruits respectively. Gong and Ong (1990, *Estuar. Coast. Shelf Sci.*, **31** (5) : 579-530) cautions that

the dynamics of leaf litter is dependent on several factors and suggest that tidal export becomes increasingly important with the amount of inundation. Puduveypu from where the data on litter production was collected was submerged during the period of study with tidal influence. The decay coefficient or rate loss (percentage loss/day) calculated for *R. mucronata* was 0.80. This is lower than the values reported for *Avicennia marina* and *Kanedlia candel*, but higher than that of *A. corniculatum*. They explain that decay rates are not only related to species, but also to different environmental conditions under which investigations are conducted.

The ecological and biological studies of *Rhizophora* dominated mangrove system at Cochin threw some preliminary light on the role of various nutrients in these ecosystems, the influence of tide and the related environmental respects.

The mangrove of the Vypeen Island in the Cochin estuarine system is vulunerably situated, sand-whiched between the Arabian Sea in the west and an urban area on the east. According to Margalef (1963, *American Naturalist*, 97: 357-374), this would lead to exploitation of the "immature" mangrove ecosystem with its low energy requirements. These mangrove support the fishery both in the Cochin Backwater and the continental shelf area of Cochin. The management and conservation of mangroves around Cochin gains importance, because the intense human pressure on coastal mangrove which may have repercussions for the productivity of marine fisheries, as pointed out by John and Lawson (1990, *loc. cit.*) may be of an unexpected and deleterious nature.

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EFFECT OF STARVATION ON BIOCHEMICAL CONSTITUENTS OF *METAPENAEUS DOBSONI*

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Introduction

Several factors *viz.* season, moulting, feeding, parasitism, bacterial or viral infections and physiological stress, can change or alter the physiology or metabolism of crustaceans. Among these, one of the most important factors is food availability to the animal. Starvation can be viewed, firstly as a stressor which evokes a physiological stress response from the animal and secondly as an effective tool to study the physiology of an animal. Crustaceans encounter starvation in periods of food scarcity and they undergo voluntary fasting during the moult cycle and seasonal dormancy. The abilities of different animals differ to withstand such stressful situations. Many decapods can survive for weeks or even months of total starvation.

An attempt, to examine the effects of starvation in *Metapenaeus dobsoni* is made in the present study, with a view to contribute to the knowledge concerning biochemical adaptation to food deprivation; to define storage materials used by prawns; to isolate effects of starvation from those of moulting, to discover which parts of the body are used for storage and to determine if metabolic rate is affected by starvation.

Material and methods

Live shrimp *Metapenaeus dobsoni*, 40-65 mm in size were obtained from the wild near Puduvyepu Light House, Cochin by using a cast net and normal healthy animals were transported to the laboratory. Prawns in the inter moult stage were used for the experiment. 80 prawns were reared in 8 separate tubs of 40 litre capacity with 10 prawns in each tub. They were starved for a period of 21 days, after which feeding was resumed

for one week. Refeeding was carried out for a single week only to find if the prawns would recover. Ten prawns each from the experimental and control tubs were sacrificed every week for biochemical studies. An experiment was also designed to study the weight loss, change in moisture content and oxygen consumption rate when prawns were subjected to starvation. Control animals also of the intermoult stage were maintained separately and fed on boiled clam meat. One third water in all the tubs was replaced with fresh filtered water, every second day and completely changed once in a week. Continuous aeration was provided throughout the experiment and the temperature ranged between 26° and 30°C.

Total carbohydrates by the phenolsulphuric acid method, glycogen by the Anthrone method, total protein by the Folin-Ciocalteu method, total free amino acids using the method Yemm and Cocking (1955, *Analyst.*, **80** : 209-213), lipids by the Sulpho-phosphovanillin method and total cholesterol by the Henlley's method were determined. The oxygen consumption rate was measured using a crude respirometer and the water samples were analysed for O_2 by the Winkler's method. Moisture content in the animals were determined by drying the weighed animals at about 60°C in a hot air oven for about 24 hours or until constant weights were obtained.

Results and discussion

The results of the biochemical analyses carried out in different tissues of starved and refeed *Metapenaeus dobsoni* are presented in Tables 1 to 5.

Results of an early study on *Hemigrapsus* led to the conclusion that there was no change in the level of glycogen after 23 days starvation (Neiland and Scheer 1953, *Physiol. Comparata et Oecol.*, 3 : 321-326). In contrast, a decrease in glycogen was noticed for fasting *Uca* (Dean and Vernberg 1965, *Comp. Biochem. Physiol.*, **14** : 29-34) and a decrease in carbohydrate for fasting *Carcinus* (Florkin 1936, *Bull. Acad. r. Belge. Cl. Sci.*, **22** : 1359- 1367). Hepatopancreas glycogen stores in *P. japonicus* was rapidly depleted during starvation presumably being converted to

TABLE 1. *Biochemical constituents (mg/100 mg) in muscle tissue of* Metapenaeus dobsoni *with starvation of three weeks duration and refeeding of one week*

	Day O		Starvation I week	Starvation II week	Starvation III week	Refeeding I week
Total Carbohydrates	1.095 ± 0.910	Exp.	0.6638 ± 0.0425	0.5114 ± 0.0360	0.3148 ± 0.0120	0.5440 ± 0.0816
		Con.	1.6750 ± 0.5135	1.7410 ± 0.5020	1.4633 ± 0.3480	1.5865 ± 0.4193
Glycogen	0.5540 ± 0.0644	Exp.	8.348 ± 0.0424	0.1592 ± 0.0521	0.0373 ± 0.0343	0.2112 ± 0.0812
		Con.	0.7796 ± 0.1023	0.8892 ± 0.2277	0.873 ± 0.2975	0.7842 ± 0.2107
Protein	22.5758 ± 3.6490	Exp.	13.074 ± 3.3710	8.9740 ± 1.245	6.4620 ± 1.0455	13.5975 ± 3.2308
		Con.	32.4774 ± 6.8009	24.5470 ± 4.375	25.8828 ± 6.8315	28.1788 ± 7.4649
Free Amino Acids	0.3322 ± 0.062	Exp.	0.2366 ± 0.0340	0.2955 ± 0.0237	0.4318 ± 0.0348	0.2980 ± 0.0299
		Con.	0.3460 ± 0.0236	0.3452 ± 0.0908	0.3348 ± 0.0246	0.3322 ± 0.0621
Total Lipids	2.9262 ± 0.1645	Exp.	2.0904 ± 0.0994	1.6878 ± 0.1326	1.0486 ± 0.1225	1.9832 ± 0.4870
		Con.	4.1700 ± 0.0994	1.6878 ± 0.1326	1.0486 ± 0.1225	1.9832 ± 1.6591
Cholesterol	0.2286 ± 0.0172	Exp.	0.1332 ± 0.0229	0.1264 ± 0.0183	0.1142 ± 0.0195	0.1722 ± 0.0235
		Con.	0.2433 ± 0.0448	0.2835 ± 0.0587	0.2230 ± 0.0295	0.2407 ± 0.0504

All values are $\bar{X} \pm$ SD of five determinations.

TABLE 2. *Biochemical constituents (mg/100 mg) in hepatopancreas of* Metapenaeus dobsoni *with starvation of three weeks duration and refeeding of one week*

	Day O		Starvation I week	Starvation II week	Starvation III week	Refeeding I week
Total Carbohydrates	2.8006 ± 0.2487	EXP.	1.6276 ± 0.0363	1.7704 ± 0.0280	2.0067 ± 0.0895	2.5120 ± 0.1789
		CON.	3.1113 ± 0.4918	3.3076 ± 0.2502	3.1028 ± 0.3126	3.1380 ± 0.3810
Glycogen	1.3832 ± 0.2403	EXP.	0.9486 ± 0.1952	0.3848 ± 0.1244	0.1432 ± 0.0525	0.6195 ± 0.1654
		CON.	2.0760 ± 0.3720	1.7702 ± 0.6625	1.9306 ± 0.6112	2.0010 ± 0.9223
Protein	10.3236 ± 1.019	EXP.	6.9960 ± 0.6807	4.7500 ± 0.8070	2.1490 ± 0.2010	4.4104 ± 0.4501
		CON.	10.8396 ± 2.6336	10.3668 ± 1.1208	10.0740 ± 0.9984	10.4255 ± 1.9497
Free Amino Acids	0.1462 ± 0.0180	Exp.	0.2595 ± 0.0238	0.3322 ± 0.0340	0.4047 ± 0.0357	0.2551 ± 0.0553
		CON.	0.1712 ± 0.0395	0.1818 ± 0.0311	0.1409 ± 0.0990	0.1407 ± 0.0238
Total Lipids	12.7276 ± 1.6508	EXP.	8.5824 ± 1.3670	4.8568 ± 0.6054	1.9654 ± 0.2739	1.1015 ± 2.0028
		CON.	14.8230 ± 1.6034	13.1416 ± 1.5945	15.6702 ± 4.4940	14.3666 ± 3.4700
Cholesterol	0.3144 ± 0.0734	EXP.	0.2759 ± 0.0175	0.2029 ± 0.0253	0.2101 ± 0.0168	0.2819 ± 0.0124
		CON.	0.3526 ± 0.0476	0.3632 ± 0.0517	0.3565 ± 0.0369	0.3445 ± 0.0538

All values are $\overline{X} \pm$ SD of five determinations.

TABLE 3. *Biochemical constituents (mg/100 mg) in haemolymph of* Metapenaeus dobsoni *with starvation of three weeks duration and refeeding of one week*

	Day O		Starvation I week	Starvation II week	Starvation III week	Refeeding I week
Total Carbohydrates	25.627 ± 4.0801	EXP.	41.0409 ± 3.8054	20.348 ± 2.7990	4.1828 ± 3.3685	26.1884 ± 4.0255
		CON.	26.7916 ± 3.9300	26.562 ± 1.9409	24.3932 ± 4.1462	25.7000 ± 4.9280
Glycogen	26.54 ± 2.990	EXP.	17.79 ± 2.420	10.70 ± 0.840	9.740 ± 1.690	16.88 ± 2.87
		CON.	35.76 ± 6.370	34.60 ± 12.25	35.26 ± 8.116	35.38 ± 8.03
Protein	6.1544 ± 1.7034	EXP.	4.6832 ± 0.4410	3.0202 ± 0.4753	1.5230 ± 0.3980	4.429 ± 0.9280
		CON.	6.5464 ± 1.8024	6.2984 ± 1.6550	6.5798 ± 1.5050	6.6228 ± 1.5361
Free Amino Acids	1.9342 ± 0.1205	EXP.	0.7196 ± 0.1176	1.8294 ± 0.5907	3.0386 ± 1.3007	2.0424 ± 0.3461
		CON.	2.2548 ± 0.2373	1.9328 ± 0.1161	2.1626 ± 0.4160	1.9909 ± 0.3807
Total Lipids	309.03 ± 46.467	EXP.	231.048 ± 43.05	154.926 ± 16.076	80.96 ± 16.759	189.718 ± 17.740
		CON.	392.958 ± 86.99	349.124 ± 102.34	353.302 ± 81.786	358.89 ± 116.383
Cholesterol	36.0554 ± 3.1714	EXP.	32.8516 ± 3.1209	20.9568 ± 2.0331	16.250 ± 2.1471	25.5352 ± 3.1749
		CON.	38.0672 ± 2.5654	36.2678 ± 1.9363	38.2524 ± 3.2182	37.5320 ± 9.114

All values are $\bar{X}$ ± SD of five determinations.

glucose. Similar results were obtained by Schafer (1968, *FAO Fish. Rep.*, **57** : 393-403) for *P. duorarum* and Cuzon and Ceccaldi (1973, *Comptes Rendus des Seances de Soc. Biol.* (Marseille), **167** : 66-69) using *C. crangon*. In the present study over 90% depletion of glycogen was observed in the hepatopancreas and muscle and over 60% in the haemolymph of starved prawn. Over 70% reduction in total carbohydrate levels were seen in the muscle and hepatopancreas and over 80% in the blood. Total carbohydrates in the blood of starved prawn increased to 60% in the first week before coming to this figure. Cuzon *et al.* (1980, *Proc. World Maricult. Soc.*, **11** : 410-423) showed decreased blood glucose levels in starved prawns due to absence of exogenous sugar supply. Results therefore indicate rapid metabolism of total carbohydrates in starved prawn, these being the immediate concern of energy supply under stress conditions.

TABLE 4. *Oxygen consumption rate in* Metapenaeus dobsoni *with starvation of three weeks duration and refeeding of one week*

Duration (Weeks)	Experimental (starved) ml/g wt/Hr	Control (fed) ml/g wt/Hr
O	1.032 ± 0.048	1.032 ± 0.048
I	0.9063 ± 0.0063	1.0003 ± 0.0416
II	0.6726 ± 0.0664	1.033 ± 0.0702
III	0.583 ± 0.07	1.054 ± 0.0585
IV (Refeeding)	1.191 ± 0.2105	1.036 ± 0.025

Renaud (1949, *Ann. inst. Oceanog. (Paris)*, pp. 259-357), one of the first to work on starvation concluded that energy metabolism in Crustacea was centered around lipids. Armitage *et al.* (1972, *Comp. Biochem. Physiol.*, **41** : 825-842) concluded that lipid was the major energy reserve in *Orconectes nair*, while Health and Barnes (1970, *J. Exp. Mar. Biol. Ecol.*, **5** : 199-233) found there was a major decrease in fat in the digestive gland of *Carcinus maenas* after starvation. None of these authors, however, examined changes is body protein during starvation.

Surendranath *et al.* (1987, *Mahasagar*, **20** (3) : 199-203) showed highest decrement in lipids (75.92% in midgut gland and 67.08% in muscle) followed by protein and carbohydrate in *Penaeus indicus* subject to starvation. He opines that it is due to a lipid oriented metabolism indicating reference of lipids to protein and carbohydrates. Barclay *et al.* (1987, *J. Exp. Mar. Biol. Ecol.*, **68** : 229-244) suggested that protein and not lipid is the major source of energy used by the shrimp *Penaeus esculentus* during starvation. The whole animal in their study lost 20% protein and 30% of its lipid after 14 days of starvation. Neil and Scheer (1953, *loc. cit.*) working on whole *Hemigrapsus nudus* showed that after 23 days of starvation, protein was the major energy source and observed no decrease in lipid. Surendranath *et al.* (1987, *loc. cit.*) found 40.6% decrement of protein in muscle and 67% in the hepatopancreas in *P. indicus*, starved for 15 days. Some studies have indicated that both lipid and protein are used (Schafer 1968, *loc. cit.*). In the present study, 75% decrement of protein and 73% decrement of lipid in haemolymph of *Metapenaeus dobsoni* starved for 21 days was observed. In the hepatopancreas protein decrement was 79% and lipid decrement 84% and in the muscle, the corresponding reduction in protein and lipid were 70% and 64% respectively. Maximum depletion of both protein and lipid occurred in the hepatopancreas, followed by blood and muscle in the starved prawn, but the percentage reduction in the amount of lipid and protein individually were more or less same in each of the tissues examined. Though it cannot be stated that metabolism is centered around lipid or protein, there is no doubt in the fact that protein, being the more dominant constituent in the body in terms of weight can be said to contribute more towards production of energy in starved prawn. Barclay *et al.* (1983, *loc. cit.*) had made similar observations in starved *Penaeus esculentus*, where lipid made a relatively smaller contribution to energy requirements inspite of their being depleted at a proportionally greater rate than protein.

It has generally been considered that the main role of free amino acids is as osmotic effectors (Dall 1975, *J. Exp. Mar. Biol. Ecol.*, **19** : 43-58; Schoffeniels 1976, *Biochem. Soc. Symp.*, **41** : 179-

204; Mccoid *et al.* 1984, *J. Fd. Sci.*, **49** : 327-330). But Torres (1973, *Comp. Biochem. Physiol.*, B **45** : 1-12) found that changes occurred during starvation in *Penaeus kerathurus*. The total FAA level rose at the end of the first week and then declined somewhat in the abdominal muscle of the prawn starved for 4 weeks. Mccoid *et al.* (1984, *loc. cit.*) comments that these free amino acids could constitute a labile pool available for energy production, since most of them are nonessential aminoacids. In the present study, the total FAA level rose continuously after an initial decline in the first week in the muscle and in the blood, while in the hepatopancreas it rose continuously. Studies of Odessey *et al.* (1974, *J. Biol. Chem.*, **249** : 7623-7629) and Fulks *et al.* (1975, *J. Biol. Chem.*, **250** : 290-298) indicated that the decline in essential cellular FAA during fasting initiates protein catabolism. It is therefore suggested that during early starvation, the existing FAAs from the pool is utilized and as starvation progresses, the protein in muscle, haemolymph and hepatopancreas is progressively hydrolysed. The liberated aminoacids enter the FAA pool and become available for energy production, the level of FAA in the pool being proportional to the amount of protein hydrolysed.

