

forms and the change in PH and formaldehyde content of the preservatives were recorded.

The results show that 2% formaldehyde in distilled water formalin neutralised with excess Sodium tetraborate—( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$ ) with 3% to 5% Potassium oxalate is the best preservative. Sea water is not used for dilution since on addition of potassium oxalate, it precipitates Calcium oxalate. Distilled water rinse is therefore preferable. The Potassium oxalate combines with Calcium carbonate of shells forming insoluble Calcium oxalate, thus preventing their dissolution. 2% formaldehyde in sea water with Calcium carbonate added to saturation is found to be another satisfactory preservative. The excess Calcium carbonate in the preservative can neutralise the acidity and also saturate it to prevent dissolution of calcit in shells. Neutralised formaldehyde is added to specimen tubes to avoid excess Calcium carbonate settling on shells. For maintaining a pH between 7.0 and 7.5, it is necessary to change the preservative once in 6 months. At low pH, owing to its acidity, Calcium carbonate in the shells tend to dissolve. At higher pH (above 8.0) calcareous plankton disintegrate because of the swelling and gelatinisation of protein binding the calcareous salts. Sea water, close to saturation in its calcium content and acting as a buffer, has less dissolution rate. This may explain why huge deposits of shells lie at the bottom of sea undissolved. Brittle nature of the shells is best prevented by the addition of a few drops of glycerine into the preservative.

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Cochin-18, Kerala, September 13, 1973.

1. Balachandran, T., "A review of the nature and causes of deterioration in zooplankton samples, 1973 (In press).
2. —, *Curr. Sci.*, 1973, 42 (9), 322.

#### ON METHODS OF COLLECTION, HANDLING AND STORAGE OF ZOOPLANKTON IN TROPICS

INVESTIGATIONS undertaken to locate the factors causing damage and deterioration in zooplankton samples under tropical conditions (Balachandran, 1973) suggest the use of the following improved

field and laboratory techniques for their better maintenance.

The plankton net with mesh size ranging from  $150 \mu$  to  $500 \mu$  is found to be the best as the larger mesh size of the gear causes damage to organisms due to increased flow of water, and the smaller size, due to clogging. The condition of the catch may be improved by lowering the speed of tow, by reducing the mouth area of the net and by increasing the area of the gauze. Hauls should be of short duration as long hauls which collect large amount of plankton may cause damage to the catch. The closed plankton buckets are preferable to the one with a side window or a bottom filter in order to prevent frictional damage. The practice of hosing down the sides of the net with a strong jet of water must be discontinued to prevent rupture or loss of appendages to zooplankton. During transfer of plankton from the bucket to the fixative, exposure to air must be avoided to prevent formation of artefacts. The fixation has to be carried out without delay to prevent lysis and bacterial growth. The volume of plankton to that of fixative should be in the ratio 1:9. Plankton must be preserved in previously numbered plastic bottles or translucent, strong, relatively unbreakable and if possible impermeable styrene jars having phenolic cap with plastic coated liners with suitable labels. Against the sample number all relevant data shall be entered in the log book. It is advisable to fill the containers completely to avoid sloshing of organisms. Separate hauls must be made for different purposes rather than splitting the same sample. Plankton samples for biochemical studies and biomass estimations are best preserved by freeze-drying. For minimal mechanical damage of the organisms, lengthy cruises must be avoided. During transport to the laboratory and on board the ship, detention of samples at improperly ventilated, warm and humid custom warehouses and on the deck for lengthy periods are best avoided.

When subsampling is inevitable, tap water should not be used without sufficient preservative so as to prevent initiation of bacterial activity and osmotic damage. Measurement of biomass by the method of displacement volume is not advisable since, during this process, the removal of interstitial fluids by shaking, blotting, filtration, etc., are found to cause damage to zooplankton. Use of plankton fractionators and dividers for subsampling may be kept to the minimum. During sorting, exposure to air must be considerably reduced. In tropics, as the laboratory temperature rises upto  $32^\circ \text{C}$ , formaldehyde evaporates causing irritation and unpleasant vapours. This can be substituted with 0.5%

