"STUDIES ON CERTAIN ASPECTS OF ECOLOGY AND BIOLOGY OF TWO PALAEMONID PRAWNS, *Macrobrachium hendersoni hendersoni* (de Man) AND *Macrobrachium hendersoni cacharensis* (Tiwari)"

by

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A D D E N D U M

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EXPLANATIONS ON THE "GENERAL COMMENT"

Regarding the statement "His preface and introduction ..... neither is carried through".

It is true that in the 'Preface' and 'General Introduction,' fishery management and aquaculture potential are dealt with, but the idea of presenting such information was only to provide the necessary background for the present work. In this context the statement that the Thesis is open ended with unclear objectives is not justified since both in the preface (Page i) and in the general introduction (Page 1) there is a clear mention of what the present Thesis contains based on what has been done.

The criticism on the calculation of correlation coefficient is explained as below.

The purpose of such statistical calculations were to establish relationships only, and <u>not</u> for the erection of any a-posteriori hypothesis.

It is admitted that there are lacunae in the methods as presented in the Thesis. However, these lacunae do not refer to the methods employed <u>per se</u>, but only rather to their descriptions that were inadequate in details. This omission further happened because of the very simple nature of most methods employed in this study. However, the detailed descriptions of these methods are provided in the present Addendum.

The criticism by the examiner that "I am unable to find much innovative work in ..... of Macrobrachium" needs an explanation.

It may be pointed out that the present work is the first of its kind in the following respects:

i) The thesis deals with the ecology and biology of two endemic prawn species that are confined only to this part of India.

ii) The present work relates to the prawn fauna of altitudinal streams.

iii) While a great deal of information is available on Indian marine prawn species from a populational and fishery points of view, the present thesis employed an ecosystem approach for the study of the fresh water prawn species.

The above views are further augmented by the commendation of the present work by the other external examiner who is one of the current Indian experts. This examiner stated that "I am well aware that it is a first study of this kind in India from a lotic environment, from hill streams at high altitudes, of species which have become totally adapted to freshwaters throughout their life cycle and also with populations of such magnitude as to contribute, may be seasonally, to the commercial catches of these regions".

EXPLANATIONS ON THE "DETAILED COMMENT"

The General Introduction was considered too long. Accordingly, the distantly related topics like Review on Marine Works and Aquaculture have been omitted and revised as below.

1. General Introduction

Prawns have great significance in the life of mankind, being an important natural source of protein (Menu-Merqua and Morales, 1974) and providing certain other useful products (Sakthivel, 1976). In view of the ever increasing demand for shrimps both for local consumption and for export purposes, the Indian prawns have attracted the attention from a rapidly developing fishing industry as well as from biologists for scientific study. But most of the notable works in India include only the marine species.

Tiwari (1955) reported the taxonomy of more than 34 species of freshwater prawn of the genus Macrobrachium from Indian inland waters, inclusive of both lotic and lentic systems. But till now, the information available does not cover the biology of all these reported species. However, in the recent years, several Indian workers have contributed to a considerable amount of information on various aspects of selected freshwater species (Rajyalakshmi, 1961, 1966, 1980, a, b; Ibrahim, 1962; Raman, 1964; Pillai, 1965; Subrahmanyam, 1966; Gupta, 1967; Nagabhushanam and Vasantha, 1967, 1968;

Pandey, 1967; Rao, 1967; Tyagi and Prakash, 1967; Koshy, 1969, 1973; Nagabhushanam and Chinnayya, 1968; Rajyalakshmi et al., 1968; Rajyalakshmi and Ranadhir, 1969, 1974; Rasalan et al., 1969; Tiwari and Pillai, 1971; Pillai and Mohamed, 1973; Jalihal and Sankolli, 1975; Goswami et al., 1977; Katre, 1976; Katre and Reddy, 1977; Nagabhushanam and Jyoti, 1977; Murthy, 1978; Sharma and Tiwari, 1978; Ghate and Mulherkar, 1979; Nagabhushanam and Kulkarni, 1979, 1981; Sukumaran and Kutty, 1979; Murthy and Saxena, 1980; Anantharaman et al., 1981; Rao et al., 1981; Saxena and Murthy, 1981, 1982).