TABLE 5. *Evolution of weights or fed and starved shrimps*

Duration (Weeks)	Starved shrimp (g)	Fed shrimp (g)
O	1.04 ± 0.12	[illegible] ± 0.07
I	1.01 ± 0.19	1.13 ± 0.13
II	0.97 ± 0.16	1.24 ± 0.01
III	0.89 ± 0.11	1.30 ± 0.08
IV (Refeeding)	0.99 ± 0.09	1.38 ± 0.43

All values are $\bar{X} \pm SD$ of three determinations

In starved *Penaeus azetecus* the cholesterol content of the blood and abdominal muscle did not alter on a wet weight basis. However there was a significant decrease in hepatopancreas after starvation of two weeks (Castell *et al.* 1976, *J. Fish. Res. Bd. Canada*, **32** : 1431-1435). In the present study however, significant

decrease was also observed in haemolymph and muscle in addition to the hepatopancreas, though the rate of decrease in the initial period of starvation was much lower. This could probably be due to an extended period of starvation in the experiment. The results presented here demonstrate that even in invertebrates such as shrimp, dietary factors influence the cholesterol levels in tissues and that starvation mobilises the levels of cholesIterol particularly in the hepatopancreas.

In general, there is a decrease in oxygen consumption following starvation (Barnes *et al.* 1963, *J. Mar. Biol. Ass. U. K.*, **43** : 213-223). Wallace (1973, *Mar. Biol.*, **20** : 277-281) observed a 40% reduction in metabolic rate of starved *Carcinus maenas* during the first week and another 20% at the end of the third week. In the shrimp *Crangon bulgaris*, the active rate of oxygen consumption declines during starvation and steadily approaches the basal rate which is unaffected by starvation over one week. Surendranath *et al.* (1987, *loc. cit.*) noted significant decrement in total metabolism from 5-15 days starvation in *Penaeus indicus*. Similar trend was also observed in *Crangon crangon* (Regnault, 1981, *J. Comp. Physiol.*, **141** : 549-555). In the present study a 43% decrement in oxygen consumption was observed at the end of three weeks of starvation. This decreased oxygen consumption rate would obviously be sufficient to oxidise low amounts of organic stores utilized during starvation for their energy demands.

Thus, it can be concluded that though lipid and carbohydrate contribute substantially towards production of energy, protein is the major source of energy used by *Metapenaeus dobsoni* during starvation. However direct comparison of these results with others in the literature are difficult to make, because of the differing duration of starvation, partial tissue analysis of test animals and differing experimental temperatures.

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LABORATORY STUDIES ON FEEDING AND WATER EXCHANGE IN THE CULTURE OF *PENAEUS INDICUS* H. MILNE EDWARDS

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Introduction

Production rate in scientific prawn farming is scaling new heights with the introduction of high energy feed and resorting to high stocking densities. Feeding and water quality management are the constraints still to be overcome in intensive prawn culture. In intensive culture systems, since about 60% of the operation cost goes to feed, feeding should be optimised to get maximum results.

On account of high density stocking, artificial feeding is a must in intensive systems. Consequently left-over feed and deposition of faecal matter in the water system is bound to be high causing pollution by toxic metabolites such as ammonia, nitrite and nitrate, total suspended solids, high BOD and lowering of pH. Water quality problems caused by excessive feeding can be resolved by exchange, which ensures supply of oxygen rich water and flush out wastes. Eventhough excess metabolites may have detrimental effects upon the cultured species, in controlled amounts they can be beneficial to the growth of the cultured organism (Millemana 1990, *Aqucult. Eng.*, 9 (3) : 143-150). Therefore, an optimum water replacement rate that will bring the toxic metabolites to the permissible level would be desirable.

The present investigation was, therefore undertaken to study different feeding and water exchange rates on water quality and growth performance of the Indian white prawn *Penaeus indicus*, in the laboratory conditions with the hope, that it would serve as a guide for such work in fields.

Material and methods

Prawns of 50-60 mm sizes were acclimatised to laboratory conditions and used for the experiment. Fibreglass tanks of 40 l capacity each containing 30 litres of water with 10 animals and continuous aeration were used. For standardisation, the experiments were done in duplicates each of 6 days duration, one with 10% feeding and 10, 30 and 50% replacement of water and the second with 30% replacement of water with 10, 30 and 50% feeding levels. Prawns were fed twice a day, 40% in the morning and 60% in the evening. Specified quantity of water was replaced daily and the water samples in the morning were analysed for ammonia. The ammonia values were plotted in graph sheet for best fit and 13.5%, 24%, 42% and 75% of water replacements on logarithmic scale were selected. Five feeding levels such as 2, 4, 8, 16 and 32% of body weight on geometric scale were used against the above replacements.

The experimental set up was the "2 factor completely randomised design" given in Snedecor and Cochran (1973, *Statistical methods. 6th Edn. IOWA State Univ. Press., Ames.*, IOWA, pp. 593). Experiments were conducted in 4 sets, each of 24 days duration. Water was replaced every day and samples were analysed for ammonia by phenol-sodium hypochlorite method, nitrite by Strickland and Parsons (1972, *Bull. Fish. Res. Bd. Canada*, **167** : 1-310), nitrate by spectrophotometric method, TSS as per standard method and pH by digital 'Elico' pH meter on alternate days. BOD assays were conducted once in 4 days with procedures and equipment described in APHA.

Close monitoring of length and weight gain was carried out to study the biological parameters such as food conversion ratio (FCR), protein efficiency ratio (PER), gross conversion efficiency (K_1%), specific growth rate and condition factor. Results were subjected to ANOVA and tested for significance.

Results

Ammonia : At 2 and 4% feeding levels against 13.5% replacement it reached the maximum by 6-8 days and then declined upto the

10th/12th day. It again increased till 22nd day and further declined. At 8, 16 and 32% feeding, the ammonia level increased upto 8-10 days and then declined sharply at 12th from where it again increased by the 16th. The ammonia concentrations exhibited high fluctuations at 13.5% replacement at all feeding levels and it approached a steady state towards higher replacements. Statistically the ammonia was highly significant between feeding and replacements.

Nitrite : At 13.5 and 24% replacements the nitrite at all feeding levels averaged around 0.3 mg/l. At 8% feeding against 42% replacement the values were initially below 1 mg/l. It reached 0.211 mg/l on 12th day, then declined and registered values below 0.1 mg/l. Though the values showed similar trend at 16% feeding, they remained totally below 0.1 mg/l throughout. But at 32% feeding it increased to a level of 0.139 mg/l on the 8th day, declined on the 10th day to 0.09 mg/l and continued at this level during the rest of the period. Irrespective of the feeding levels, the nitrite values remained much below at 75% replacement. The variation in nitrite was hightly significant between replacements, but not at all significant between feeding levels.

Nitrate : The nitrate at all feeding levels against 13.5% replacement had the same trend of an increase from the initial to a peak on 8th day, fall on 12th day and a subsequent levelling. At 24% replacement at all feeding levels, there was a sudden increase from the initial on the 4th day and a subsequently steady, if not a gradual upward trend along with the levelling. At 42% also, the values were almost steady, but 75% replacement showed fluctuations at all feeding levels indicating the lowest on 12th day. Statistically the variation in nitrate showed the same trend as in intrite.

H^+ Concentration : There was a gradual decrease in pH from the Ist day to the last at all feeding levels. But it was more at lower replacements, against higher feeding levels. At 75% replacement the pH at all the feeding levels was above 8. The variation in pH was highly significant between feeding and replacements.

Total Suspended Solids : TSS showed higher concentration at higher feeding levels. The concentrations at 2, 4 and 8% feeding were below the permissible level of 15 mg/l at all replacements. But at 32% feeding even at higher replacements it was near to the permissible level.

Biological oxygen demand : With the advancement of days, BOD increased at all feeding levels, but the variation was less towards higher replacements at 2, 4 and 8% feeding. The BOD was highly significant between feeding and replacements.

Consumption : Prawns consumed the entire feed given at 2 and 4% levels. Above these, left over food was seen. The daily consumption showed an increase from 8 to 32%, but the percentage consumption showed a decreasing trend towards higher feeding rates. The percentage consumption is significant between feeding, but not significant between replacements.

Growth rate per day : There was an increase in daily growth rate with corresponding increase in ration size from 2 to 16% feeding and thereafter at 32% it decreased at all levels of replacements. The maximum growth (40.12 mg/d) was at 16% feeding and 75% replacement. In the case of 8% feeding, the growth rate at 42 and 75% replacement was 27.83 and 29.70 mg/d respectively, indicating not much difference between them. The growth rate was highly significant between feeding and replacements.

Specific growth rate : In weekly observations, specific growth rate showed negative values at 2% feeding, but increased and obtained positive values at 4% feeding. This increase continued upto 16% feeding, but declined at 32%.

The maintenance, optimum and maximum rations at each replacements arrived at from this is as follows :

Ration (in % of body weight)	Replacements			
	13.5%	24%	42%	75%
Maintenance	2.65	2.50	2.70	2.65
Optimum	7.00	6.10	5.65	6.35
Maximum	27.50	24.00	14.30	26.00

Food conversion ratio : FCR decreased from 2% feeding, to a minimum at 8% feeding (2.76 against 42% replacement and 2.74 against 75% replacement). The variation in FCR is highly significant between feeding levels.

Protein efficiency ratio : The PER increased at all replacements from 2% feeding upto 8% and then declined.

Gross conversion efficiency (K_1%) : At food rations below and above 8% the K_1% was low. The highest value (36.45) was obtained at 8% feeding at 75% replacement. The variation in gross conversion efficiency was highly significant between feeding levels.

Condition factor : The condition of the animals at 2% feeding decreased from the initial at all replacements. At 4% feeding it went slightly above the initital and reached the peak at 16%, but showed slight decline thereafter at all replacements.

Survival rate : The survival rate elevated parallel with increase in all replacements at 8, 16 and 32% feeding and against 75% replacement. It was as high as 90 at 8 and 16% feeding. The survival at all replacements was low at 2% feeding. Statistically it was highly significant between replacements.

Discussion

The increase in ammonia with increase in feeding rates observed in the present study can be equated to ammonia accumulation at higher feeding levels reported by earlier workers. In the present study the total ammonia (un-ionized and ionized) calculated at 13.5% replacement at 8, 16 and 32% feeding rates were 1.240, 1.106 and 1.205 respectively. These being much higher than Wickins' (1976, *Aquaculture*, 9 : 19-37) value may explain the poor growth of the animals.

In the present study also, reduction in appetite due to higher levels of ammonia can be attributed to the lower level of consumption at 13.5% and 24% replacements at 8, 16 and 32% feeding levels, as observed in previous investigations.

In the present study, the treatments in which the feeding levels were 8, 16 and 32% against 13.5 and 24% replacements can be compared to conventional ponds characterised by high fluctuations in ammonia and nitrite. The steady level of nitrite at higher replacements may be due to the continuous supply of oxygen. The sudden fall of nitrate after a peak on 7th day at 13.5% replacement is due to the denitrification process. In the present study, at lower replacements mortality having occurred only during ecdysis might have been influenced jointly by ammonia and nitrite toxicity.

There is little evidence that nitrate concentrations (25-100 mg NO_3-N/l) likely to be encountered in intensive culture systems are toxic to fish. The maximum concentration of the nitrate obtained was only 0.83 mg/l and it appears to do no harm. But accumulation of nitrate was reported to cause undesirable pH decrease, by replacement of carbonate and bicarbonate ions, as well as nitric acid fromation (Honig 1934, *J. Mar. Biol. Ass. UK.*, **19** : 723-725). Also, the reduction in pH may be due to the high concentrations of TSS. A reduction in pH was observed at lower replacements. At higher replacements due to dilution of nitrate concentration and reduction in TSS by additions of fresh medium the pH showed an increase.

BOD increases with increase in feeding level. Millemana (1990, *loc. cit.*) in laboratory experiments with *P. monodon* postlarvae observed high BOD and low survival at higher feeding levels. The low survival rate obtained in the present study at 13.5% replacement can be attributed to reduction in dissolved oxygen concentration which may increase the toxic effect of ammonia.

Food supply is probably the most important factor affecting the growth in fish and ration levels above the "optimal level" does increase the growth rate, although at a lower conversion efficiency.

In the present study the growth rate didnot increase at higher feeding levels eventhough the consumption was very high. But it improved towards higher replacements, because the

metabolic expenditure of energy due to stress on account of toxic metabolites is less and the energy from ingested food is directed towards growth.

The maintenance ration arrived at all replacements was around 2.5% of body weight. At low replacements due to stress the requirement for optimum and maximum food ration may increase and at higher replacements the increase may be due to the higher metabolic activity associated with higher consumption. According to Brett *et al.* (1969, *J. Fish. Res. Bd. Canada*, 26 : 2363-2393) the ration level which maximises both growth rate and conversion efficiency is intermediate to the feeding level yielding the maximum conversion efficiency and the feeding level providing the maximum growth rate. Thus in the present study this ration may be inbetween 8% feeding which gave maximum conversion efficiency and 16% which gave the highest growth rate.

In the present investigation a decrease in FCR from 2% to 8% feeding and then an increase towards 32% was observed. The initial decrease in FCR with increasing feeding levels according to Niimi and Beamish (1974, *Can. J. Zool.*, **52** : 447-456), is due to the increased utilization of energy for growth from zero at maintenance, to maximum at optimum level. At higher feeding levels the utilization of energy for growth decreases resulting in an increase in FCR. The rise in food conversion values at high feeding rates was attributed to high ammonia concentrations (Boyd 1985, *CMFRI Spl. Publ.*, **22**, pp. 96). The decrease in FCR at 8, 16 and 32% feeding against higher replacements as observed in the present study, may be due to the low ammonia concentrations since stress due to undesirable metabolites are low.

A steady increase in condition factor upto 16% feeding as observed in the present study is in accordance with the results obtained by Chua and Teng (1982, *Aquaculture*, **27** : 273-283). The increase in condition factor may be due to the increased utilization of energy for growth. Since the metabolic activity of the animals is high at 32% feeding, the condition is bound to

come down. The increase in condition factor towards higher water replacements as observed in the present study may be due to the decreased utilization of energy for maintenance on account of the metabolites remaining below the permissible levels. The reduction in condition factor at 2% ration may be due to the utilization of fat to meet the energy requirements at below maintenance level and thus the animals lose weight.

A combination of the adverse environmental conditions such as high BOD, low DO, high NH_3-N and N at lower replacements in the present study may be the reason for the low survival rate. Survival was not significant between feeding levels, while it was significant between replacements.

The desirable feeding level may be inbetween 8 and 16% and replacement in between 42 and 75%. Further work within this range is required to find out the exact levels. The study at present being in the laboratory conditions requires varification in field for application in farming.

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STUDIES ON THE ENZYME PHENOL OXIDASE IN *PENAEUS INDICUS*

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Introduction

The exoskeleton of prawn is a rigid calcareous structure. Its basic structure consists of a thin outermost layer of lipoproteinous epicuticle followed by a procuticle. The exoskeleton of a newly moulted prawn will be soft and pale in colour, but becomes harder over a period of few hours. This hardening process is called sclerotization. In this process quinones of the tyrosine metabolism react with the free amino groups of the structural proteins of the exoskeleton called Arthroidin, to give structures cross-linked with covalent bonds. The quinones are formed from the O-dihydric phenols by the action of O-dihydroxy phenol oxidase. It has also been found that in the tyrosine metabolism conversion of tyrosine to the final substrate of the enzyme phenol oxidase is triggered by the moulting hormone ecdysone. It also removes the inhibition of the phenol oxidase enzyme. So it can be concluded that hardening of the new cuticle is initiated by phenol oxidase enzyme which inturn is influenced by ecdysone.

In the present study, the enzyme phenol oxidase was partially purified and some of its properties were studied followed by experiments on the effect of steroid hormones ecdysone, progesterone and estradiol on this enzyme. The study was also extended to observations on the enzyme activity in soft prawns. The Indian white prawn *Penaeus indicus* was used for the study.

Material and methods

The haemolymph was collected by piercing the heart with a No. 26 stainless steel injection needle and withdrawn into a

1 ml syringe and stored in a polyethylene vial and syringes were rinsed with 5% trisodium citrate solution before use. The haemolymph and exoskeletel extracts of exoskeletel parts namely rostrum, carapace, abdomen, telson and uropod were used for protein and enzyme assay. Protein was estimated by the method of Lowry *et al.* (1951, *J. Biol. Chem.*, pp. 193-265). The enzyme assay was done by the procedure of Preston and Taylor (1970, *J. Expt. Zool.*, 71 : 489) with slight modifications.

The enzyme was partially purified from 50 ml of haemolymph by ammonium fractionation method. The purified enzyme was used for studying the properties. In all the studies 0.33 mM adrenalin was used as the substrate.

The experiments were done in triplicates. In the first experiment, 20-hydroxy beta ecdysone was injected at a rate of 1 µg/gm of body weight continuously for three days. At the end of third day animals were sacrificed for enzyme assay. In the second experiment out of three groups of premoult animals, two groups were given 17-beta estradiol and progesterone injections and the third group was used as control. The experiment was continued for three days as in ecdysone experiment.

Results

Partial purification of the enzyme phenol oxidase resulted in 5 fold purification with an overall recovery of 52%. The time of incubation is 3 minutes and optimum temperature of incubation is 50°C. The enzyme was stable upto 45°C. It lost 60% of its activity on heating to 70°C for 10 minutes. The optimum pH of phenol oxidase was recorded at 8.5. A slight decrease in activity was observed from pH 5.0 till 6.5 indicating the possibility of a minor peak below pH 5.0. The enzyme showed varying degrees of activity towards the bi-phenolic substrates differing in their side chain with the order of preference as Adrenalin > Dopamine > Catechol > Dopa. Monophenolase activity was totally absent. From the Line weaver Burk plot of the enzyme with adrenalin as the substrate k_m was

found to be 0.129 mM and V_{max} for the enzyme was observed to be 0.870 μ moles/mg protein/min.