phenoxetol in distilled water as a good sorting medium (Balachandran, 1973) and for sorting, special types of brushes, needles, forceps and fillers have to be used. Specimen tubes having screw caps with plastic coated liners are preferable for storing the sorted specimens. The common practice of immersing tubes of specimens in a jar of preservative has to be discouraged. The volume of plankton to that of storage fluid has to be in the ratio of 1:5 and the containers should be selected accordingly. The concentration and types of additives and the diluents used in the preparation of storage fluids shall depend on the nature of plankton stored. As polythene is permeable to air, glass containers are preferred. Use of rubber washers and liners must be avoided as they melt and swell in due course. Container lids should be rust proof and air tight and must be filled to the brim to avoid air bubbles and drying up of plankton sticking to the sides. Periodic topping up after checking pH can add to improved preservation. Change of preservatives occasionally can be of additional benefit. Specimen jars properly labelled and catalogued are best stored in air-conditioned rooms and preferably in darkness, to avoid damage caused by light and temperature.

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Indian Ocean Biol. Centre, T. BALACHANDRAN,  
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1. Balachandran, T., *A Review of the Nature and Causes of Deterioration in Zooplankton Samples*, 1973. (In press).
2. —, *Propylene Phenoxetol as a Good Sorting cum Preservation Medium in Tropics*, 1973 (In press).

#### A PRELIMINARY NOTE ON THE OCCURRENCE OF CEPHALINE GREGARINES (PROTOZOA : SPOROZOA) IN INSECTS OF KALYANI, WEST BENGAL

THE cephaline gregarines have been the object of intensive study in many parts of the world since Watson<sup>1</sup> and Kamm<sup>2</sup> published their monographs on these parasites. A new classification was proposed by Grassé<sup>3</sup> who also recorded the then known

species of the group. In India, Ray<sup>4</sup> and Ray and Chakravarty<sup>5</sup> first started work on this group of protozoa and more accounts have been published from time to time by other authors. Recently we have undertaken a comprehensive survey work on the occurrence of cephaline gregarines (Protozoa : Sporozoa) in insects in and around Kalyani, West Bengal, India, and also to study the morphology, life-history and bionomics of this group of parasites. The present communication records our survey work on three orders of insects comprising 11 families and 26 species (including one nymphal stage, which could not be identified beyond family level). A brief note on some of our earlier work on the same line has already been published<sup>6</sup>.

For studying the presence of the gregarines, the host insects are collected from fields and gardens in and around Kalyani and brought to the laboratory alive, their gut contents smeared, fixed in Schaudinn's fixative and Bouin's fluid, and subsequently stained in Heidenhain's iron alum-haematoxylin. The entire mid-gut of the parasitized insects is fixed in Bouin's fluid, cut into 5 microns thick sections and stained as above for observing the intra-cellular stages of the parasites. Cysts collected from mid- and hind-gut of insects are kept in moist chambers for development of spores in living condition.

The present work was initiated on February 12, 1973, and our observations upto December 20, 1973 have been recorded in this paper. The orders, families and species to which the host insects belong, the number of specimens examined as well as infected and the percentage of infection have been indicated in Table I. It is noted that while all the six species of the order Orthoptera are infected, none belonging to the order Hemiptera is parasitized, whereas out of the 17 species of coleopteran insects more than 50% carry protozoan parasites of the group. As regards seasonal intensity, infection is very scanty during summer, increases greatly during monsoon and decreases gradually with the fall of temperature during winter.

So far, we have recorded a new genus *Phleobum* with the type-species *P. gigantinum*<sup>7</sup> and a new species *Quadruspinospora chakravartyei*<sup>8</sup>, both cephaline gregarines, from *Phleoba antennata* Brunn. and *Spathosternum* sp. respectively. Preliminary studies show that parasites obtained from other insects belong to the genera *Hyalospora* Chakravarty, 1935, *Gregarina* Dufor, 1828, *Stenophora* Labbé, 1899, *Stylocephalus* Ellis, 1912 and *Quadruspinospora* Sarkar and Chakravarty, 1969, and are likely to be new species. Detailed study of their life-histories, intra-cellular development and sporulation is now being worked out.