It is common knowledge that in order to understand the bionomics of locally available species, investigations on the basic ecology and biology of different species become imperative. Most of the investigations today take into account these view points depending upon the particular need of a region, state or even a country. As such a vast amount of literature on few selected freshwater prawns has accumulated over the years in different parts of the world (Schmitt, 1933; Gunter, 1937; Mori, 1939; Hedgneth, 1949; Holthuis, 1949; Riek, 1951; Maglhaes and Pintu, 1959; Parry, 1961; Johnson, 1963, 1966, 1967, 1968, 1973; Tobia, 1964; Lewis and Ward, 1965; Costa, 1966a, b, 1970; Lewis et al., 1966; Mistakidis, 1966; Carrillo, 1967; Antheunisse et al., 1968; Costlow, 1968; De La Cruz, 1968; Denne, 1968; Apollonio, 1969; Kwon and Uno, 1969; Choudhury, 1970; Chung, 1970; Fielder, 1970; Fujimura

and Okamoto, 1970; Holthuis and Provenzano, 1970; Bailey and Crichton, 1971; Kamiguchi, 1972a, b; Yu and Mijake, 1972; Fujeno and Baba, 1973; Ruello *et al.*, 1973; Stoffel and Huschman, 1974; Wickins and Beard, 1974; Fielder *et al.*, 1975; McVey, 1975; Sandifer *et al.*, 1975; Ngoc-Ho, 1976; Martin, 1976; Thebault and Le Gal, 1978; Lee and Fielder, 1979, 1981, 1982a, b, 1983; Mcbride and Muguire, 1979; Beard and Wickins, 1980; Peebles, 1980; Yasuda and Kitoa, 1980; Fair and Fortner, 1981).

Keeping these in view, studies on certain aspects of the ecology and biology of two palaemonid prawns, *Macrobrachium hendersoni hendersoni* (de Man) and *Macrobrachium hendersoni cacharensis* (Tiwari) from the East Khasi Hills of Meghalaya, India, were undertaken. The present study includes the habitat structure, limnological parameters, population dynamics and their relationships. Other detailed biological studies include sexual dimorphism, maturation and spawning, brood size and reproductive efforts, larval development, food and feeding habits, digestive physiology and neuro-endocrine regulation of blood chloride.

Continuation of Explanations on other Comments

2. Study Area

- In page 27 of the Thesis Table 1 is given. Kindly read this Table 1 after the text explained in page 29.
- The recording of vegetational data using symbols (*, -, +, ++, +++) was followed after the quadrat method (Misra, 1968).
- All the species listed in Table 1 (Page 27) as macro-vegetation are terrestrial flora and as such only indirectly influence the functioning of the stream ecosystems.
- The overall criteria for selecting the study area was given in page 19 and this may kindly be referred. The criteria for choosing the sampling stations (A_1, A_2, A_3, B_1, B_2 and B_3) were detailed from page 29, of which the following two criteria were most prominent.
    i) These were the perennial portion of the streams which could be sampled throughout the year by standard stream benthos techniques
    ii) These stations were also found to have relatively adequate prawn populations.

- Regarding the sampling stations, detailed description on depth of water and width of stream at each station were not provided as these parameters were always variable during the annual cycle. However, some of the available data for a particular month (July 1980) is provided below.

The depth of the stations A_1, A_2, A_3, B_1, B_2 and B_3 were 0.60 m, 0.75 m, 0.80 m, 0.82 m, 1.0 m and 1.15 m while the width of the stations A_1, A_2, A_3, B_1, B_2 and B_3 were 8.0 m, 9.2 m, 6.5 m, 6.1 m, 7.2 m and 7.0 m respectively.

- Description of Samoling Sites: Though the sampling stations are described in detail commencing from page 29 of the Thesis (Section 2.8, Sampling Sites), it needs further clarity in the context of the querries raised.

Each sampling station (e.g. A_1, A_2, A_3, B_1, B_2 and B_3) covered a stretch of stream length. Thus, stations A_1, A_2, A_3, B_1, B_2 and B_3 were 18.0 m, 20.5 m, 25.0 m, 20,0 m, 18.0 m and 24.0 m long respectively.

- Page 29: Unit of Length: Please read 18.3 m instead of 60 ft.

3. Material and Methods

The descriptions of the methodology was considered to be inadequate. Accordingly details of the methods used are provided with a view to clarify the ambiguities.

3.1.1. Physico-chemical analysis:

Water samples for physico-chemical analysis were collected usually in the forenoon between 1000-1200 hrs and always at a distance of a metre away from the margin of the streams. Since the depth is a variable factor at each of the sampling stations, samples were always collected from close to the bottom to a height equivalent to the mouth diameter of a plastic bucket of 10-litre capacity. Such samples were obtained at monthly intervals for a period of two years from January 1979 to December 1980. Immediately after collection, each sample was passed through a 0.45 μ filter (Crowther and Hynes, 1977) and stored in a 500 ml plastic bottle. Various parameters were analysed and estimated on the same day.