Salts of magnesium, manganese and calcium were observed to stimulate enzyme activity. Cupric salts at lower (10^{-3}M) and higher (10^{-4}M) concentrations and iron at 10^{-4}M concentration, was found to be inhibitory. Sodium metabisulphite and mercaptoethanol very strongly inhibited the enzyme. Inhibition of activity was also observed with 1-10 Phenanthroline, EDTA and 8-hydroxy Quinoline. No inhibition was observed with cysteine - HC1.

The haemolymph and exoskeleton extracts obtained from the animals injected with beta ecdysone, progesterone, estrogen as well as control group (Intermoult stage and premoult stage respectively) was assayed for phenol oxidase activity. The results are expressed as specific activity (OD units/mg protein/min).

In the haemolymph of the ecdysone injected animals, a highly significant increase in enzyme activity (0.0505 ± 0.022) was observed when compared to the intermoult control (0.031336 ± 0.099). Similarly a highly significant increase in enzyme activity was observed in the extracts of rostrum (0.14327 ± 0.08516), carapace (0.16398 ± 0.1461), telson (0.41324 ± 0.02239) and uropod (0.05366 ± 0.0346). The exoskeleton at the abdominal region was found to be detached and hence change in the enzyme activity was found to be statisticaly not significant in the abdomen.

In the haemolymph of the progesterone and estradiol injected prawns, no significant change in enzyme activity was observed. In the exoskeletal extracts of the progesterone and estradiol injected animals a highly significant decrease in enzyme activity was observed in rostrum, carapace, telson and uropod.

In the haemolymph of soft prawns a highly significant increase in enzyme activity was observed when compared to

intermoult control from the same pond. The exoskeleton of the soft prawns also showed a highly significant increase in enzyme activity. In the exoskeleton rostrum showed highest enzyme activity when compared to other parts.

Discussion

Properties of phenol oxidase from different sources vary considerably. In the case of phenol oxidase from *Fasciola hepatica,* the reaction rate was linear for approximately 20 minutes. In the present study the reaction was linear for approximately 3 minutes. The enzyme clearly preferred adrenalin as the substrate. The enzyme in bivalve *Modiolus demissus* have a pH range between 8.0 - 8.5. *P. indicus* had 8.5 as the optimum pH.

Increase in enzyme activity was reported in the liver fluke when copper sulphate was added to the reaction mixture. In the present study copper salts inhibited the enzyme activity. The enzyme phenol oxidase was found to be severely inhibited by Mercaptoethanol and sodium metabisulphite. This shows the enzyme to be having a disulphide bridge for its activity. The enzyme was also severely inhibited by metal chelators such as EDTA and 1, 10 Phenanthroline. This shows the requirement of certain metals as cofactors by the enzyme for its activity. To support this it has also been observed that overnight dialysis of the enzyme reduced its activity.

Injection of ecdysone into *Calliphora erythrocephala* larvae, which have been ligated shortly before pupation, induces synthesis of several enzymes particularly of dopadecarboxylase. Since the blockage of DNA-dependent RNA synthesis prevents this effect, the hormone appears to control the transcription of a specific gene coding for the enzyme in question.

The RNA isolated from ecdysone treated insects had about six times higher template activity for the dopadecarboxylase than RNA from controls. Exposure of cells or nuclei to ecdysone induces the formation of specific puffs occurring normally in the course of the moulting process. This cytological

observation complements the biochemical findings that ecdysone stimulates the synthesis of specific messenger RNAs. The hypothesis that ecdysone acts on the level of transcription thus rests on the assumption that induction of enzyme synthesis or occurrence of the puffs are the primary effects. In the present study there was a highly significant increase in phenol oxidase activity in the haemolymph of beta ecdysone injected animals when compared with the control. In the exoskeleton the increase in the enzyme activity was highly significant with regard to rostrum, carapace and telson whereas the increase was significant with regard to uropod and non-significant with regards to abdomen. At the end of the experiment the exoskeleton of the abdomen was found to be detached from the underlying body tissue. This explains the results obtained in the case of abdomen.

The crustacean ovary seems to have the ability to metabolise steroids. Ovariotomy blocks vitellogenin synthesis in *O. gammerella* despite the fact that in such females Y-organ, the source of ecdysteroids was intact and active (Junera *et al.* 1977, *Gen. Comp. Endocr.*, **31** : 457-462). Hence experiments were conducted to know the effect of hormones of reproduction (progesterone and estradiol) on the enzyme phenol oxidase that hardens the exoskeleton under the influence of ecdysterone. In the present study there was no significant change in the phenol oxidase activity in the haemolymph of the hormones (progestrone and estradiol) injected animal and the premoult control. However a highly significant decrease in phenoloxidase activity was observed in the exoskeleton (rostrum, carapace, telson and uropod) of progesterone injected animals. In the exoskeleton (carapace, telson and uropod) of estradiol injected animals there was a highly singificant decrease in enzyme activity, though the enzyme activity *per se* in the rostrum of estradiol injected animal was found to be less than that of the control. Due to the effect of progesterone and estradiol whose function is towards reproduction, the enzyme (phenol oxidase) expressed due to the moulting hormone get retracted from the exoskeleton into the haemolymph to be subsequently carried to the target tissue for getting metabolised into aminoacids.

No significant change in the enzyme activity was observed in the abdomen of either the control or hormone treated animals. This may be because of the already rising levels of ecdysone which increases the synthesis of the exoskeleton hardening enzyme phenol oxidase in premoult animals. Because of the hormones progesterone and estradiol that are injected the further increase in the enzyme level is prevented.

A highly singnificant increase in phenol oxidase activity was observed in the haemolymph of the soft prawns compared to control. The same trend could be seen in the exoskeleton of the soft prawn. This may be because of the need of the body to produce more phenol oxidase to harden the soft exocuticle of the soft prawns. But in reality inspite of the high enzyme level the exoskeleton remains soft. This may be because of the non-availability of the substrate by certain inhibition of the substrate producing mechanism.

Rostrum being used in the defense mechanism of the prawns, more of the enzyme as well as the substrate is being pumped into that region as a sequence to the body's need to keep the portion hard. But other parts of exoskeleton are found to be soft. This may be because of the inadequate supply or damage of the substrate production mechanism in those regions. More studies are needed to confirm this view.

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A COMPARATIVE STUDY OF METAL IN WATER, SEDIMENT AND BIOTA FROM SELECTED AQUACULTURE SYSTEMS IN THE COCHIN AREA

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Introduction

Aquaculture has been chosen as a source for augmenting production which will also help in creating employment opportunities and the improvement of rural economy. Since many of the traditional culture farms are situated in the vicinity of industrial, municipal and estuarine areas the possibility of contamination of the water is apparent, besides this the inputs used in the culture system may also contribute pollutions resulting in poor quality of water.

The safety of products for consumption is of prime concern from the point of view of managing aquaculture, as well as ensuring public health. In the light of strict quality control measures prevalent for exported marine food products, it is essential to have information on metal levels, in the aquaculture products. Both in enclosed farms as well as in open water, metals are persistent and have the capacity of bioaccumulation. As aquaculture has been located in inland or inshore waters, there is a greater chance of contamination through domestic agricultural and industrial water.

The present study has been undertaken to draw some attention on the heavy metal contents leading to pollution in aquaculture systems which play a vital role in determining the quality of the product. Other aspects such as seasonal variations, sediment characteristics and size differene in accumulation rate, etc. were also studied.

Material and methods

Study area : Taking into consideration the varieties of culture practices prevailing in the region, three different types of culture systems have been selected for this study *viz.* (i) seasonal pokkali field at Cherai (st. 1) having an area of 5 acres with depth ranging 0.5-1.0 m, (ii) perennial culture system at Narakkal (st. 2) with an area 4 acres having an average depth 1.5 and (iii) a canal system at Puduvyppu (st. 3) having a depth of about 1 m.

Collection of samples : The samples (water, sediments and prawns) were collected from each stations at fortnightly intervels from April-September 1992.

Estimation of parameters : Water temperature was measured by a mercury thermometer. Turbidity was recorded with a secchi disc. pH of water was measured using an ELICO digital pH meter. Salinity was determined by the classical Mohr titration method and total hardnes was determined as per standard guidelines given in APHA-AW WA-WPCF. Sedimentological parameters such as grain size, organic carbon and pH were also studied.

Heavy metals : The metals C, Cu, Zn, Pb and Cd were determined from water, sediment and prawns. The heavy metals in water were determined by simultaneously extraction of their complexes with APDC into MIBK and the subsequent analysis using atomic absorption spectrophotometer. 1 gm of sediment sample was digested with HNO_3 and $HClO_4$ (10 : 2) and analysis was done in a Perkin Elmer 2380 AAS (FAO 1971-1975) on an air acetylene flame. 10 gm of the abdominal muscle of prawns was taken and digested with HNO_3 and H_2O_2 and subsequent analysis was done in Perkin Elmer 2380 model AAS on an air acetyline flame. Besides this bioaccumulation factors were also calculated using the formula.

$$BAF = \frac{\text{Concentration of an element in the organism}}{\text{Concentration of element in the sea water}}$$

Statistical analysis : The collected data from three stations were analysed (ANOVA - two ways) to find out whether there is any significant difference in the heavy metal level among the stations and dividing the period of sampling as well to correlate the relationship between the environmental and biological parameters.

Results

Hydrological parameters : Water temperature in all the three stations covered, ranged between 27.5°C and 34.0°C. Lowest temperature was recorded during August at Narakkal and highest during April at the same station. In general water temperatures were high during April. The pH ranged between 6.67 and 9.2. The highest value was recorded during August at Narakkal and lowest was recorded from Puduvyppu. In general, at all stations pH of water were on the alkaline side. Salinity ranged from 0.81 ppt during August to 38.4 ppt during third week of April. Lowest was recorded from Narakkal and the highest was recorded from Cherai. Total hardness varied from 146.7 ppm during 3rd week of August to 738.35 ppm during third week of April. Highest value was recorded from Narakkal and lowest was recorded from Cherai. The values were high during premonsoon and low during monsoon seasons. Turbidity values varied from 30.5 cm during 3rd week of August to 49 cm during first week of September. Highest value was recorded from Puduvyppu whereas the lowest (30.5 cm) was recorded from both Puduvyppu and Narakkal.

Sedimentological parameters

Soil pH varied from 6.67 to 8.65 during September and April respectively. Both these values were recorded from Cherai whereas in other two stations the reported pH was within the above range. The soil was found to be more alkaline in monsoon months. The soil at Cherai is predominantly composed of sand (53.79%), silt (27.726%) and clay (18.48%). At Narakkal the composition was sand (61.12%), silt (29.177%) and clay (9.703%). At Puduvyppu grain size analysis revealed a composition of sand (51.07%), silt (25.48%) and clay (23.448%). Organic carbon

content varied from 0.27% to 2.73%. Highest value was recorded from Puduvyppu during April and lowest at Narakkal during August. At all the three stations organic carbon content decreased from April-September *i. e.* 1.425% - 0.84% at St. 1, 2.73% to 1.23% at St. 3 and 0.6% - 0.27% at St. 2.

Heavy metals

Water : Off the four metals studied lead showed the highest value in water (319 ppb at Puduvyppu followed by Zn (53.5 ppb at Cherai) followed by Cu (17.25 ppb at Cherai). At Cherai the heavy metal levels were as follows : Copper 2.75 - 17.25 ppb, zinc 29.25-53.5 ppb and lead 0-38 ppb. At Narakkal, the metal levels were : copper 3.25-10.0 ppb, zinc 8.75-51.5 ppb and lead 0.0-0.235 ppb. At Puduvyppu, the values were : copper 4.25-14.0 ppb, zinc 5.75-47.75 ppb and lead 0.0-319.0 ppb.

Sediment : Off the four metals studied in sediment, highest value was recorded for Zn (38.5 ppm at Puduvyppu) followed by lead (18.25 ppm at Narakkal), copper (13.45 ppm at Puduvyppu and then cadmium (9.86 ppm at Puduvyppu). At Cherai, metal levels were : copper below detectable limit to 9.75 ppb, zinc 7.5-22.0 ppm, lead below detectable level-7.5 ppm and cadmium below the detectable level to 2.88 ppm. At Narakkal, the metal levels were copper below detectable level-9.99 ppm, zinc 2.5-31 ppm, lead below detectable level to 18.25 ppm and cadmium 0.0 ppm-2.47 ppm. At Puduvyppu, the metal levels were : copper 1.25 to 13.45 ppm, zinc 1.75 ppm-38.5 ppm, lead below detectable level 14.21 ppm and cadmium below detectable level-9.86 ppm.

Prawns : Abdominal muscle of *P. indicus* and *M. dobsoni* were analysed and of the four metal analysed zinc was found to be higher (18.31 ppm) followed by copper (9.91 ppm), lead 8.612 ppm and cadmium 0.113 ppm in *M. dobsoni*. At Cherai the metal levels were : copper 1.98-9.91 ppm, zinc 7.78-15.08 ppm, lead below detectable level to 2.378 ppm and cadmium below detectable level to 0.029 ppm in *M. dobsoni*. In *P. indicus* cadmium was always below detectable levels in all the three stations. At Narakkal the metal levels were : copper 2.71-

5.89 ppm, zinc 8.33-15.081 ppm and lead below detectable level to 1.226 ppm and cadmium from below detectable level to 0.101 ppm. At Puduvyppu, the metal levels were : copper 1.44-9.43 ppm, zinc 6.66-11.688 ppm, lead below detectable level to 8.612 ppm and cadmium was always below the detectable level in *M. dobsoni* and *P. indicus* at St. 3.

Bioaccumulation factors : Bioaccumulation factor recorded was highest for copper (3049 ppm) then lead (2375.5 ppm) and then zinc (1967.83 ppm). At Cherai the bioaccumulation factors were : copper 139.29 ppm - 3049 ppm, zinc 182.07-1251.42 ppm and lead 0.478.25 ppm.

At Narakkal the values were as follows : copper 255.07-1795.38 ppm, zinc 182.97-1251.42 ppm and lead 0-2375.5 ppm. At Puduvyppu canal system the metal levels were : copper 160.97-1110 pm, zinc 175.61-1697.82 ppm and lead 0-274.70 ppm.

Discussion

The culture fields and canal system studied are directly connected to the brackishwater and inshore water and are therefore subjected to wide variations in the environmental conditions.

Water temperature prevailing in the culture system influences the biochemical reaction, microbial release of nutrients, redox conditions and also the physiology of fauna and flora including the growth and metabolic activities. The monthly variation in temperature in different culture field were approximately 2-3°C. Since these fields are shallow, the entire water coloumn was warm through out the study period. The earlier studies also indicated the same trend as in the present observation. The variation in water temperature may be due to variation in air temperature and also due to influence of tide. pH in these culture fields showed alkaline character during all months except for the slightly acidic value of 6.6 during the first week of July noted in Puduvyppu which may be due to increased sedimentation and subsequent reduction of organic debris resulting in relatively more reducing conditions near the

bottom. Earlier reports by Subhash Chander (1986, Ph. D. Thesis. *Cochin Univ. Sci & Tech.*, pp. 325), Prasad (1982, M. Sc. Dissertation. *Cochin Univ. Sci & Tech.*, pp. 80), while investigating the ecology of brackishwater culture system near Cochin, high pH values were observed for water and sediments during monsoon and postmonsoon, same situation was noted in these culture fields, during the present study period also.

High salinity (upto 38.5 ppt) were recorded during premonsoon. The precipitation and fresh water discharge into the ecosystem during monsoon reduced salinity and the minimum values were recorded mostly during monsoon period (0.81 ppt). In general higher salinity values were recorded during April and May and lower salinity was recorded during July and August observation agress with that of earlier works of Gopalan *et al.* (1982, *Proc. Symp. Coast. Aquacult. MBAI*, **1** : 151-159) and Prasad (1982, *loc. cit.*).

High organic content in soil is probably due to the exchange of water and the presence of suspended vegetation. Pokkali fields showed a high organic content and this may be due to the death and decay of benthic animal during the prawn culture season and the subsequent decay of paddy stumps after cultivation.

The metal levels were always higher in the sediment when compared to the metal levels in water and prawns. This increased metal concentration may be due to the flocculation and settling of colloidal particles in the water to the bottom according to changes in hydrological parameters.

The lowest level recorded for Cu was 2.75 ppb (May) and the highest was 17.25 ppb (July). Joshi (1990, Ph. D. Thesis. *Cochin Univ. Sci & Tech.*, pp. 192) reported similar trends 3.44 ppb during premonsoon and (12.64 ppb) in August. In the perennial fields the value reported by the same author was slightly more (8.69 ppb) during August and minimum of 3.44 ppb which is similar to the present data obtained for the perennial fields. During the present study, non-conservative behaviour of copper was quiet apparent.

Proximity to Udyogamandal Industrial Complex and other industrial activity in the vicinity, discharging their effluents into the Cochin Backwater may account for higher concentration of copper during runoff period as effluent rich sediment are flocculated and rushed out (Subhash Chander 1986, *loc. cit.*; Venugopal *et al.* 1982, *Mahasagar*, **15** : 205-214). It may be also due to land runoff from the agricultural fields where large scale application of copper containing fungicides also noted.

Zinc concentration generally varied between 7.75 ppb (June) and 53.5 ppb (September). Generally high values were recorded during August and September. Maximum concentration was recorded in August and September in Pokkali fields during the study period. Joshi (1990, *loc. cit.*) also reported a similar trend. Observation by Ouseph (1992, *Proc. Int. Dept. Seminar Mar. Poll. India*, DOD, New Delhi, pp. 86-105) a) in the water of Cochin Estuary also showed high value for Zn during monsoon seasons. When compared with the reports of Joshi (1990, *loc. cit.*) and Subhash Chander (1986, *loc. cit.*) much higher value were found during August and September.

Higher value recorded for lead was 319 ppb during April. Generally highest values were noted before monsoon showers. Ouseph (1992, *loc. cit.*) noted highest value for the Pb during premonsoon. The high concentration during premonsoon may be due to evaporation and increased dissolution from sediments and also due to higher summer temperature and larger content time in summer (Paul and Pillai 1983, *Water Air Soil Pollt.*, **19** : 63-73).

Cadmium values were always below the detectable levels during the study period in all the three stations, probably due to limitations in the equipment used.

Low values of heavy metal levels were noted in the water during premonsoon when the salinity of water were high and high concentration of heavymetal were observed during low saline conditions. This may be due to the predominant chemical form of complexed heavy metal in high saline waters and also

due to the precipitation of heavy metal at higher salinites. Ouseph (1992, *Mar. Poll. Bull.*, **24** (4) : 186-192) also noticed that maximum precipitation occurs for more than salinity for 10 ppt.

Copper values in sediment were high (9.75-13.45 ppm) before monsoon and it decreased towards September. The value obtained for July, August (6.8-11 ppm) is similar to the observation of Subash Chander (1984, *loc. cit.*) and in prawn culture field the reported level was 8.62 ppm during August. The high values recorded during premonsoon may be due to precipitation of these metals at high salinity followed by settling.

High values of zinc was reported during May and low values were reported during September. This observation is similar to that of Ouseph (1987, *loc. cit.*) who reported that in general monsoon season showed the lowest and premonsoon showed the highest concentration in sediments.

High values for lead is noted during the monsoon season. This may be due to the result of precipitation.

Low values for cadmium were recorded (0.018 ppm) during May and higher levels (9.86 ppm) during August. The high concentration of Cd in sediments during monsoon season might have resulted from the runoff containing domestic and industrial wastes. This view can be supported by the findings of Ouseph (1992, *loc. cit.*). He reported high cadmium pollution in Cochin Estuary and that it might be partly due to industrial and domestic pollution as Zn factory located near the bank of Periyar River discharges effluent which may contain zinc and cadmium.

High concentration of the metals was noted in the sediment during April and May, the concentration of element showed a direct relationship with the organic matter of the sediment. The organic carbon content decreased in the sediment from April-September.

Relatively higher concentration of copper was noticed in the muscle tissue during premonsoon (9.91 ppm at Cherai and 8.9 ppm at Narakkal). At Puduvyppu it was highest (9.438 ppm) during June.

The bioaccumulation factor for Cu was very high and it may be due to the imbalance between metal excretion and metal uptake. The bioaccumulation factor noted here corresponds to the high level in tissues since the metal concentration in water was comparatively low.

The Zn values were high (18.316 ppm) during April and a gradually decreasing trend was noted from April to September. But the fluctuation in values were not high. The range of Zn concentration, similar to present study have been reported by earlier workers.

High concentration for lead is noted during May and also during September. High concentration in May may be due to the mobilisation of this metal from industrial and domestic wastes as noted in the case of zinc by earlier workers from this area. The increase in metal level during September may be due to high concentration in water which the animal might have absorbed through the body surface.

Cadmium concentration was very low in the tissues when compared to other metals. The values reported during monsoon season may be due to slightly increased concentrations in the sediments during monsoon season.

As pH values recorded in all stations were almost similar, it will not be having much relation with variation in the heavy metal levels in muscles. The concentrations of heavy metal were high in prawns of smaller size when compared to bigger ones.

Since the culture systems used in the region are situated in the vicinity of industrial area, it is essential to have a baseline data on the levels of toxic substance such as metals in the environment and cultured species. The present study highlights the significance of such information for the management of shrimp culture in the region.

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SOCIO-ECONOMIC STUDY OF PRAWN FARMERS IN ERNAKULAM DISTRICT

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Introduction

The socio-economic improvement of farmers is the primary objective of all fishery development schemes. Coastal villages in Ernakulam District are still backward and farming of naturally available shrimp resources by simply trapping them, has to some extent, improved the living conditions of these people through income generation. Improved culture techniques are also accepted and practiced in Narakkal area.

The present study is confined to Narakkal Village, covering a large number of farms of different sizes to compare and analyse the relative advantages of scientific prawn farming in terms of the betterment of their socio-economic conditions and general standards of living. Only through this kind of surveys the problems faced by the farmers in field conditions and also the extent of innovations in culture techniques alongwith the comparative advantages of these practices could be analysed.

Along with the development in advanced technology, the unit productivity has been increasing steadily. But it remains to be analysed why after more than three decade of such developmental work, still, poverty, unemployment, malnutrition, low-living standards and inequalities in income persist among the coastal-folk. Technological improvement has indeed overcome low yields and other production constraints. But socio-economic aspects have not so far been given due weightage in the process of aquaculture development. Poverty and indebtedness in the rural areas are very common and wide spread especially among marginal and small farmers which inhibit them in applying required inputs for farming.

The ultimate goal in the development of aquaculture, aims to the removal of poverty, malnutrition attainment of self-reliance and employment generation among the coastal people. The major steps taken to elaborate on aquaculture development, include a well conducted socio-economic evaluation and a comparative study of different types of management systems that will lead aquaculture development in the right direction.

The primary cause for the developments in shrimp farming falls back on the problems and needs of the prawn farmers. Inorder to identify them and then proceed with the need based research projects, a socio-economic survey of existing farms is very important which will provide a basic, information about the current status, problems and constraints of shrimp culture in particular.

The main aim of the study was to analyse the socio-economic condition of the different types of prawn farms namely marginal, small and large. Practical suggestions for the socio-economic improvement of farmers are indicated in the present study and the results will be significant for future planning of aquaculture development programmes.

The survey

A schedule was prepared covering all the family particulars, religion and caste, details on literacy level, type of house, material possessions, occupation, both main and subsidiary, household consumption, and also details covering the entire economics of prawn culture, animal husbandry and other cultivations, if any. The constraints, general problems and farmers' opinions on the feasibility of prawn culture in improving their socio-economic conditions were collected, covering a large number of farms at random, through a personal interview. The formats were pooled into three groups based on the size of the respective water-holdings with upto 1 acre being marginal, more than 1 to 20 acres being small and more than 20 acres being large farms. The different socio-economic parameters were then analysed on a comparative basis, with a sample size of 13 marginal, 45 small and 13 large farms each.

Analysis of variance (ANOVA) was used to find out the difference between the three categories of families for different parameters such as farm income, inputs, consumpion, indebtedness, stocking density, yield and farm size. Correlations were also worked out for the above cited parameters.

Results and recommendations

In Narakkal where a case study was conducted, prawn farming is carried out in seasonal ponds while year-round culture was limited to some areas of Vypeen Island. The livelihoods of people in Narakkal area mainly depends on paddy cultivation, fishing, growing coconuts and prawn farming. The people are by and large poor. Majority of the population belongs to socially and economically backward communities.

The average family sizes were 7.7, 5.5 and 5.6 for marginal, small and large farmers respectively. The male : female ratios showed that except among marginal farmers the males outnumbered the female population contrary to the trend in Kerala State.

The literacy level was analysed based on the educational status of each family member in the three categories of farms. Illiterates were more among marginal farmers and almost the some number among small and large farmers. By illiterates it is meant that apart from those who know only to recognise alphabets and sign their names, children below 5 years of age are also included. Majority of the marginal farmers have less than high school qualification while among small farmers 6% comes under the category of graduates and postgraduates.

The dwellings under the category of marginal farmers are kutcha houses, with a set of one room and a kitchen. More than 1/3rd of the houses occupied by small farmers are having kutcha/pukka walls and thatched roof, and the remaining are pukka houses with one or two rooms with a provision of latrine and bathroom outside the house. In the category of large farms about 80% families have pukka houses and the reamining kutcha

houses. The necessary material possessions were wanting among marginal farmers and they had very limited luxury items. The water and electric connections are yet to be provided to their houses. Small farmers are fairly equipped with the luxury items like television, refrigerator, radio and furniture. In certain pockets of the surveyed area, there is no provision of electricity. The position of large farmers can be safely equated with the small farmers. Though gross income for the large farmers is more than that of small farmers, the profit realised per unit of farm area is comparatively lesser.

The farmers excluding a few have taken water-bodies on lease at the rate of about Rs. 10,000/ha. Their main occupation includes prawn farming, animal husbandry and other alternate jobs. For about five months, they are involved in prawn farming which earns them 25% of their total annual income. The credit facilities for the culture are available from different sources like HDFC, Cooperative Societies, Commercial Banks, Matsyafed, Private prawn buyers, friends, money lenders and relatives. The total annual gross income of the marginal farmers is worked out at Rs. 24,761. Since the farm area for culture is only 0.53 acres, the income from prawn farm is about Rs. 6,140 per family.

For small farms annual income from all sources worked out to be Rs. 1,00,000 which includes Rs. 14,000 from other sources and rest from prawn farming. Similarly, the gross income for large farms averages at Rs. 1.6 lakh including Rs. 12,000/- from subsidiary occupations. Thus, the prawn farming contributes more than 80% to the gross income in case of small and large farmers. If the income of all the 71 families is analysed, per family income comes to about Rs. 98,0000, out of which more than 80% comes from prawn farming.

The house-hold expenditure, in case of marginal farmers is worked out at Rs. 20,000/annum. The essential consumable items like cereals, pulses, fats, vegetables, egg, meat, fish, sugar, salt, tea, etc. account for about 70% of the expenditure. The semi-essential items like clothes, foot-wear, education fees, drugs and medicine, refreshment and toilet items account for 14% while

non-consumable items like conveyance, hair dressing, laundry, entertainment and rents account for 16% of total expenditure.

The costs and returns have been analysed separately for the three categories. For small farmers, the average annual house-hold expenditure amounts to Rs. 45,187 of which 46% is incurred on essential items, 28% on semi-essential items and 26% on non-consumable items. In case of large farms, family expenditure is Rs. 46,292 per annum. Of total amount spent on house-hold items, essential items share 45% semi-essential items 27% and non-consumer items 28%.

In Narakkal area majority of the farms surveyed under this study are using inadequate quantities of inputs required per unit area of farm, which is responsible for over all low yield of prawns. The farms are predominantly stocked with *P. indicus* seeds. Other varieties that enter these farms by tidal filtration include *M. dobsoni, M. monoceros* and *P. monodon.* The main sources of seed availability are collection centres which collect seeds from wild, especially from the surf-zone of the sea and from the brackishwater canals, the former ones are in the postlarval stage measuring 8-12 mm in size and the latter are juveniles of the size 20-40 mm. Apart from these two sources, hatchery reared larvae are available from CIBA, Narakkal. The stocking density in marginal farms averaged at 30,842 No. The seed cost is found to be Rs. 1002.5/acre in these farms. On small farms, the stocking density is of a little lesser magnitude and the total investment on seed is Rs. 8746. On large farms, an amount of Rs. 23921.2 is incurred for an average size of 34.13 acres. Thus, it is found that larger the farm, lesser is the stocking density and the amount incurred on seeding per unit of area.

The use of feed in the farms was not significant owing to the unavailability of high quality feeds in the market. Feeds such as clam meat, tapioca powder, ground-nut oil cake and prawn meal are used. Some of the farmers also use home-made feeds like cooked rice which is given to postlarvae and juveniles in the farm of a paste.

An average amount of Rs. 40 is taken as wage for one man-day labour. The hired labour is mainly used for preparing the pond before starting the culture, like repair of bunds and fixation of sluice gate and also for harvesting. Family labour is mainly engaged in feeding, maintenance of stock, watch and ward, etc.

The total variable cost for marginal farmers is calculated at Rs. 3699/acre whereas for small and large farmers it comes to Rs. 2236.9 and Rs. 1655.2/acre respectively. The main components of fixed costs in prawn farming include sluice-gate, nets and other equipments and land lease. The details on variable and fixed costs in all the three types of farms are given in the original Dissertation.

Due to more use of inputs on marginal farms the yield per acre was higher than the other two categories. The overall average yield on marginal farms (380.76 kg) and small farms (312.3 kg) has been noted more than the average yield of 71 farms (223.54 per acre) whereas on large farms (142.13 kg) it is less than the average yield.

For calculation of gross income from prawn farming, production per farm has been multiplied with the 1 kg price of prawn. The price of the prawn is decided based on the count of the prawn-per kg. The overall average income has been calculated at Rs. 83,379.3. Price realised per kilogram of prawn comes to Rs. 31.35. Since there is a significant difference in the productivity and yield between different type of farms, the revenue realised on different farms, per unit of area also significantly differs. The net income per farm averages Rs. 21,685.7. Among the three types of farms studied here, per acre net income is highest for small farms (Rs. 3610.1) and lowest for large farms (Rs. 241.0) and low income is realised from marginal farms also (Rs. 1377), which is attributed to improper management practices.

A Cobb-Douglas type of production function has been used for calculating Marginal Value Productivity of inputs. The co-efficients of regression analysis, mean values of revenue and

cost of inputs and maximum profitable level values were analysed and given in detail in the original Dissertation. The analysis showed that the important factors of production such as seed and labour have been under utilised and that these factors of production can be enhanced about 3 to 4 times to get the maximum profitable level.

The general and significant constraints in Narakkal area and recommendations to profitable, prawn farming are highlighted below.

(i) The squid processing unit near CIBA, Narakkal has been disposing the wastes including squid ink-sacs, which depletes oxygen in the feeder canal, leading to mass mortality of prawns and fishes. The problem remains to be thoroughly checked.

(ii) Popularisation and incentives to start hatchery by culturists to make them self-sufficient to overcome irregularities in seed availability is wanting.

(iii) Soft-shell syndrome, which is a disease, thought to be due to decrease in salinity during monsoon is very common which seriously affects market value and consumer preference. The exact reason has to be identified and remedy should also be suggested.

(iv) Quality feed that are both cheap and efficient should be made available to the farmers.

(v) 'Poaching' is a social problem faced by farmers. Watch and ward arrangements should be tightened especially during the period close to harvest. The political influence must be done away with.

(vi) The prawns are sold to the local peeling sheds at Narakkal at different rates based on the counts. A competitive market as such for the cultured prawns does not exist in Narakkal.

(vii) The role of Co-operatives in providing inputs in proper time and quantity is recommended.

The proper implementation of the technology and its impact on the socio-economic conditions of farmers has to be assessed from time to time. Practical suggestions for the socio-economic improvement of farmers are indicated in the present study and the results will be significant for future planning of aquaculture development programmes.

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INFLUENCE OF DIFFERENT LEVELS OF AMBIENT OXYGEN ON GROWTH AND METABOLITE CHANGES IN LABORATORY REARED *PENAEUS INDICUS*

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Introduction

Water temperature and dissolved oxygen are two parameters that are vitally important for optimisation in aquaculture production. If DO levels fall below 2 mg/l and water temperature less than 22°C, warm water species of fishes and invertebrates become stressed. This leads to decreased growth and survival, increased aggression, lower quality product and thus a lowered income. In shrimp culture, there is a critical DO level above the lethal and the incipient limiting level, necessary to sustain a commercial growth rate.

This study reports on the effects of various levels of ambient oxygen on growth and mobilisation of metabolites such as proteins, lipids, carbohydrates, glycogen and cholesterol in the hepatopancreas, haemolymph and muscle of laboratory reared *Penaeus indicus*.

Material and methods

Penaeus indicus of 50-60 mm TL obtained from Puduvyppu and transported to the laboratory were acclimated in well aerated seawater of 15‰ salinity for a period of seven days prior to the start of the experiment. During this period they were fed with dried clam meat.

Four DO levels were selected for the study *viz.* 5, 4, 3 and 2 ml O_2l^{-1}. While aeration was used to obtain the higher levels of DO of 4 and 5 ml O_2l^{-1}, nitrogen stripping by bubbling nitrogen gas through water for 30 and 45 minutes was done to achieve and maintain a DO level of 3 and 2 ml O_2l^{-1} respectively.

Twenty four healthy intermoult animals, weighed to the nearest 0.1 mg were subjected to each treatment. To prevent sudden respiratory stress to prawns kept at the lowest DO level, they were kept in unaerated water for a period of 24 hours, before start of the experiment.

The experiment was carried out for a period of thirty days during which they were fed with dried clam meat @ 5% of body weight daily. The left over feed was collected daily before the morning meal and used to calculate food consumption rate. To maintain the water quality and remove harmful metabolites 40% of the water was exchanged daily with water of appropriate DO concentration.

After 30 days, final weights were recorded and healthy intermoult animals subject to each treatment were sacrificed. Organic reserves in the hepatopancreas, haemolymph and muscle included protein, lipids, cholesterol, total carbohydrates and glycogen and estimated by Folin cio calteu method, Sulphophospho vanillin method, Henly's method, Phenol Sulphuric acid method and Anthrone method respectively. All the organic reserves were estimated on a fresh weight basis. Growth studies concentrated on the percentage increase in wet weight at the end of the study period. Moulting rates were also calculated during the study period using the formula

$$\text{Moulting rate} = \frac{\text{Moulting percentage}}{\text{Mean life of the group}}$$

Food consumption rate was also recorded daily using the formula

$$\frac{\text{100 - Feed wasted}}{\text{Feed given}} \times 100$$

Results

Mean DO levels for various treatments were 2.05 ± 0.07 ml/l, 3.15±0.05 ml/l, 5.35±0.05 ml/l and 4.29±0.03 ml/l. After a period of 30 days shrimps in each treatment were

weighed to the nearest 0.1 mg and recorded by tank. Comparisons of the percentage variations in these organic constituents were also made with the levels observed at a DO level of 4.29 ml O_2l^{-1} where growth was best.