In addition to the air and water temperature readings measured in the field using a mercury bulb thermometer, maximum and minimum air temperatures, relative humidity, rainfall and wind velocity of the area under study were

collected regularly from the local metereological stations located close to the study areas. The metereological data were primarily collected to infer the overall influence on the study areas in addition to the specific parameters that were measured in each of the streams.

Turbidity was expressed as percentage volume of total suspended matter, transparency as Secchi disc readings (Welch, 1948) and the rate of water flow in cm^3/sec by using a metre tape, stop watch and a simple cork (Leitritz, 1959). pH and conductivity were measured with the use of a Toshniwal pH meter (Model CAT.CL-43) and Elico-conductivity bridge (Type CM-82) and the values expressed as pH units and µmhos/cm respectively.

For oxygen estimation, samples were taken directly from the bottom of the stream in glass bottles of 125 ml capacity and the dissolved oxygen fixed immediately. While sampling, care was taken not to disturb the bottom and air bubbles avoided. Modified Winkler's method (APHA, 1965) was employed for the oxygen estimation. Carbon dioxide content was determined using phenolphthalein indicator and N/44 NaOH, while total alkalinity was measured by using the Standard method (APHA, 1965). Nitrate Nitrogen was estimated by using phenol disulphonic acid method and Ammonia Nitrogen by Nessler's reagent method (Mackereth, 1963). Phosphate Phosphorus was determined by stannous chloride and molybdate

method and chloride by Silver nitrate titration technique (APHA, 1965). Calcium and magnesium were estimated spectrophotometrically. Sodium and potassium ions were estimated by Flame photometry and oxidisable organic matter by Permanganate method. Silicate content was measured by Silico-molybdate method and total iron content by using the method after Mackereth (1963).

3.1.2. Phyto- and Zooplankton

The water samples for plankton analysis were collected from the same sites using the same procedures as for physico-chemical analysis. At each station, samples were collected in triplicate at monthly intervals for a period of two years (January, 1979 to December, 1980). Water sample was collected from the bottom by the 10-litre plastic bucket facing upstream. Each sample consisted of five buckets making up a total of 50 litres of water. The water thus collected was poured through a plankton net made of No. 25 xxx nylon bolting silk (60 μ mesh size). Finally, the actual sample of plankton and water was reduced to 50 ml of water and preserved by adding a few drops of Lugol's iodine. A Sedgwick rafter plankton counting cell of 1 ml capacity was used, having 1000 squares marked on its bottom (Utermohl, 1958). After a thorough stirring of the sample, one ml sub-sample was taken in the counting cell. Nine such sub-samples (three from each

of the triplicate samples) were counted for the estimation of diversity and density of each group of plankton. However, for each sub-sample only 100 squares were counted. The average of these 100 counts for all the nine sub-samples were calculated and these values multiplied by a factor of 10 to make up for the 1000 squares. From this, the computation of the numbers of phyto- and Zooplankton per litre at different stations of the two streams was done by using the formula:

$$n = \left(\frac{a \times 1000}{l}\right) \times c$$

where, n = number of plankton/l of original water.

a = average number of plankton in all counts in the Sedgwick rafter cell.

c = volume of original concentration in ml.

l = volume of original water expressed in litre.

The data were presented in terms of percentage composition of the respective planktonic group.

The generic identification of phyto- and Zooplankton was done with the help of monographs after Smith (1950), Pennak (1953), Edmondson (1959) and Needham and Needham (1962).

General Clarification on the Comments Regarding Section 3.1.1. (Benthic organisms) and Section 3.2 (PRAWN POPULATION STUDY)

The author accepts the comments on the above items gratefully and offers clarity and explanation as given below. These explanations have also necessitated the change in the order of sequence. Thus Section 3.2 on prawn population study is presented first followed by Section 3.1.3. on benthic organisms. This is necessary since the sampling techniques of both these groups involved the same sampler.

3.2. PRAWN POPULATION STUDY

In the present study for the purpose of population analysis, prawns were collected at monthly intervals for the period January 1979 to December 1980. Animals were collected from five sites within each sampling station. The sampler used had a wrought iron frame of one metre square (length x breadth : 1.0 m x 1.0 m) and a height of 1.5 m. The top and the bottom of this box sampler were open, while three of its sides were covered with wire netting of 3.2 mm mesh size. The fourth side was fitted with a removable cloth net of similar mesh size with its tail end extending 1.5 m length. This side of the sampler always faced opposite to the direction of the current. In actual sampling, the sampler was first firmly inserted on to the substratum thus enclosing an area of one square metre of stream bottom. Initially, all the prawns in

the overlying water were scooped out by a hand net, while most others were collecting at the tail end of the cloth net. Further, in order to ensure effective sampling, all the boulders, pebbles on the substratum were also manually disturbed with a view to dislodge the remaining animals.