Growth studies : The mean wet weights, growth rate, percentage increase in body weight, moulting rate and food consumption are given in Table 1. Growth in terms of percentage increase in body weight was highest at the DO level of 4.29 ml O_2l^{-1} being 22.93% and a growth rate of 12 mg day^{-1}. At a DO level of 3.15 O_2l^{-1} a growth rate of 9 mg day^{-1} was observed and at 5.35 ml O_2l^{-1} DO level, a growth rate of 7 mg day^{-1} was observed. At the lowest DO level, there was no increase in weight.

ANOVA showed that while growth did not differ significantly among DO levels of 5.35, 4.29 and 3.15 ml O_2l^{-1}, when compared to the lowest DO level of 2.05 ml O_2l^{-1}, the difference was significant.

Food consumption rates too varied with the ambient DO levels. While it was 59% at the DO level of 4.29 ml O_2l^{-1}, it was only 45% at 2 ml O_2l^{-1}, showing a difference of nearly 15% in consumption. Moulting rate was also observed to be low at a DO level of 2 ml O_2l^{-1} when compared to the control.

Organic reserves

Proteins : In the hepatopancreas at the lower DO levels of 3 and 2 ml $O_2\ l^{-1}$, protein content was observed to decrease by 48 and 50% respectively. In the muscle also, protein catabolism was evident as the protein content had declined by about 11-12%. However, in the haemolymph, protein levels increased by about 113-123% at the lower DO levels of 3 and 2 ml O_2l^{-1}.

Lipids : In the hepatopancreas, at the lowest DO level of 2 ml O_2l^{-1}, lipid levels increased by 177% while at all the other DO levels, they showed a decline only. In the haemolymph lipid levels did not show any general trend. In the muscle, lipid levels declined by 42-48% at the DO levels of 3 and 2ml O_2l^{-1}.

TABLE 1. *Observations on growth, food consumption and moulting rate in laboratory reared* Penaeus indicus *exposed to various DO levels*

DO level ml $O_2 l^{-1}$	Mean weight		Increase in		Growth rate mg day^{-1}	Moulting rate	Average food consumption (%)
	Initial (g)	Final (g)	Mean weight (g)	% Mean weight			
5.35 ± 0.05	1.47 ± 0.05	1.68 ± 0.44	0.21	14.29	7	2.2	54.70
4.29 ± 0.03	1.57 ± 0.01	1.93 ± 0.08	0.36	22.93	12	2.34	58.59
3.15 ± 0.05	1.44 ± 0.07	1.72 ± 0.22	0.28	19.44	9	2.23	47.28
2.05 ± 0.07	1.42 ± 0.06	1.40 ± 0.17	Negative	Negative	− 0.001	1.38	45.33

Total carbohydrates : Carbohydrate levels declined significantly (13%) in the hepatopancreas only at the lowest DO level of 2 ml O_2l^{-1}. In the heamolymph however they declined at the various DO levels (2ml O_2l^{-1}, 56.5%, 3 ml O_2l^{-1}, 49%; 5 ml O_2l^{-1}, 32%). As seen, the decline was highest at the lowest DO level. In muscle also, carbohydrate levels declined by 66-72% at the DO levels of 3 and 2 ml O_2l^{-1}.

Glycogen : Glycogen levels declined at all the DO levels in the hepatopancreas, but it was not significant at the lowest DO level of 2 ml O_2l^{-1} where it declined by 60%. In the haemolymph however glycogen value at the lowest DO level was 64% higher. In the muscle, glycogen levels varied directly with DO levels. While the highest level was observed at a DO level of 5 ml O_2l^{-1}, it declined by 50% at the lowest DO level of 2 ml O_2l^{-1}.

Cholesterol : Cholesterol levels in the muscle did not show any general trend with the various DO levels. In the haemolymph, cholesterol levels declined significantly at the higher DO levels only (5 ml O_2l^{-1}, -67% 2 ml O_2l^{-1}, -13%). This shows a difference of more than 50% in the mobilisation of this important organic reserve.

Discussion

Below a critical oxygen level crustaceans show aerobic shut down (Burke 1979, *Biol. Bull.*, **156** : 157-168) and switch over to anaerobic metabolism, where glycogen the storage form of energy, is metabolised to lactate. In the present study, while muscle glycogen levels declined by 50%, it decreased by 59 and 70% in the hepatopancreas at the DO levels of 3 and 2 ml O_2l^{-1}. This difference in the utilization pattern could be due to the fact that specific tissues do not necessarily have equivalent energy requirements nor utilize the same pathways to the same extent during anaerobiosis.

Lactate is the end product of glycogenolysis and its levels decrease during recovery to normoxia (Lowery and Tate 1986, *Comp. Biochem. Physiol.*, **85** (1) : 689-692) indicating its selective utilization during low DO conditions.

Glycogen levels in the haemolymph were high under low DO conditions (3 ppm : + 64% 2 ppm : + 77%) when compared to a DO level of 4 ppm. During the moulting process, glycogen is mobilised from the hepatopancreas *via* haemolymph to the epidermal cells where it is converted to glucose and then to n-acetyl glucosamine and finally chitin (Travis 1955, *Bid. Bull. Mol. Biol. Lab. Woods Hole*, **108** : 88-112). Under low DO levels however, moulting process is impaired and this may be the cause for glycogen accumulation in the haemolymph.

The principal lipid storage site is the hepatopancreas and in general reflects total lipid content and composition of the whole animal. In the present study under the lowest DO levels, of 2 ml O_2l^{-1}, an increase of 178% was observed. Fatty acids undergo oxidation to yield energy only in the presence of oxygen. In the hypoxic conditions, this may not be possible, resulting in accumulation of lipids in the hepatopancreas.

Muscle lipid levels declined by 42-48% at DO levels of 3 ml O_2l^{-1} and less. This indicates that lipids are also being utilised for immediate energy demands during stress and the source is muscle rather than hepatopancreas or haemolymph.

Proteins are found to be increasingly catabolised during hypoxia in fishes such as *Tilapia mossambica* and crustaceans like *Penaeus semisulcatus* and *Macrobrachium malcolmsonii.*

In the present study, protein levels in the hepatopancreas declined by 48 and 50% and in the muscle by 11 and 12% at DO levels of 3 and 2 ml O_2l^{-1}. At the same time, protein levels in the haemolymph showed an increase of 113-122%.

Protein depletion in hepatopancreas followed by blood and muscle during reduced feeding conditions has been reported earlier in starved *Metapenaeus dobsoni.* Thus increased protein catabolism during hypoxic stress, coupled with reduced feeding at the low DO levels might be responsible for lowered protein levels. At the same time this may be causing an increase in the free amino acid (FAA) content of the blood.

Carbohydrates participate in processes of energy production in crustaceans (Hu 1958, *Arch. Biochem. Biophys.*, **75** : 387-395). Carbohydrate levels were found to decline most in the muscle (-66 to 72%) followed by haemolymph (-49 to 56%). In the hepatopancreas, the decline was less marked being only 13%, indicating that carbohydrate stores in muscle and haemolymph are utilised more for energy production than hepatopancreas storage.

Cholesterol, the important precursor of the moulting hormone ecdysterone, is present in all the crustacean tissues throughout the moulting cycle (Kanazawa *et al.* 1976, *Bull. Jap. Soc. Sci. Fish.*, **42** : 1003-1007). Higher cholesterol content in the hepatopancreas (+91% and +141%) at the lower DO levels of 3 and 2 ml O_2l^{-1}, indicate that less of it is utilised. This is directly reflected in the lower moulting rate at DO level of 2 ml O_2l^{-1}. At the same time, haemolymph cholesterol levels were lower at the normoxic levels indicating that cholesterol is being increasingly drawn from the hepatopancreas into the haemolymph and subsequently used for moulting purposes.

Growth studies : Percentage increase in wet weight and growth rates were higher at the DO levels of 3, 4 and 5 ml O_2l^{-1} when compared to the lower DO levels of 2 ml O_2l^{-1}. While no significant difference in growth was observed between the higher DO levels, when compared to the lowest DO levels of 2 ml O_2l^{-1}, significant difference was observed.

Below a certain DO levels, shrimp respiration rate is dependent on DO levels and might involve some compensatory depression of metabolism. Food consumption is also found to be adversely affected being nearly 15% lower at 2 ml O_2l^{-1}. Similar decreased feeding and lower growth rates have been reported in Channel catfish reared at low DO levels, *Macrobrachium rosenbergii* and *Penaeus vannamei* by earlier investigators. Starvation and other stress factors which cause a drain on the animals' organic reserves also inhibit moulting and thereby growth. Besides, in the face of an external stress, more energy is utilised for maintenance of homeostasis than for growth.

In the present study, highest growth rate was recorded at a DO levels of 4.29 ml O_2l^{-1}. Again, at a DO level of 5.25 ml O_2l^{-1} growth was not significantly different, but growth rate was lower. Similarly, DO levels below 3 ml $O2l^{-1}$ were inhibiting growth. Thus there seems to exist a critical DO level between 3.15 and 5.35 ml O_2l^{-1}.

Critical DO levels for growth was derived for 50-60 mm sized individuals only and is likely to vary for a different size group. Some validation is also required from the field. Ultimately the effects of salinity and temperature on these DO levels will also have to be determined.

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A STUDY ON THE BLACK CLAM *VILLORITA CYPRINOIDES* (GRAY) AS PROTEIN SOURCE IN PRAWN DIET

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Introduction

In the high-density culture systems, complete or supplementary feeding becomes inevitable for better farm production and feed takes upto even 50-80% of the total production cost depending on the type and efficiency of the culture system. Proper formulation of nutritious feeds with little production cost is now one of the major priority areas in aquaculture research. This requires a knowledge of the nutritional requirements of the species under farming and evaluation of locally available raw materials for feeding is essential. In this context, evaluation as well as optimum inclusion level of locally available black clam *Villorita cyprinoides* (Gray) in the diet of one of the foremost cultivable prawn *Penaeus indicus* has been undertaken for investigation. Molluscs like clams, mussels, squid and snails have been proved to be among the best diets for prawn. The black clam *Villorita cyprinoides* selected for the present study, supports a regular fishery in many estuaries in Kerala, Karnataka and Goa providing cheap source of protein by way of meat.

Objectives

1. To evaluate the proximate composition of the clam meal with a view to use it as supplementary source in prawn feed.
2. To study the effect of different levels of clam meal in semi-purified diets on growth, protein efficiency ratio and food conversion ratio to determine its optimum inclusion level for *Penaeus indicus*.
3. To asses the overall biological value of the clam protein through nitrogen balance studies.

Material and methods

Villorita cyprinoides was used as animal protein source for *Penaeus indicus* by making it one of the ingredients in the semi-purified diet. The feeds used in the experiment included one control feed [standard purified diet recommended by Kanazawa *et al.* (1982, *CMFRI Spl. Publ.*, 8 : 90-94) having casein as the main protein source, Cod liver-oil as the lipid source and glucose, starch and cellulose as carbohydrate source], five experimental feeds (numbered F_1-F_5, included in addition to casein and gelatin, clam as the protein source) and a zero-protein feed.

Clam was used in the feeds in the form of clam meal prepared from the meat of *Villorita cyprinoides*, by drying the meat extracted from them in an oven at 60°C for 12-15 hrs followed by powdering and sieving. This powder was used in various proportions as 10%, 20%, 30%, 40% and 50% in experimental feeds F_1, F_2, F_3, F_4 and F_5 respectively. The composition of control and experimental feeds are given in Table 1. The pelleted feeds were stored in plastic containers during the experiment.

Experiment was carried out in circular tubs of 50 litre capacity for a period of 45 days. The clam used was collected from Nettur, situated about 13 km southeast of Cochin Harbour. Juveniles of *Penaeus indicus* having an average weight of 1 ± 0.3 g and an average length of 55 ± 2 mm were collected from backwater canals located in Vypeen Island near Cochin. Before introducing them to the experimental tanks, the prawns were acclimatised to the laboratory conditions as well as to artificial feeds. The quantity of water in the tubs was maintained at the rate of 2 litres per prawn. One-third water was replaced every day and complete replacement once in four days. Left over food and faecal matter were collected every morning. The shrimps were measured for length and weight at the start of the experiment and were introduced at the rate of 10 prawns per tub. They were fed twice daily (one-third ration between 0900 and 1000 hours and the rest between 1600 and 1700 hrs) at the rate of 10% (dry matter basis) for the first week and 8% of the

body weight for the succeeding weeks. They were weighed individually every ten days to determine the weight gain and feed allowance.

Metabolic faecal nitrogen (MFN) (refers to the nitrogen in faecal matter due to the metabolic activity of the body and is required for the calculation of true digestibility), was determined by feeding the animals with a known quantity of nitrogen-free diet and the nitrogen appearing in the faeces is therefore considered as the metabolic faecal nitrogen.

TABLE 1. *Composition (%) of control feed and experimental feeds*

Ingredients	Control feed	Experimental feeds				
		F_1	F_2	F_3	F_4	F_5
Casein	55	45	35	25	15	5
Clam meal	0	10	20	30	40	50
Glucose	4.5	4.5	4.5	4.5	4.5	4.5
Sucrose	8	8	8	8	8	8
Starch	3.5	3.5	3.5	3.5	3.5	3.5
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Cod liver oil	10	10	10	10	10	10
Cellulose	1.7	1.7	1.7	1.7	1.7	1.7
Gelatin	4.0	4.0	4.0	4.0	4.0	4.0
Mineral mix	8.5	8.5	8.5	8.5	8.5	8.5
Vitamin mix	3.2	3.2	3.2	3.2	3.2	3.2

For calculating MFN, animals were individually held seperately in six rearing containers and fed with zero-protein diet or in otherwords nitrogen-free diet (F_0) 'ad libitum' for 20 days. Faeces were collected every day, and the nitrogen and

chromic oxide in the diet and faeces were determined. At the end of the experiment the carcasses of the animals were analysed for determining the net protein utilization.

Water stability of the pellets was evaluated by employing the method described by Jayaram and Shetty (1981, *Aquaculture*, **23** : 355-359) with minor modifications. The loss of weight of pellets [Packed in cone shaped pouches made with bolting silk cloth (1 mm mesh)] kept under water after 2, 4, 6 and 8 hours was determined.

Analysis : Proximate composition of the feeds were determined following methods given by AOAC (1975, Official Methods of Analysis. *Ass. Off. Anal. Chem. Washington, D. C.*, pp. 1094), protein in the feed and faeces by micro-kjeldahl method, crude fat by soxhlet extraction method.

Ash content in the feeds was found out by keeping pre-weighed sample in muffle furnace at 600°C for 6 hours and crude fibre by doing acid and alkali digestion followed by keeping in muffle furnace at 500°C for 3 hours (AOAC 1975, *loc. cit.*). The chromic oxide in the feed and faecal matter was estimated by the method suggested by McGinnis and Kasting (1964, *J. Agri. Food Chem.*, **12** : 259-262). Salinity was estimated by Mohr-Knudsen method and dissolved oxygen using the modified Winkler method. The pH of water was measured using a digital pH meter. Ammonia concentration in the water was determined by phenol hypochlorite method.

Parameters studied

$$\text{\% growth in length/weight} = \frac{\text{Final length/weight - Initial length/weight}}{\text{Initial length/weight}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Average weight of food consumed (dry weight)}}{\text{Average live weight gain}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Average live weight gain}}{\text{Average protein consumed}}$$

$$\text{Gross conversion efficiency } (K_1\%) = \frac{\text{Increase in average wet weight}}{\text{Consumption}} \times 100$$

$$\text{Net conversion efficiency } (K_2\%) = \frac{\text{Increase in average wet weight}}{\text{Assimilation}} \times 100$$

$$\text{Net protein utilization (NPU)} = \frac{\text{Body nitrogen of test group animals} - \text{Body nitrogen of animals receiving zero-protein feed}}{\text{Nitrogen consumed}}$$

$$\text{True digestibility} = 100 - \frac{\text{\% Indicator in diet}}{\text{\% Indicator in faeces}} \times \frac{\text{\% corrected protein in faeces}}{\text{\% protein in diet}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Initial number of animals} - \text{Final number of animals}}{\text{Initial number of animals}} \times 100$$

$$\text{Biological value} = \frac{\text{Net protein utilization}}{\text{True digestibility of protein}}$$

Moulting rate was calculated using the formula given by Petriella (1990, *J. Aqua. Trop.*, 5 (1) : 77-85)

$$\text{Moulting rate} = \frac{\text{Moult percentage}}{\text{Mean life of the group}}$$

$$\text{Moult (\%)} = \frac{\text{Number of moults}}{\text{Initial number of animals}} \times 100$$

Mean life of the group was calculated by adding the number of days each individual survived and then taking the mean.

The data obtained with various parameters were subjected to Analysis of Variance (ANOVA) to find out the significance between treatments and mean values were compared by least significant difference (LSD) in both cases.