All animals from the five sites in each station were pooled which comprised a single population sample. The animals were fixed in the field with 5% formalin and brought to the laboratory. The organisms in each sample were then measured and grouped into size categories and sexed. The population density was calculated per square metre of bottom by taking the mean of the total of the five sites in each Station, while the entire sample was used to estimate the sex ratio and length frequency measurements. The berried condition of females were also noted to distinguish the ovigerous from the non-ovigerous individuals.

Having explained the sampling procedure, it is necessary also to explain the discrepancies in Figs. 34-39 as pointed out by the examiner. The N values given in each of the figures (Figs. No. 34-39) refer only to the population density/m^2 while the actual histograms for the length frequency distribution were based on the entire sample from all the five sites in each station. The author regrets for having given the N values in the figures which represents the values per m^2 only without clarifying the above differences.

3.1.3. Benthic Organisms

Benthic samples were collected at monthly intervals for two year period (January 1979 to December 1980). The sampling stations in both streams and the five sites within each station were the same as for the prawn population study. However, in view of the anticipated diminutive size of most benthic organisms, a special dip net of 135 μ mesh size was employed. The net was placed within the box sampler referred earlier in Section 3.2 and most of the organisms in the various stones, pebbles and boulders were dislodged and caught by kicking and raking up the stream bottom.

Each sample thus obtained represents one square metre of the stream bottom. This method may be considered as a further modification of the modified kick sampling method of William and Hynes (1976). The animals collected were then fixed in 5% formalin. In the laboratory, the different groups of benthos were sorted out, counted and their percentage composition computed. Organisms were identified only upto genera wherever possible with the help of treatises of Pennak (1953), Edmondson (1959) and Needham and Needham (1962).

3.1.4. Vertebrate Fauna

The source of material for this study were mostly from the catches of the fishermen, while direct physical observations were always made to supplement the data. The collected

material were fixed in 10% formalin and stored in 70% ethanol after the methods followed by Williams and Coad (1979). Preserved fishes were identified using the key in Day (1978), Hora (1951), Hubbs and Lagler (1964) and Scott and Crossman (1973).

3.3.1. Accessory Habitat Analysis

A number of depressions or in other words 'Supplementary habitats' were located adjacent to both the Umshing and Pongtung main streams. The length of the major axis, minor axis and depth of the depressions were measured with a metre tape. The analysis of their physico-chemical and biological properties were done by employing the same procedures as for the streams though no detailed graphs were presented to express the data. Also, no attempt was made to establish detailed correlations between the abiotic and prawn popula[illegible] as in the case of the streams.

3.3.2. Condition Factor

Individual variations in length-weight relationship have been used to determine the "condition" (Le Cren, 1951). Such factors like 'condition', condition factor or Ponderal index have been calculated by using different formulae by various workers. However, in the present study, the 'condition factor' has been determined by using the following

formula (Hile, 1936; Beckman, 1948):

$$K = \frac{W \times 10^5}{L^3}$$

where K = condition factor; W = dry weight of the prawn; and L = length of the prawn. The number 10^5 is a factor to bring the Ponderal index (K) to near unity (Carlander, 1970).

The examiner has questioned the validity of estimating the condition factor, while suggesting that it may be meaningful if dry weights of the animals are used for calculation. The author has omitted adding the word 'dry' in explaining the formula, whereas only dry weight was used in actual calculations. This omission is regretted. It is also true that apart from the present author, an earlier worker had determined the condition factors in another species of Macrobrachium (Rao, R.M. 1967. Studies on the biology of Macrobrachium rosenbergii (de Man) of the Hooghly estuary with notes on its fishery. Proc. Nat. Inst. Sci., Vol. 33(B), No. 5 & 6, 252-279).

4. Results

4.1.1. Physico-chemical analysis

Regarding the general comments by the examiner expressing concern about most of the physico-chemical analyses, a completely revised chapter on these aspects as provided above may kindly be referred.

4.1.2a. Phytoplankton

In page 58 of the Thesis (bottom 2 lines) it was reported that there is a distinct summer maxima and winter minima in all stations, while the examiner has inadvertently quoted "a distinct summer minima".