Results

The results of the experiments conducted to evaluate the clam meal, its comparative efficiency at different inclusion levels as feed for *Penaeus indicus* are as follows :

Proximate composition of clam meal : Showed a high protein (50.82%) as well as lipid content (8.5%) in the clam meal

indicating that clam meal is suitable to the nutritional requirements of the prawn.

Increase in length, live weight and dry weight : In respect of six feeds (denoted as Control, F_1, F_2, F_3, F_4 and F_5) the prawns fed on the feed F_3 (30% clam meal) registered the highest growth of 30.22% in length, 52.50% in live weight and 96.13% in dry weight. Animals fed with F_4 (40% clam meal) obtained the second highest growth of 28.34% in length, 44.48% in live weight and 92.66% in dry weight. *Penaeus indicus* fed on F_5 (50% clam meal) showed the third best growth rate with a growth of 25.77%, 39.5%, 88.0% in length, live weight and dry weight respectively. F_2 (20% clam meal) provided a growth of 16.9% in length, 39.33% in live weight and 87.58% in dry weight. Among the experimental feeds, the feed with 10% clam meal (F_1) had shown the lowest growth of 9.6% in length, 37.05% in live weight and 77.56% in dry weight. The lowest growth was recorded in the case of the control feed with a growth of 3.9% in length, 4.11% in live weight and 34.44% in dry weight. Analysis of variance (ANOVA) showed that the growth in length, live weight and dry weight differ significantly between treatments at 1% level ($P < 0.01$).

Food conversion ratio (FCR) : Except the control feed, all other feeds gave good conversion rates. However, F_3 recorded the best FCR (1.6) followed by F_4, F_5, F_2, F_1 with the ratio values of 1.70, 1.83, 2.10, 2.11 respectively. The control feed obtained a FCR of 10.09, thus showing that inclusion of clam meal helps in reducing the FCR at least by 5 times. Analysis of variance showed that treatments differ significantly at 1% level ($P < 0.01$).

Survival rate : The survival rates of the prawns fed on control and experimental feeds were found the comparatively low, ranging from 40% to 60%. Among all the feeds F_2 recorded the maximum survival rate (60%). While F_3 recorded the lowest survival rate of 40%. The process of moulting was observed to be one of the major factors contributing to the mortality of shrimps. Those which were soft, probably just moulted within 24 hours, accounted for 36% of the total mortality.

Gross conversion efficiency (K_1%) and Net conversion efficiency (K_2%) : Feed F_3 showed the highest value of K_1 as well as K_2 of 0.60 and 0.71 respectively. F_4 showed the second best K_1 and K_2 values (0.58 and 0.69 respectively). Though F_2 showed the next higher K_1 value (0.52) its K_2 value was found to be lower than that of F_5. The other feeds F_5, F_1 and control obtained K_1 value of 0.51, 0.48 and 0.40 respectively, their respective K_2 values being 0.57, 0.51 and 0.41. Analysis of variance showed that in the case of K_1% the treatments differ significantly at 5% level ($P < 0.05$).

Moulting rate : It was found that the moulting rate values increased as the percentage of clam meal in the feed increased upto a certain level and after that it started decreasing. The moulting rate increases upto 30%, with higher percentages, the value reduces gradually. Analysis of variance of the data showed that treatments differ significantly at 1% level ($P < 0.01$).

Protein efficiency ratio (PER) : The best value of PER was shown by F_3 (0.62) followed by F_4 (0.61), F_5 (0.55), F_1 (0.49) and F_2 (0.48). The control feed showed the lowest value (0.10). Analysis of variance showed that treatments differ significantly at 1% level ($P < 0.01$).

Net protein utilization (NPU) : The NPU values exhibited no correlation with the increasing amount of clam meal in the diet. It is important to note that all experimental feeds had shown a NPU value greater than that of control diet. Among feeds, F_4 showed the maximum value (68.44) followed by F_5 (57.98), F_3 (56.01), F_1 (39.47) and F_2 (37.50). F_3 showed a sudden increase in value when compared to F_1 and F_2 feeds. ANOVA showed that all treatments differ significantly at 1% level ($P < 0.01$).

Biological value (BV) : Maximum BV was shown by the feed F_4 (80.79), followed by F_5 (68.44), F_3 (64.41), F_1 (41.63), F_2 (41.34) and control (31.19). As in the case of NPU here also all experimental feeds showed a higher value than the control. Analysis of variance showed significant difference between treatments at 1% level. LSD showed that feeds F_1 and F_2 differ significantly at 5% level ($P < 0.05$), all the rest at 1% level ($P < 0.01$).

Water stability of the feed : The pellet stability was inversely related to the dietary level of clam meal. The percentage of dry matter remaining decreased with increasing clam meal level in the feeds. After an exposure of 8 hours the feed remains were 77.5% of the feed before leaching for control, 75% for F_1, 74% for F_2, 73% for F_3 as well as F_4 and 72.5% for F_5. In the case of all feeds the leaching rate was found to be higher in the initial stages (upto 4 hours) and then slowly coming down.

Discussion

The results obtained from the proximate analysis of clam meal in the present study are comparale to the values obtained by Ali (1988, *Ph. D. Thesis.* Cochin Univ. Sci. & Tech., pp. 243) for *Sunetta scripta* meal (protein 48.10%, lipid 13.55%, nitrogen-free extract 11.69% and ash 7.62%) and by Gopal (1986, *Ph. D. Thesis.* Cochin Univ. Sci & Tech., pp. 306) for *Meretrix casta* meal (protein 56.6%, lipid 8.2%, nitrogen-free extract 20.8% and ash 10.50%).

Sedgwick (1979, *Aquaculture*, **16** : 7-30) studied the growth of *Penaeus merguiensis* using 69% of freeze-dried mussel *Mytilus edulis* in diet in which the protein content was 39.5% and reported a weight gain of 57.14% in 8 weeks. Colvin (1976, *Aquaculture*, 7 (4) : 315-326) observed a growth rate of 44 mg/ day in *P. merguiensis* of 0.95 g when fed with a combined meal of fresh mussel and fresh juvenile prawn in equal ratio. Though the highest growth increase recorded in the present study was only 52.55%, is comparable to the result obtained by Sedgwick (1979, *loc. cit.*). The higher growth rate obtained by Colvin (1976, *loc. cit.*) may be due to the combination of two protein sources (with amino acid composition similar to prawn) since a mixture of two or more protein sources, invariably show better growth than single source (Deshimaru and Shigueno 1972, *Aquacultura*, **1** (2) : 115 - 133). The following reasons can be attributed to the low growth rate : Variation in size of prawns, form of protein used and the protein content of the feed. Another reason that can also be attributed to lower growth rate obtained in the present study is that certain essential aminoacids are lost during

drying, because of the reactions with reducing sugar and carbonyl compounds present in the diet as suggested by Swaminathan (1967, *Newer methods of nutritional Biochemistry*. Academic Press, New York, 3 : 197-241).

Ali (1988, *loc. cit.*) has reported a Food conversion ratio (FCR) of 1.83 using a diet containing 51% *Sunetta scripta* meal in *Penaeus indicus* of 10-20 mm length. Gopal (1986, *loc. cit.*) obtained a value of 0.92 with *Penaeus indicus* of 20±5 mm using 51.2% *Meretrix casta* meal and Ali (1982, *loc. cit.*) a value of 1.46 with compounded diets having 38% *Villorita* meal in *Penaeus indicus* juveniles. The highest FCR (1.6) obtained in the present study is comparable to these values, taking into account the variation in size, experimental duration and protein source.

The highest PER of 0.62 obtained in the present study was for the group having 30% incorporation of clam meal in the diet.

The higher moult percentage values obtained with F_1, F_2 and F_3 when compared to the control feed may be due to the moult inducing factor reported in fresh *Sunetta scripta* by Ali (1982, *loc. cit.*). The same had found that the high moulting rate resulted in high mortality. Gopal (1986, *loc. cit.*) reported that prawns fed on clam meal (*Meretrix casta*) had significantly lower calcium and phosphorus levels compared to those fed on diet with fish meal, crab meal and shrimp meal. This may indicate that lower calcium and phosphorus metabolism may perhaps affect these prawns resulting in comparatively high mortality rates, especially during post-moult stages (New 1976, *Aquaculture*, 9 (2) : 104 - 144).

Metabolic faecal nitrogen (MFN) in *Penaeus indicus* was determined for the first time by Ali (1988, *loc. cit.*) and the value obtained in the present study are comparable to the value obtained by Ali (1988, *loc. cit.*). Here also varying values were obtained with 6 different experiments. The values obtained on true digestibility in the present study are comparable to that of earlier findings.

Net protein utilization (NPU) of clam meal (*Sunetta scripta*) at 51% inclusion level was found to be 60.91, while a NPU of 28.68 was obtained with casein-based purified diet (Ali 1988, *loc. cit.*). In the present study the value obtained with 50% clam meal diet was found to be closer to the value obtained by Ali (1988, *loc. cit.*), and a value higher than that was obtained with 40% clam meal diet. Agreeing with other's results, the casein-based diet showed a lower value (30.11).

Ali (1988, *loc. cit.*) recorded a biological value (BV) of 74.60 for *Sunetta scripta* meal when used at 51% level in *Penaeus indicus*, the same had obtained a value 61.93 and 53.10 for 86% prawn waste and 68.5% mantis shrimp meal respectively in the same species. This shows that clam meal used in the present study has got higher biological value than prawn waste and mantis shrimp meal.

When NPU, TD and BV are taken together, it was seen that 40% clam recorded the maximum value as far as NPU and BV are considered, but at this level, the TD was found to be the lowest. All the NPU, BV and TD values obtained show that 30% clam meal was the best giving a combination of BV and NPU values close to the maximum, and a good TD value. At lower levels of clam inclusion, though the TD was found to be good, the BV and NPU were found to be very low. The inverse relationship between the clam percentage in the diet and pellet water stability, is totally agreeing with the study carried out by Lim and Dominy (1987, *Aquaculture*, **87** (1) : 53-63) where they found that percentage of dry matter remaining, after exposure to water decreased with increasing soyabean meal level in diets.

Conclusion

30% clam meal level is the best inclusion level.

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EFFECT OF NUTRIENTS ON THE PHOTOSYNTHETIC RATE OF SOME PHYTOFLAGELLATES

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Introduction

It is well known that the micro-algae acquire nutrients from their environment in order to sustain growth and for conducting photosynthesis. On the basis of their quantitative requirements, it has been classified into two groups, *viz.* macro-nutrients or essential nutrients such as nitrate, phosphate and silicate and micro-nutrients or trace elements such as copper, zinc, cobalt, manganese and molybdenum.

The dynamics of micro-algal growth in nature depends on the behaviour of individual species. The latter can be understood only by laboratory studies on unialgal cultures.

The growth rate is usually calculated from cell number, cell carbon or the amount of chlorophyll *a*. In this account an attempt has been made to study the influence of two nutrients *viz.* nitrate and phosphate, on the rate of growth and photosynthetic response of five species of phytoflagellates.

Material and methods

Five species of phytoflagellates such as *Isochrysis galbana*. *Pavlova lutheri*, *Chromuline freiburgensis* (Haptophyceae), *Dicrateria inornata* (Chrysophyceae) and *Tetraselmis gracilis* (Chlorophyceae) were selected for the present study. They were maintained as stock culture in the Algology Laboratory of the CMFR Institute, using Conway or Walne's culture medium. Three different nitrate concentrations of the medium (KNO_3) were prepared (half nitrate - 50 gm of KNO_3 and double concentration of nitrate - 200 gm of KNO_3) and treated with each species along with the normal nitrate (100 gm of KNO_3), for a

period of 15 days. Similarly, three different phosphate concentrations of the medium also prepared (half - 10 gm of Na_2HPO_4; $1^1/_2$ times of phosphate 30 gm of Na_2HPO_4 and double concentration of phosphate 40 gm of Na_2HPO_4) along with the normal (20 gm of Na_2HPO_4) and treated with each species for a period of 15 days. A fixed amount of stock culture was added to each culture flask which contain different concentrations of nitrate and phosphate. Data were collected on parameters such as cell count on all days using Haemocytometer, production rate *i. e.* gross, net and respiration rate on every alternate days using Winkler method, chlorophylls and nutrients (NO_2, NO_3 and PO_4) were Analysed once in every 5 days.

A similar experiment was conducted with natural population of phytoplankton dominated by diatoms such as *Chaetoceros* spp., *Thalassiosira subtillis, Thalassiothrix* sp. and *Nitzschia* spp. and all parameters were determined for comparative studies and cells were counted by using Sedgewick rafter chamber by the settling method. A statistical analysis also made by using the computer to verify the data collected whether the two nutrients are playing a significant role for the growth and photosynthetic activity of these flagellates.

Results

The enriched experiment revealed that the nutrients concentrations affected the amount of photosynthetic pigments especially Chl. *c* particularly on the exponential phase of the algae. But the value of Chlorophyll reduced considerably in high concentration of NO_3 and PO_4, when compared to lower and optimum concentrations except control, other concentrations of nutrients on natural population show very low values of Chl. *a* within a week time.

In *Chromulino freiburgensis* with varying concentrations of NO_3 maximum gross production on the 14th day of culture, except in double where maximum on the 10th day, but in different concentration of PO_4, the gross production was high on the initial day and some phases a gradual increase of production was noticed.

In *Dicrateria inornata* on the 14th day were observed peak values of gross production, but in different concentration of PO_4, in control and lower concentration, gross production was high on the 12th day whereas in other concentration on the initial days.

In *Isochrysis galbana* with varying concentration of NO_3 and PO_4 in control showed a maximum value on 12th day of culture and in others high gross productivity on 10th day. Whereas in $1^1/_2$ times of NO_3, a gradual increase in production upto 8th day. Whereas in double PO_4, maximum on the 12th day of culture. In lower PO_4 growth enhanced gradually from 6th to 12th day of culture.

In *Pavlova lutheri* gross productivity values showed a gradual increase, where as in lower concentration, a decreasing trend was noticed. In different concentration of PO_4, peak values of production was observed on the 10th to 14th day of culture.

The different concentration of NO_3, in *Tetraselmis gracilis* maximum production was on 8th to 12th day. Whereas in different concentration of PO_4, in control and double peak values on the 14th day. In other concentration high production was on the initial days.

In natural population with different concentration of NO_3, except in control maximum production on the 2nd day and production declined gradually. Whereas in different concentration of PO_4 a gradual increase was observed till 12th day with some fluctuations.

Effect of varying concentration of NO_3 and PO_4 on the growth phases of flagellates

In *Chromulina freiburgensis* with different concentration of NO_3, an initial lag phase with a gradual increase of cells except in double growth declined on the 13th day, whereas in different concentration of PO_4 , all concentration reached the peak value on the 13th day and growth declined gradually. Whereas in double concentration peak values were on the 15th day.

In *Dicrateria inornata* with different concentration of NO_3, a gradual increase in cells was noticed except in $1^1/_2$ times of NO_3, the growth started on the 9th day only. In different concentration of PO_4 in double concentration growth declined on the 11th day.

In *Isochrysis galbana* with different concentration of NO_3, peak values were noticed on all the concentrations on the 15th day of culture except in lower concentration. Whereas in different concentration of PO_4, maximum values were on the 15th day of culture.

In different concentration of NO_3, of *Pavlova lutheri* maximum values were on the 11th day of culture. Whereas in different concentration of PO_4, high values were on the 13th day.

In *Tetraselmis gracilis* in $1^1/_2$ times and double concentration of PO_4 peak values were observed on the 15th day of culture. But in different concentration of PO_4, peak values were on the 13th day expect in control.

In the varying concentration NO_3 and PO_4, the uptake of nutrients depends upon the exponential phase of the algae and high absorbtion rates were noticed on the exponential phase of the each species, *i. e.* inbetween 10th and 15th day of culture and high absorbtion was noticed in the optimum and slightly higher concentrations.

The statistical analysis (2-way ANOVA) was carried out to test whether there is any effect in chlorophyll, productivity and cell counts, with change in NO_3 or PO_4 concentration.

The analysis revealed that change in nitrate levels has significant effect at 5% level in Chl. *c* concentration and change in PO_4 has significant effect on respiration of *P. lutheri*. In varying concentration of NO_4 and PO_4, the cell count was significant at 5% level in *C. freiburgensis* and *T. gracilis*. Whereas *D. inornata* significance was in different concentrations of NO_3 and *I. galbana* and *P. lutheri* significance was at different concentration of PO_4.

Discussion

As the chlorophyll content is an index of photosynthetic potential, its decrease or increase represents the multiplication or decline of the micro-algae in a culture system. As the growth phases gradually increase, the absorption of nutrients also increase with some exceptions. Raymont (1980, *Plankton Productivity in the Oceans,* **1** : 1-489) states that the rate of photosynthesis of micro-algae under conditions of nutrient limitations, can be described by Michaeles Menten kinetics, but the rate of growth is related to the concentration of the medium. Ketchum (1939, *J. Cellular Comp. Physiol.,* **13** : 375-381) pointed out that if the media contain different concentration of nutrients, the culture shows different growth rates.

The enrichment experiments conducted in the laboratory revealed that the varying concentrations of nitrate and phosphate affected the amount of photosynthetic pigments, especially chlorophyll *a* considerably, particularly on 10th and 15th day of culture period. The exponential phase of growth of some flagellates in this experiment was found to be extended to 10th or 12th day of culture period. However, the quantum of chlorophylls was found to be reduced considerably in high concentrations of nitrate and phosphate when compared to the optimum and lower concentrations of these macro-nutrients. Earlier observations of Ammini Joseph (1983, *Ph. D. Thesis.* Cochin Univ. of Sci & Tech.) on the culture of *Isochrysis* and *Tetraselmis* also showed similar results.

In the natural population of phytoplankton, except control, all the varying concentrations of nitrate and phosphate indicated very low values of chlorophyll *a* within a week productivity value and were influenced by varying concentration of nitrate and phosphate in a culture system.

The effect of varying concentrations of nitrate and phosphate on the growth phases of these flagellates indicated 1-3 days of lag phase, except in *Dicrateria* where the lag phase has extended upto 5-6th day. An exponential phase of growth reached upto 10-13th day in most of the cases except in double

concentrations of nitrate and phosphate when the culture showed symptoms of declining on 12th to 13th day. All the Haptophycean and Chrysophycean flagellates showed the continued exponential phase upto 15th day in the control and slightly high concentration of nitrate and phosphate levels. In low and double concentration levels of the nutrients, the culture showed symptoms of stationary and declining phases even from 8th to 14th day.

The estimation of the uptake of nutrients by the flagellates indicated that from 5th to 15th day of culture period, an enhancement levels were noticed in most of the flagellates where the concentration was optimum or slightly in higher rates of these nutrients. However, in lower levels of these nutrients, the uptake of nitrate was found to be slightly faster than the uptake of phosphate upto 15th day of the culture period. Briefly, the absorption of nitrogenous products by these flagellates were found to be in an increasing trend when compared to the phosphate contents.

The results showed most of the flagellates prefer nitrates for their growth and multiplication than the phosphate. However, according to Guillard (1963, *Symp. Mar. Microbiol,* **3** : 93-104) that some flagellates have not shown expected growth on nitrate. Similar results were obtained in experiments conducted by Vijayaraghavan *et al.* (1975, *J. mar. biol. Ass. India,* **17** (1 & 2) : 206-212) and Gopinathan (1984, *J. mar. biol. Ass. India,* **26** (1 & 2) : 89-94) with the batch cultures of nanoplankters.

In all the varying concentration of nitrate and phosphate, the culture showed a limited time of lag phase, except in *Dicrateria* may be due to the nature of inoculum. In some cases, the growth declined on the 10th to 13th day due to the high concentrations of nutrients. Eppley and Strickland (1968, *Advances in Microbiology of the Sea,* **1** : 23-62) pointed out that cells acquire different concentrations of nitrate and phosphate at different stages of growth in cultures, where nutrients are not limiting.

The productivity estimates were found to be high between 10th to 14th day of culture in most of the flagellates. In the 'control' of most of the flagellates in varying concentrations of nitrate and phosphate caused wide fluctuations in gross and net production rates. However, in optimum and slightly high levels of nitrate and phosphate, the productivity values were found to be moderately high when compared to very low and very high concentrations of these macro-nutrients in the culture medium. In the natural population of phytoplankton, lower and very high levels of nitrate and phosphate indicated low to negligible rates of gross and net production. The respiratory values of all these phytoflagellates were found to be slightly in higher levels during the initial exponential phase of growth of the culture period.

The present investigations clearly indicated that nitrates support good growth of Chrysophycean and Haptophycean flagellates than a Chlorophycean member *Tetraselmis*, than phosphate in a culture system. According to Strickland (1960, *Fish. Res. Bd. Canada, Bull.*, 122 : 1-172), the nitrate has not shown any significant growth of micro-algae in a culture system in few cases. In most of the cases, the initial uptake increases in terms of cellular concentrations, but such variation may be related to the growth requirement of different species in different growth phases. In some cases, growth occur at lower rate, may be due to the nutrient depletion in the medium or in the reduction of cellular concentration of nitrate and phosphate.

The statistical interpretations conducted in this account revealed that there is little effect in chlorophyll contents due to the changes in concentrations of nitrate and phosphate level in the medium. However, the 2-way analysis indicated that changes in nitrate levels has significant effect in Chlorophyll *c* values in the flagellate *Pavlova lutheri*. Similarly, the changes in phosphate levels in the medium has also showed significant effect in respiratory rates of the above species. However, most of the flagellates indicated non-significant effects on the rate of gross and net production and in chlorophyll contents. As regards the effect of these nutrient changes in the medium, the statistical analysis proved that there is significant effects on the

total number of cells at the time of exponential growth phases of most of the flagellates.

From these accounts, it can be concluded that though the macro-nutrients are the essential elements for the growth and development of phytoflagellates in general, the optimum and slightly higher levels of nitrate and phosphate are quite acceptable for the normal growth of these flagellates and thereby for the mass culture operations in a hatchery system for the use as live-feed while rearing the larvae of economically important cultivable organisms.

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STUDIES ON THE INTERSTITIAL SALINITY AND RELATED ENVIRONMENTAL PARAMETERS OF CERTAIN BRACKISHWATER PRAWN CULTURE ECOSYSTEMS

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Introduction

Prawn culture has been taken up by India in a big way in order to augment the produce, as prawns occupy an important place in India's seafood export trade and earn valuable foreign exchange. The backwaters in Kerala form the nursery grounds of many of the commercially important species and they are cultured in mangroves, coconut groves, culture ponds, etc. The prawns are found to survive in these systems even when the salinity drops to almost freshwater ranges during the southwest monsoon. Hence it is quite possible that the substratum where most penaeids remain burried during the day, affords these prawn a comparatively better range of environmental condition particularly salinity that helps them to survive and grow.

The present investigation aims at comparing the interstitial water of the substratum with the column water as regards the parameters - salinity, temperature, pH, nitrate and nitrite. Besides, growth studies were simultaneously conducted in 4 pens erected in the culture pond by growing the juveniles of *Penaeus indicus* in them. The aim of the growth studies was to study the effect of the hydrological parameters, particularly salinity of the column and interstitial water on its growth.

Material and methods

All the three stations - the mangrove, coconut grove and culture pond are situated on Vypeen Island, Cochin at about 2-3 km distant from each other. While the mangrove station is

dominated by *Acanthophora* spp., the other two stations influenced by tide, were fed by backwater system with dilution from freshwater influxes.

The pens in the culture pond were of 1 m^2 at the centre of each side of the culture pond. The inner net of the pen had a mesh size of 5 mm while the outer, used to prevent the entry of predators, had a 2 cm mesh. The column water and interstitial water data from one pen alone (which was used as the control in the growth studies) was used for comparison with the other two ecosystems.

The study period had a duration of 60 days extending from August and September 1992 at an interval of 5 days in the forenoon hours between 8 and 12 hrs. The column water was sampled from the surface and bottom while the interstitial water was sampled from 5 different depths *viz.* 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm and 20-25 cm using a specially designed interstitial water sampler.

The interstitial water sampler : This is a light and leak proof instrument of 144.5 cm total length consisting of 4 detachable parts : (i) *The basal cone* consists of a driving cone of twisted into shape from a 2 inch diameter GI pipe onto which were welded successively 2 adaptors of 2 inch and 2.5 inch diameter respectively. (ii) *The inner ventillated PVC pipe* is 1.25 m in length that is threaded on the outside at one end and at a distance of 10 cm from this end ventilations are made as 6 longitudinal slits of 5 cm length and 9 cm width which are equidistantly placed along the circumferance. This PVC pipe threads into the lower coupling of 2 inch diameter. (iii) *The outer GI pipe casing* of 1 m length and 2.5 inches diameter that is threaded on the outside at one end which helps to fasten it to the upper coupling the basal cone. (iv) The *'filter candle'* is the actual filterine and collecting unit. It is the 'filter candle' made of ultra high porosity ceramic material used in domestic filters. A copper wire fastened to the steel cap helps in lowering and lifting the filter into the instrument during the operation.

Operation : The GI pipe casing is fastened to the basal cone by threading it to the upper coupling and the water depth is marked on the exterior measuring from the tip of the cone. From this point 5 markings are made upward at 5 cm intervals to mark the different sampled depths from the soil surface downwards. The cone together with the GI pipe is driven into the substrate with a hammer and when the desired depth is reached, known from the external markings, the PVC tube is lowered into the GI pipe and threaded into the lower coupling of the basal cone. Now the filter is lowered into the PVC pipe and the copper wire is fastened at its upper end such that the top of the filter coincides with the upper end of the ventilations. After leaving the instrument undisturbed for 10 minutes, the filter is taken out, the water collected and poured out into labelled 50 ml plastic bottles.

The column water and interstitial water samples were analysed and their salinity, pH, nitrate and nitrite contents analysed using standard methods.

The temperature was measured, using the PVC pipe as a core and pushing the soil to the ventilated end with a piston and measuring the temperature of the consecutive 5 cm cones of soil as they coincide with the ventilations by inserting a thermometer at the ventilations.

The growth studies were conducted using the juveniles of *Penaéus indicus* of the size range of 4-7 cm, stocked at the rate of 25 animals/m^2. 5 specimens from each pen were sampled at 5 day intervals to record the length and weight. The prawns in station I were kept as control with no supplementary feed provided while those in the other 3 pens were fed with a diet of 36% protein at 12% body weight per day and fed once daily in the evening hours.

The relation between the various parameters were statistically analysed using two-way ANOVA and correlation matrix procedures.

Results and discussion

Salinity : In all the three environments it was found that the salinity of the bottom, later in the column was higher than the surface though not markedly. The interstitial water was found to be more saline than the column with the salinity in the 5-10 cm depth samples lower than that in the 0-5 cm depth. Below the 5-10 cm depth the salinity mostly increased by about 1 ppt per 5 cm depth reaching a maximum in the 20-25 cm depth. Among the stations the mangrove site showed higher salinities than the other two ecosystems with the coconut grove showing lower values.

The two-way ANOVA showed that the salinity values from the different depths and those from different ecosystems were significant at 1% level.

The higher salinity in the mangrove station could be due to the direct connection with the tidal canal. The coconut grove being further away from the sea than the culture pond, could be the reason that it has the least salinity, along with the fact that the backwater system feeding these two ecosystems is diluted by freshwater inflows.

The higher density of higher saline water must make the bottom water of the column more saline than the surface.

According to Mortimer (1941, *J. Ecol.*, **29** : 280-329 & 1942, *J. Ecol.*, **30** : 147-201) various trapping mechanisms such as adsorption and complex formation in the superficial sediments prevent the transport of materials into the water. This could be the reason that a marked increase in salinity is noticed in the 0-5 cm depth. Lack of mixing in the interstitial water causes the increasing salinity in the lower layers.

The lowest salinity values in all the sampled depths were observed in one single sampling day during the first week of August and highest values during the third week of September. This is notable and clearly explains a limited and definite interaction between column and interstitial waters. The low salinity in the 5-10 cm compared to the 0-5 cm strata above and 10-15 cm strata below is intriguing.

Temperature : The bottom water samples had a lower temperature than the surface water. There was an increasing trend of temperature from the soil surface downwards with the 20-25 cm depth having the highest temperature. But a significant exception is that the temperature in the 5-10 cm depth layer had lower values than the zones above and below it. Among the three ecosystems the coconut grove had the least temperature values and the culture pond had the highest.

The two-way ANOVA of the temperature data from the different depths including the atmospheric temperature and those from the different ecosystems were found to be significant at 1% level.

The higher temperature in the culture pond station could be due to the lower water depth and also because the pen is directly exposed to sunlight. The lower temperature in the coconut grove could be due to the canopy of trees preventing the direct heating of water.

The higher temperature in the surface water compared to the bottom water could be due to the direct heating of surface water by sunlight and that water at lower temperature is demen and sinks to the bottom. A lowering of temperature in the first 2 depths is similar to the observation of Ganapati *et al.* (1962, *J. mar. biol. Ass. India,* **4** (1) : 44-57). The increase in temperature at the lower depths could be due to the slower loss of heat from these layers as we move away from the surface due to lack of any mixing forces.

pH : The pH in the 0-5 cm depth was more or less the same as that in the column water, but in the lower depths (below 0-5 cm) generally an increase in pH is noticed. While comparing the different ecosystems the culture pond had comparatively higher values of pH than the other two ecosystems.

The two-way ANOVA of the pH values from the different depths were found to be insignificant even at 5% level while those from the different ecosystems were found to be significant at 1% level.

The comparatively higher pH in the culture pond could be due to the higher photosynthetic activity caused by the fertilising effects of the feed used and the closed nature of the pond with less water exchange acting as a nutrient sump. The low pH in all the stations in the first week of August could be due to the decomposition of organic matter carried in by the flood water of the rains. The increase in pH noticed in the later collections could be due to the photosynthetic activity of phytoplankton and aquatic vegetation which removes the CO_2 from the water column.

Nitrate and nitrite : The nitrate and nitrite values in all the ecosystems showed a general increase in the column water from the surface to bottom and in the interstitial waters if increased in the first two layers reaching a peak in the 5-10 cm layer. A progressive decrease was noticed in the lower layer with the drop being more prominent in the nitrite values from the 5-10 cm depth to the 10-15 cm depth. The nitrite values in the mangrove station did not show any regular trend.

The two-way ANOVA of nitrate and nitrite values from the different depths and those from the different ecosystems were found to be significant at 1% level.

Higher values of nitrate and nitrite in the mangrove station could be due to its high amount of organic discharge from the human settlement around.

Lerman and Brunskill (1971, *Limnol. Oceanogr.*, **16** (6) : 880) have indicated the presence of an upward flux across the sediment water interphase which is responsible for the chemical budget of the overlying water. Interruption of this flux must result in a decrease in the concentration of dissolved components compared to the sediments, the low values of nitrate and nitrite obtained could be due to their depletion by the postmonsoonal bloom of phytoplankton. Bender *et al.* (1977, *Science*, **198** : 605-609) have indicated that the nitrate values reach a maximum at the base of the zone of aerotic respiration and decreases in the zone of denitrification which is in accordance with the data observed in the present study where the depth upto 10 cm could be the zone of aerobic respiration.

Growth data : A steeper increase in length was observed between the 10th day and 35th day in the experimental pens while in the control it was more pronounced between the 20th and 35th day and later between the 45th day and 50th day.

The weight data showed a steep increase between the 20th and 35th day while the growth in weight was more pronounced in the control between the 25th and 40th day and later beyond the 45th day.

From the initial stocking rate of $25/m^2$ in all the pens the highest survival rate after 60 days was noticed in the experimental pen I and experimental pen III which was $21/m^2$, while the experimental pen II registered a survival of $18/m^2$ and the control had the least of $16/m^2$.

The salinity in all the pens had their minimum values in the first week of August and the maximum values were in the third week of September. The salinity in all the 4 pens were more or less uniform.

According to the correlation matrix the growth data and salinity values from the surface, bottom and 0-5 cm depth were correlated with very high significance.

Previous works on the tolerance of salinity of juveniles of *P. indicus* reveal that the lowest tolerance limit of salinity is 3.9 ppt (Shylaja 1989, *M. Sc. dissertation*. Cochin Univ. Sci. & Tech., pp. 48). But the present study reveals that the culture prawns tolerated a low salinity of 1.16 ppt in the bottom water. This could be due to the higher saline water of the 0-5 cm depth and the burrowing habits of the particular species. The higher correlation of the growth parameters *viz.* length and weight with the salinity of the interstitial water than that with the salinity of the bottom water strengthens the possibility of prawns seeking the sub-soil environment in adverse conditions.

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STATISTICAL EVALUATION OF PLANKTON DIVERSITY IN MANGROVE ECOSYSTEMS IN COCHIN AREA

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Introduction

Mangrove forests are complex intertidal, soft substrate habitats that occur circumtropically and comprise a substantial portion of protected coasts and estuaries. As a unique protective margin between land and sea, mangrove swamps attract faunal components from adjoining terrestrial and aquatic ecosystems in addition to harbouring many indigenous animal species.

In almost all studies of mangrove ecosystems the role of zooplankton has been neglected or mentioned only briefly, because most of the studies in mangrove ecosystems are related to either productivity or their associated benthic fauna. A wide variety of holoplanktonic and meroplanktonic organism appears in the zooplankton of mangroves.

How healthy an ecosystem can well be explained by the richness of the species and the evenness of the distribution of the species to which it provides habitat niche. One of the prime objectives of the present study is to focus on this pivotal problem of the richness of the mangrove system in respect of various groups of phytoplankton and zooplankton and how it varies in relation to hydrographic characteristics. This is accomplished by constructing what are called diversity indices and by studying their behaviour in relation to hydrographic parameters.

Diversity indices are statistical tools for analysing ecological patterns and this help to understand , in a given community,

how many species or groups are there and what are their relative abundance and how many are rare and how many are common.

Diversity is composed of two components, the total number of species or groups and how the abundance of data are distributed among the species. The number of species in the community is often termed as 'species richness' by ecologists. The second component is species evenness on the equitable distribution of the species or groups to the total population.

Indices that attempt to combine both richness and evenness into a single value are refered as diversity indices.

Material and methods

Based on the extent of area, tidal influx and dominance of species of mangrove flora, two stations were selected. The station I is located in 'Mangalavanam' adjacent to CMFRI and station II is in Puthuvyppu in Vypeen Island. Only 3 species of mangrove flora are noticed in station I whereas 7 species in station II.

A total of 21 water and plankton samples each from both the stations were collected during the southwest monsoon in June and continued upto September 1992. Time of sampling was maintained uniformly between 0700 to 1100 hours. The hydrological parameters examined were water and air temperature, salinity, pH, dissolved oxygen and inorganic nutrients such as nitrate, phosphate and silicate. Water samples were collected from the surface in narrow 250 ml polythene bottles. Weekly and monthly rainfall data were obtained from Daily Weather report issued from the Indian Meteorological Department. Since both the sampling stations were shallow and less than 1 m deep, only surface samples were taken.