Figs. 28-29 were given only as percentages, in order to give an over view of the phytoplankton composition, though the actual numbers are available with the author and are herewith presented in Annexure I. Further, the total phytoplankton data were used elsewhere (Table 13-18) when computing regression equations along with the prawn population data. A similar treatment of the data on Zooplankton and benthos was followed and their actual numbers are also appended in Annexure I.

4.1.2b. Zooplankton

The detailed description of sampling procedures given in Section 3.1.2 shows that the plankton sampling was done close to the bottom of the stream. Therefore, the occurrence of organisms like Difflugia and Vorticella could be explained as having originated from the bottom or from associated vegetation.

4.2.1. Seasonal fluctuation

- Comments refer mostly to correlations, although this aspect was not dealt with under this Section of the Thesis. Nevertheless, as pointed out at the very beginning of this Addendum (Page 1) correlations were worked out only to find out the degree of relationships among the various parameters and nothing more.
- Prawns of less than 20 mm length were never recorded at the sampling stations per se, though considerable numbers were caught in accessory habitats (please refer Fig. 41).

4.2.3. Length frequency distribution

Please refer to explanations provided under Section 3.2.

4.3.1. Accessory habitat analysis

During the rainy season (June-July), the depressions are filled with water from the main stream and serve as suitable places for the prawns to breed. As compared to males, the number of mature and berried females were always found to be higher in these habitats. During September, prawns less than 20 mm size occurred abundantly in these depressions (Fig. 41). This clearly shows that these depressions do serve

as convenient places for egg laying and hatching of larvae. It was also observed that most of the juveniles were restless and had a tendency to escape out of these depressions. This probably indicates that most of the prawns ultimately swim back to the main stream when they reach 20 mm or more, before the drought actually sets in. As an evidence it may be mentioned that the author has noticed the streamward migration of juveniles on several occasions.

4.3.2. Condition factor

This aspect has already been explained in Section 3.3.2.

5. Discussion

The general criticism on the 'correlations' had already been explained earlier (Page 1) as to justify the need to establish relationship only.

2.1. Sexual dimorphism

The method for measurements of total length and carapace length was followed after the standard work of Truesdale and Mermilloid (1979) on Macrobrachium ohione (Smith) as it was felt adequate for the present study.

2.2. Maturation and spawning

Individuals of size frequency from 35.0 mm to 55.0 mm in *Macrobrachium hendersoni hendersoni* (de Man) and size range of 35.0 to 50.0 mm of *M. hendersoni acharensis* (Tiwari) were used for calculating Gonad Index (GI) (Table 23). Animals in post breeding phase (Table 24) were never included in the above analysis.

2.3. Food and feeding habits

For calculation of Gastrosomatic Index (GSI), the whole gut was always used by dissecting out, whereas for the quantitative analysis of the food, only the foregut contents were considered. Such measurements were also employed by earlier workers (Venkataraman, 1960; Marte, 1980).

2.6. Digestive physiology

pH indicator paper was always used to directly measure in all parts of the alimentary canal. However, some of these readings were confirmed and verified by a sophisticated pH meter with a very tiny electrode. Even in these cases, the washings of parts of the gut were only minimal just to remove the food particles if any.

4.6. Digestive physiology

The reference to appendages as parts of the alimentary canal was included unfortunately during typing and this error was overlooked. The author acknowledges this mistake. At the same time the lack of including the terms 'mouth' and 'oesophagus' may also be condoned as a gross omission.

Addendum: Dissolved Oxygen (Fig. 12)

The author is aware of the significance of dissolved oxygen fluctuations and the contributing factors as discussed in detail from page 130 to 131. However, the apparent supersaturated values in Fig. 12 could be due to any of the causative factors and difficult to pinpoint precisely. On re-examining the raw data, it is found that the June to September period was also the time of phytoplankton maxima (Annexure I). In addition to this, June to September period is also the high rainfall season with maximum flow rate. It is likely that anyone of the above causes could be attributed to the supersaturated condition, since precautionary measures were always taken while sampling for dissolved oxygen.

Finally, regarding the suggestion of the examiner to include an analytical section on the "recommendation for management or aquaculture", it may be pointed out that the

main objective of this work was only to gather basic and fundamental data that may be of eventual use for aquacultural and management practices. Therefore the author feels reluctant at this stage to offer any recommendations which may sound far-fetched. Such recommendations are also unwarranted in view of the limited scope of the Title of the present Thesis. Nevertheless, ample discussions are provided for both sections of the Thesis and relationships established for the factors studied.

Please refer to the Thesis for all the literature cited in this Addendum.