Salinity, dissolved oxygen and nutrients were estimated by standard methods.

For the study of phytoplankton settling method was used. A 10% neutral formalin was used to fix the phytoplankton at the time of collection itself and was shaken well for uniform mixing.

The phytoplankton were counted using the Sedgewick Rafter chamber.

Zooplankton samples were collected from both the stations by filtering, 1 m^3 (1000 liters) of water through a handnet made of bolting silk of mesh size 0.33 mm, with a collecting bucket. The filtering was carried out with a plastic bucket of 10 litre capacity, by pouring quickly drawn 100 buckets of water through the net. The zooplankters collected in collecting bucket were preserved in 5% formation. Results are expressed as total numbers of organisms of each broad taxonomic group per cubicmeter.

Samples were analysed for qualitative and quantitative study of zooplankton groups. As the plankton samples from mangroves contains detritus, leaves, twigs, fruits, etc. the displacement volume did not give satisfactory results. Quantitative estimation was therefore made in terms of number of zooplankton present, zooplankton seperated from the debris were counted and grouped into major categories.

The results obtained through the investigation were statistically treated by suitable statistical methods to obtain diversity indices, richness indices and evenness of phytoplankton and zooplankton seperately and to relate them to the ecological parameters. Correlation analysis was carried out to ascertain the association of various hydrographic parameters with the counts of zooplankton and phytoplankton. Students 't' test was applied to test the hypothesis whether the mean counts of the two stations are the same. Meteorological parameters were correlated with various plankton groups.

Results

The temperature and pH variations showed a similar trend in both stations. Salinity variations were comparatively low in station I. The minimum value of 0.11 ppt was obtained in the first week of August and a maximum of 4 ppt was recorded by the end of September. In station II highest salinity was recorded during second week of June which declined gradually and increased by second week of August. The values

raged between 8 ppt and 11.3 ppt. In the 't' test conducted for obtaining the equality of the mean values of the two stations salinity differ significantly. ($t = 8.54$, $p = 0.0000$).

The dissolved oxygen content in station I was higher than that in station II, on almost all observations. But the 't' test showed that dissolved oxygen did not show significant difference in two stations ($t = 0.4484$, $p>0.65$).

Among the nutrients the phosphate readings were fluctuating between 3.8 and 16.0 µg at/l in station I. The maximum value obtained in the third week of July and the minimum in the third week of September. A maximum of 19.4 µg at/l was recorded in the 4th week of August and a minimum of 5.33 µg at/l in the third week of September was obtained in station II.

The silicate in station I, fluctuated between 10.0 µg at/l and 109.6 µg at/l. The values recorded in June were lower, but steadily increased and attained the maximum in the third week of July. Comparatively higher values were recorded in September in Station I. The values recorded in station II did not show high variations, the minimum of 30.3 µg at/l was recorded in the fourth week of July and the maximum of 98.7 µg at/l during the 4th week of September.

The 't' test conducted for nutrients showed that only phosphate mean value differed significantly.

The water depth ranged between 0.28 m and 0.95 m in station I and 0.40 - 0.95 m was obtained in station II.

Rainfall : A total of 2409 mm rainfall occurred during June to September. A maximum of 84 mm of rain occurred during the last week of June.

Biological parameters : Diatoms were the most important phytoplankton observed at both the stations. They were constituted mainly by 5 genera : *Coscinodiscus, Fragilaria, Navicula, Pleurosigma* and *Nitzschia.* In station I, among the major diatoms, *Coscinodiscus* was low during most of the observations with an exception in the observation 6 (in the middle week of

July) and was found 52%. A similar pattern was also observed in station II, with maximum contribution in observation 2 (last week of June). The cell count of *Fragilaria* ranged from 60-6210 at station I and from 412 to 82,700 at station II.

Fragilaria at station I was maximum in observation II. (94%) and observation 15 (81%). The cell count of *Navicula* ranged from 20 to 364,000 at station I and from 40 to 14,400 at station II. The peak values were observed in last week of September. A peak count of 50,000 cells per litre for *Pleurosigma* was observed in September last at station I and 72,500 cells per litre at station II in the middle week of September.

Cyanophyceae was represented by *Oscillatoria*. The peak value of cell count was found to be 3,66,300 cells/litre in station I in the first week of September and 2,55,000 in the last week of August in Station II. The percentage was low upto observations 14 and maximum percentage were recorded in observations 16 (First week of September) at station I and observations 15 and 20, first and last week of September at Station II.

Minor phytoplankton : The groups which occurred few in number and absent in most of the observations are regarded as minor phytoplankton groups. They include, dinoflagellates and diatoms like *Thalassiosira, Skeletonema* and *Biddulphia.*

Zooplankton : Based on abundance and the total number of zooplankton 2 groups were recognised. The major groups existed are copepods, mysids, Amphipods, decapod larvae and fish eggs and larvae. Cladocera, tanaids and brachyuran larvae were treated as minor groups. Among the major zooplankton copepods are the most abundant in terms of both number and percentage of occurrence. Next abundant group is the fish eggs and larvae.

The important group of the minor group of zooplankton that dominated at the station I were cladocera and 'others'. At station II, brachyuran larvae were observed in almost all observations and were the dominant group.

The indices of diversity 1.1' showed the trend similar to richness index R in respect of major groups of phytoplankton. The trend is almost similar in both stations.

The fluctuations are also similar in the case of zooplankton. This showed that as the evenness index is more or less stable, the diversity is expected to follow the trend of richness.

In order to ascertain whether the number of groups in the stations bear any associationship with any of the nutrients, correlation coefficients were worked out. It is found that the number of groups was correlated with nitrate. ($r = 0.406$, $p < 0.01$). And also tested whether the diversity index bears any relationship with nitrate. It is observed that diversity index H' is correlated with nitrate ($r = 0.50$, $p < 0.01$).

Linear correlation coefficients were estimated between major groups of zooplankton and phytoplankton, in order to test whether the distribution of zooplankton any way influenced the distribution of phytoplankton. In some cases it was observed that some of the groups of phytoplankton are perfectly independent of zooplankton. Significant correlation exists between fish eggs and larvae and some of the phytoplankton groups like *Navicula, Pleurosigma* and *Nitzschia.*

None of the other zooplankton groups exhibited any correlation with any of the phytoplankton group.

Discussion

The hydrological parameters showed wide fluctuations, probably because the study was undertaken during the south-west monsoon period. Since Mangalavanam being located in the mainland, decrease in salinity may be due to the greater influence of land runoff. Salinity plays an important role in zonation and distribution of mangrove trees. The higher salinities reported at station II may therefore contribute to the high species diversity.

Dissolved oxygen and pH are similar to the observation reported from other mangroves. The pH values are close to

neutral with slightly alkaline conditions during certain observations. Dissolved oxygen values tend to be low in mangrove ecosystems, probably due to the presence of high oxidisable matter organising from mangrove litter. The reduced dissolved oxygen at both the stations indicates that there is an increased sediment bacterial activity on the organic matter and a consequent utilization of oxygen from overlying waters. The mean values observed at station 2 is almost twice as that of stations I. This suggests that ground water is probably an important source of phosphorus in mangrove areas. The values of nitrate are similar at both the stations indicating that ground water, estuarine water and mangrove pore water are all equally important. Silicate at station I was found to have linear relationship and hence conservative while at station II the relationship was a week one. There was no distinguishable pattern for phosphate at both the sites while nitrate also show conservative nature at both the stations. Wattaykorn *et al.* (1990, *Esturine Coast. Shelf Sci.*, **31**: 667 - 688) did not find a relationship for both nitrite and nitrate at a mangrove swamp in Thailand, while the other observations are similar to the present observation.

The major hydrological parameters that influenced phytoplankton were salinity, phosphate, nitrate and silicate. Most important among the nutrients was nitrate which influenced 3 genera, while silicate showed relationship with 2 and phosphate effected the abundace of *Fragilaria*. Lee and Choa (1990, *Bull. Mar. Res. Inst. Cheju Natl. Univ;* **14** : 9 - 24) identified water temperature, dissolved oxygen, nitrate and phosphate as the environmental factors that influenced phytoplankton. Durate *et al.* (1992, *Deepsea Res.*, **39** (1): 45-54) concluded phytoplankton heterogeneity is limited in hydrographically complex systems. It was observed that the phytoplankton count at station II was more when compared to Mangalavanam. This may be due to higher phosphate concentrations in station II.

Zooplankton was related to only 2 ecological parameters. Fish eggs and larvae showed a significant relationship with salinity while copepods and amphipods were related to

phosphate. The groups of larvae of finfishes and shellfishes obtained were mainly freshwater and estuarine forms. It showed that the seasonal presence of riverine water may be important to larval fish. The abundance of mature egg bearing copepods in the samples may be an indication that mangrove areas are the breeding sites for important zooplankton groups. In both the stations it was found that zooplankton density was less when compared to adjacent waters like estuaries and backwaters. Qasim (1977, *Proc. Symp. Warm Water Zoopl., NIO Spl. Publ.*, pp. 226 - 241) opined that the representation of zooplankton organisms in the mangrove swamps are few in numbers and groups and their role in the food chain was meagrely known. The low numbers of zooplankton in the mangrove may be due to the shallowness of the area and the high turbid nature of water.

Fundamentally the species or group diversity is a function of the number of species occurring in an environment. It is reasonable to assume that this number of speices or groups is correlated with hydrographic parameters. Correlation coefficient between the number of groups of phytoplankton and other parameters such as nitrate, phosphate, silicate, etc. were estimated. Among the nutrients only nitrate is found to have significant, correlation with number of groups. Since diversity draw information from the number of groups, it is expected that diversity index H' also may be related with nitrate and the study estabilished this assumption, showing correlation coefficient of 0.500 ($p < 0.01$).

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STUDIES ON THE EFFECTS OF EUTROPHICATION IN CULTURE PONDS IN COCHIN

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Introduction

Eutrophication signifies the nutrient enrichment and increased biological productivity of an aquatic system. The problem that arises following the discharge of wastes containing various nutrients into any aquatic ecosystem comes not from the nutrients themselves, but from the changes they induce in the primary productivity of the aquaculture pond. The term eutrophication denotes the lowering or deterioration of water quality for domestic, recreational and other uses. The continuous deposition of organic debris and its degradation by the benthic bacteria, in an eutrophic pond is so great that a variety of nutrients become released into the water which can thus support large population of algae and higher plants.

Of the nutrients entering the pond, a major portion become incorporated into algae which release the nutrients back into water either through phytoplankton excretion and/or dissolution after death. In fact certain blue-green algae can serve to indicate eutrophic conditions. Eutrophic waters rich in selected species of phytoplankton, have large numbers of individuals and phytoplankton blooms will occur frequently. The common algae of such waters belong to Cyanophyta, Bacillariophyta and Chlorophyta. Eutrophic ponds are generally turbid, muddy and enriched due to heavy nutrient load and phytoplankton density. In heavily eutrophic ponds, dissolved oxygen level will deplete to lethal limits during night hours, owing to the utilization of available dissolved oxygen by the existing living biomass for their respiration.

Eutrophication is always accompanied by a change in speices composition of the aquatic biota, which may be either directly due to nutrient enrichment or may be caused by some underlying homeostatic factors such as change in grazing pattern of animals. The nutrients once deposited in sediments may continue to diffuse into and enrich the pond water long after further inputs of nutrients have been checked into the pond.

Objectives

The main objectives of the present study are to compare two systems, one eutrophied and other non-eutrophied based on changes in its physico-chemical and biological characteristics, and also to assess the effect of eutrophication in culture ponds in and around Cochin.

Material and methods

Preliminary survey : Prior to the commencement of the work, a preliminary survey was conducted in and around Cochin during first week of March 1992, to fix the sampling stations for regular fortnightly samplings. Initially more stations were sampled to get a general idea about entrophied and noneutrophied stations in the area. Based on the preliminary study four stations were selected for regular sampling.

Study area : The present study was confined to the perennial and seasonal ponds at Narakkal, Cherai and Cheranelloor Villages of Ernakulam District during March to September 1992.

Station I : A seasonal pond having an area of 0.5 ha with an average depth of 68.7 cm and located at Cheranelloor.

Station II : One of the seasonal ponds having an area of 0.4 ha with an average depth of 52.0 cm and is located near Cherai junction.

Station III : The Coconut channels located near Matsyafed farms at Narakkal. These Coconut channels having an average depth of 64.9 cm, was stocked with fish seeds by the farmers.

Station IV : KVK Ponds at Narakkal. The ponds having an area of 0.2 ha with an average depth of 56 cm were under management practices like fertilization and stocking.

Laboratory experiments : Induced eutrophication experiment was conducted in outdoor glass tanks of 500 l capacity each. Control tanks as well as experimental tanks were kept in duplicate. All the tanks were filled with pond water. The experimental tanks were fertilized with phosphate and nitrate fertilizers, (FACT super phosphate 150 g/500 l water, FACT urea 100 g/500 l water and sodium silicate 5 g/500 l water). While the control tanks were kept as unfertilized systems, both control and experimental tanks were provided with a layer of pond sediment at the bottom.

Collection of experimental data : Control and experimental tanks were monitored for a period of 30 days. During the experimental period primary productivity, chlorophyll *a* pigment concentration and nutrient levels from control and experimental tanks were analysed based on standard procedures.

Physico-chemical and biological parameters studied : Nutrients in water and sediments (PO_4, NO_3, NO_2, SIO_3 in pond water and reactive phosphorus and nitrate in sediments), Chlorophyll *a* pigment concentration in water, dissolved oxygen concentration in water, pH of water and sediments, biochemical oxygen demand (5 Day BOD), turbidity of the water, water temperature and water depth and phytoplankton group were studied.

Collection of field data : Biweekly sampling was made regularly from these four stations in duplicate from the last week of March to 1st week of September 1992.

Sample analysis : Water samples brought from the field were analysed on the same day for hydrological properties. Dissolved oxygen by winkler method, reactive phosphate by the method of Murphy and Riley (1962, *Anal. Chem. Acta.*, **27** : 31) where the extinction was measured in spectrophotometer at 885 nm, nitrite by Bendschneider and Robinson method (the absorbance was

measured at 545 nm), nitrate by the method of Morris and Riley as described in Strickland and Parsons (1968, *Bull. Fish. Res. Bd. Canada*, **167** : 310) and silicon by the method of Crow N Robinson as reported by Strickland and Parsons (1968, *loc. cit.*) were estimated.

Phytoplankton groups : From the samples collected, major groups of phytoplankton were identified for their relative abundance using Sedgewick-Rafter Counter.

Phytoplankton pigments : Chlorophyll *a* was estimated according to the method prescribed by Parsons (1984, *A Manual of chemical and biological methods for sea water analysis.* Pergamon Press, pp. 101-104).

Primary productivity : Primary productivity was made by light and dark bottle method.

BOD : was estimated by "unseeded dilution method".

Reactive phosphorus in sediments : Extraction of reactive phosphorus was done by the method described by Jackson (1973, *Soil Chemical Analysis*. Prentice Hall of India, pp. 498).

Nitrate in sediments : The extraction solutionn used was that described by Jackson (1973, *loc. cit.*).

Statistical analysis : The values of each parameter for eleven collections for each stations were fed to the computer for two way analysis of variance and the test of significance between stations and also between sampling periods were made.

In order to find the correlation between each parameters, correlation matrix method was followed.

Results and discussion

Water depth in the ponds studied was found to be influenced by tidal inflow, fresh water discharge and land drainage. Temperature and reactive phosphorus had an inverse relationship with depth in all the stations. Temperature had a positive correlation with Chlorophyll *a*, nutrients and pH of water. Turbidity of water showed direct relationship with

silicate and Chlorophyll *a*. Dissolved oxygen showed a positive correlation with nutrients, pH and Chlorophyll *a*. Bacillariophyceae and Chlorophyceae formed the predominant group over Cyanophyceae in all the stations sampled. BOD was always well below permitted level, indicating less pollution level in all sampling stations. A direct relationship was obtained between BOD and turbidity, nutrients and dissolved oxygen in water. Station IV (KVK pond, Narakkal) was significantly different from other three stations at 5% level in Chlorophyll *a* concentration. Changes in Chlorophyll *a* concentration over time was due to varied synergistic effects of turbidity, temperature and other physico-chemical factors. Phosphate level in water had a direct relationship with Chlorophyll *a* and pH of water as well as in sediments. Station I was found to be significantly different in nitrate level from other three stations. Significant negative correlation was obtained between nitrate level and water temperature and positive correlation was obtained with turbidity of water. Distribution of silicate showed no significant difference between stations. Direct relationship existed between silicate level and Chlorophyll content, BOD, dissolved oxygen and pH. Silicate showed an inverse relationship with temperature and depth. Direct significant relationship was obtained between Chlorophyll *a* concentration and net primary productivity in induced eutrophied system, but a less significant correlation existed between Chlorophyll *a* and net primary productivity in the control system. A negative correlation existed between Chlorophyll *a* concentration and number of days in declining phase.

The results of the present investigation showed a good fertilizer response to make the system eutrophic in experimental tank and synergistic effect of physico-chemical and biotic factors lead to the development of eutrophied conditions in experimental tanks as well as in culture ponds sampled during the investigation period.

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

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

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

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

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

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

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

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

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* Out of print.